Structures of New Aromatics Glycosides from a Japanese Folk Medicine, the Roots of *Angelica furcijuga*

Toshio MORIKAWA, Hisashi MATSUDA, Norihisa NISHIDA, Teruki OHGUSHI, and Masayuki YOSHIKAWA*

Kyoto Pharmaceutical University; Misasagi, Yamashina-ku, Kyoto 607–8412, Japan.
Received July 14, 2004; accepted August 25, 2004

Three new aromatics glycosides, hyuganosides II, IIIa, and IIIb, were isolated from a Japanese folk medicine, the roots of *Angelica furcijuga* KITAGAWA. The structures of the new glycosides were determined on the basis of chemical and physicochemical evidence.

Key words *Angelica furcijuga*; hyuganoside; hyuganol; Japanese folk medicine; phenylpropanoid; neolignan

The Umbelliferae plant *Angelica furcijuga* KITAGAWA is indigenous to Japan (Japanese name, hyugatouki) and the roots have been used for the treatment of hepatopathy, allergosis, inflammation, diabetes, and hypertension as a Japanese folk medicine. During the course of our characterization studies on Japanese folk medicines,[1—6] we have reported the structure elucidation of four acylated khellactone-type coumarins called hyuganins A—D and the vasorelaxant activities of the principal constituents from the roots of *A. furcijuga*.[1] Furthermore, we communicated that the methanolic extract and principal constituents including hyuganosides II and III from this folk medicine showed nitric oxide (NO) production inhibitory and hepatprotective activities.[2] In this paper, we describe a full account of the isolation and structure elucidation of hyuganosides II (**1**), IIIa (**2**), and IIIb (**3**).

The methanolic extract from the fresh roots of *A. furcijuga* cultivated in Miyazaki prefecture, Japan, was subjected to Diaion HP-20 column chromatography to give H_2O-, MeOH-, and acetone-eluted fractions as described.[1,2] The MeOH-eluted fraction was additionally purified by ordinary- and reversed-phase silica gel column chromatographies and finally HPLC to give three glycosides, hyuganosides II (**1**, 0.0030% from the fresh roots), IIIa (**2**, 0.0008%), and IIIb (**3**, 0.0010%) together with 4-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyloxy]-3-methoxypropiophenone[7] (**4**, 0.0009%), hymexelsin[1] (**5**, 0.0006%), and (*R*)-peucedanol 7-*O*-β-D-glucopyranoside[1] (**6**, 0.004%).

Structure of Hyuganoside II (1) Hyuganoside II (**1**) was isolated as a white powder with positive optical rotation ($[\alpha]_D^{25}$ +8.7°, MeOH). The IR spectrum of **1** showed absorption bands at 3410, 1719, 1708, 1613, 1560, 1509, 1075, and 812 cm^{-1} ascribable to glycosidic and carbonyl functions and aromatic ring, while its UV spectrum showed absorption maxima at 230 (sh, log ε 3.82), 285 (3.43), and 323 (3.24) nm. The positive-ion FAB-MS of **1** showed quasimolecular ion peaks at *m/z* 857 $(2M+H)^+$ and 451 $(M+Na)^+$, while quasimolecular ion peaks were observed at *m/z* 855 $(2M-H)^-$ and 427 $(M-H)^-$ in the negative-ion FAB-MS. The molecular formula $C_{20}H_{28}O_{10}$ of **1** was characterized from the positive- and negative-ion FAB-MS and by high-resolution MS measurement. Acid hydrolysis of **1** with 1.0 M hydrochloric acid (HCl) liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[8,9] The aglycon of **1**, hyuganol II (**1a**) with positive optical rotation ($[\alpha]_D^{25}$ +47.3°, MeOH), was obtained by enzymatic hydrolysis of **1** with naringinase. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra[10] of **1** and **1a** indicated the presence of two methyls [**1**: δ 1.22, 1.24 (3H each, both s, 3′-*gem*-CH_3); **1a**: δ 1.21, 1.23 (3H each, both s, 3′-*gem*-CH_3)], three methylenes {**1**: δ [2.55 (1H, br dd, *J*=*ca.* 7, 16 Hz), 2.60 (1H, br dd, *J*=*ca.* 8, 16 Hz), 8-H_2], [2.89 (1H, ddd, *J*=7.6, 7.9, 14.4 Hz), 2.97 (1H, ddd, *J*=7.0, 7.3, 14.4 Hz), 7-H_2], [3.29 (1H, dd, *J*=8.8, 16.2 Hz), 3.35 (1H, dd, *J*=9.5, 16.2 Hz), 1′-H_2]; **1a**: δ 2.51 (2H, t, *J*=7.2 Hz, 8-H_2), 2.78 (2H, t, *J*=7.2 Hz, 7-H_2), [3.03 (1H, dd, *J*=8.5, 15.6 Hz), 3.07 (1H, dd, *J*=9.2, 15.6 Hz), 1′-H_2]}, a methine bearing an oxygen function [**1**: δ 4.55 (1H, dd, *J*=8.8, 9.5 Hz, 2′-H); **1a**: δ 4.54 (1H, dd, *J*=8.5, 9.2 Hz, 2′-H)], and two aromatic protons [**1**: δ 6.43 (1H, d, *J*=8.1 Hz, 6-H), 6.91 (1H, d, *J*=8.1 Hz, 5-H); **1a**: δ 6.19 (1H, d, *J*=7.9 Hz, 6-H), 6.79 (1H, d, *J*=7.9 Hz, 5-H)] together with an β-D-glucopyranosyl part [**1**: δ 4.86 (1H, d, *J*=7.3 Hz, Glc-1-H)]. The planar

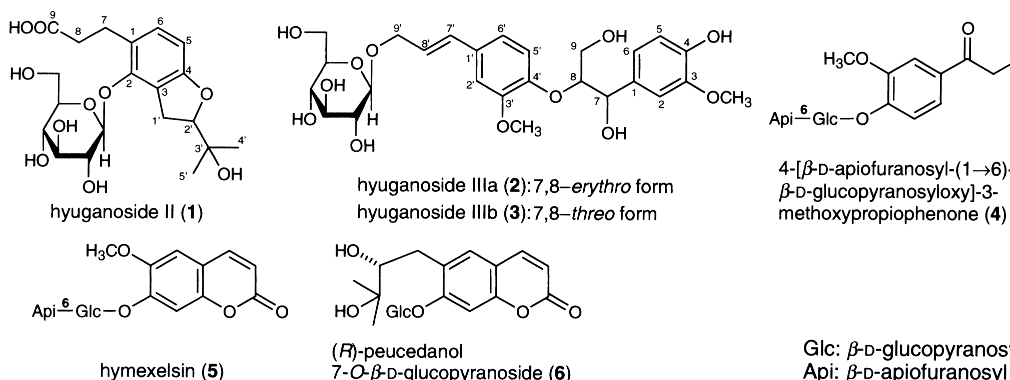

Chart 1

* To whom correspondence should be addressed. e-mail: shoyaku@mb.kyoto-phu.ac.jp
© 2004 Pharmaceutical Society of Japan

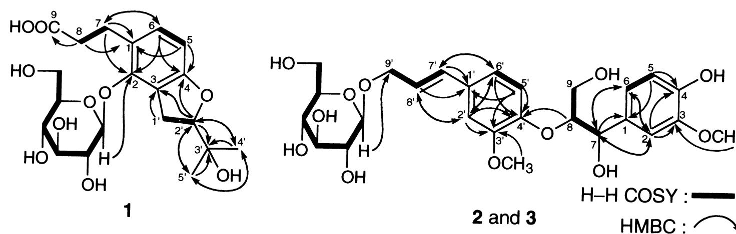

Fig. 1

structure of **1** was constructed on the basis of the $^{1}H–^{1}H$ correlation spectroscopy ($^{1}H–^{1}H$ COSY) and heteronuclear multiple bond correlation (HMBC) experiments as shown in Fig. 1. Thus, the $^{1}H–^{1}H$ COSY experiment of **1** indicated the presence of partial structures in bold lines (C-5—C-6, C-7—C-8, C-1′—C-2′, and Glc-C-1—Glc-C-6). In the HMBC experiment of **1**, long-range correlations were observed between the following protons and carbons: 5-H and 1, 4-C; 6-H and 2, 4, 7-C; 7-H_2 and 1, 2, 6-C; 8-H_2 and 1, 9-C; 1′-H_2 and 3-C; 2′-H and 3, 4, 3′—5′-C; 4′-H and 2′, 3′, 5′-C; 5′-H and 2′—4′-C; Glc-1-H and 2-C (Fig. 1), so that the connectivities of the quaternary carbons, the substitution pattern of the aromatic ring, and the position of the glycosidic linkage in **1**, were clarified. Consequently, the structure of hyuganoside II (**1**) was determined.

Structures of Hyuganosides IIIa (2) and IIIb (3) Hyuganosides IIIa (**2**) and IIIb (**3**) were obtained as a white powder with negative optical rotation (**2**: $[\alpha]_D^{25}$ −6.1°, **3**: $[\alpha]_D^{25}$ −16.9°, both in MeOH), respectively. The IR spectrum of **2** showed absorption bands at 3410, 1607, 1508, 1076, and 1032 cm^{-1} ascribable to hydroxyl, aromatic, and ether functions. In the UV spectrum of **2**, an absorption maximum was observed at 267 (log ε 4.16) nm. The IR and UV spectra of **3** resembled those of **2** [IR: 3432, 1603, 1508, 1076, and 1030 cm^{-1}, UV: 270 (3.96) nm]. The positive- and negative-ion FAB-MS of **2** and **3** showed the same quasimolecular ion peaks at *m/z* 561 $(M+Na)^+$ and 537 $(M-H)^-$, and high-resolution MS analysis revealed the molecular formula of **2** and **3** to be $C_{26}H_{34}O_{12}$. Acid hydrolysis of **2** and **3** with 1.0 M HCl liberated D-glucose.[8,9] The ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (Table 1) spectra[10] of **2** showed signals assignable to two methylenes and two methines bearing an oxygen function [δ 3.60 (2H, br s, 9-H_2), 4.18, 4.40 (1H each, both br dd, *J=ca.* 6, 13 Hz, 9′-H_2), 4.31 (1H, br q, *J=ca.* 5 Hz, 8-H), 4.70 (1H, br s, 7-H)], two methoxyl protons [δ 3.72, 3.73 (3H each, both s, 3, 3′-OCH_3)], two *trans*-olefinic protons [δ 6.21 (1H, ddd, *J*=5.8, 6.1, 15.9 Hz, 8′-H), 6.54 (1H, br d, *J=ca.* 16 Hz, 7′-H)], and six aromatic protons [δ 6.67 (1H, d, *J*=7.9 Hz, 5-H), 6.77 (1H, dd, *J*=1.5, 7.9 Hz, 6-H), 6.86 (1H, dd, *J*=1.5, 8.5 Hz, 6′-H), 6.92 (1H, d, *J*=8.5 Hz, 5′-H), 6.99 (1H, d, *J*=1.5 Hz, 2-H), 7.00 (1H, d, *J*=1.5 Hz, 2′-H)] together with an β-D-glucopyranosyl part [δ 4.21 (1H, d, *J*=7.6 Hz, Glc-1-H)]. As shown in Fig. 1, the $^{1}H–^{1}H$ correlation spectroscopy ($^{1}H–^{1}H$ COSY) experiment on **2** indicated the presence of partial structures written in the bold lines and the HMBC experiment were observed between the following proton and carbon pairs of **2** (2-H and 3, 4, 6, 7-C; 5-H and 4, 1-C; 6-H and 2, 7-C; 7-H and 1, 2, 6-C; 8-H and 4′-C; 2′-H and 3′, 4′, 6′, 8′-C; 5′-H and 1′, 3′, 4′-C; 6′-H and 2′, 4′, 7′-C; 7′-H and 1′, 6′-C; 8′-H and 1′, 2′-C; Glc-1-H and 9′-C; 3-OC$\underline{H}_3$ and 3-C; 3′-OC$\underline{H}_3$ and 3′-C). The stereostructure of the 7 and 8-positions in **2** was clarified by the nuclear Overhauser enhancement spectroscopy (NOESY) experiment on the acetonide derivative (**2b**). Namely, enzymatic hydrolysis of **2** with β-glucosidase followed by treatment with 2,2-dimethoxypropane in the presence of *p*-toluene sulfonic acid (*p*-TsOH) yielded the 7,9-acetonide derivative (**2b**). The NOESY experiment on **2b** showed NOE correlations as shown in Fig. 2, so that the stereostructure of the 7 and 8-positions in **2** was determined to be the *erythro*-form.

Table 1. ^{13}C-NMR Data for Hyuganosides II (**1**), IIIa (**2**), and IIIb (**3**), and Hyuganol II (**1a**)

	1[a]	**1a**[a]	**2**[b]	**3**[b]
C-1	126.2	121.7	133.1	132.8
C-2	153.1	152.8	111.6	110.9
C-3	118.7	114.4	146.9	146.9
C-4	161.8	161.2	145.4	145.3
C-5	105.6	101.7	114.6	114.6
C-6	130.4	130.4	119.5	118.9
C-7	26.7	26.8	71.7	70.9
C-8	36.4	37.3	83.8	84.2
C-9	178.1	179.9	60.2	60.0
C-1′	30.9	29.4	129.7	129.6
C-2′	90.8	90.7	110.2	109.8
C-3′	72.6	72.6	149.7	149.6
C-4′	25.3	25.1	147.9	148.1
C-5′	25.4	25.4	115.7	115.3
C-6′			119.3	119.3
C-7′			131.3	131.3
C-8′			124.0	124.0
C-9′			68.7	68.7
3-OCH_3			55.5	55.3
3′-OCH_3			55.6	55.5
Glc-1	103.8		102.0	102.0
Glc-2	75.6		73.5	73.4
Glc-3	78.1		76.7	76.7
Glc-4	71.5		70.2	70.0
Glc-5	78.2		76.8	76.8
Glc-6	62.7		61.1	61.0

Measured in *a*) CD_3OD and *b*) DMSO-d_6.

On the other hand, the proton and carbon signals of **3** in ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (Table 1) spectra[10] were almost superimposable on those of **2** {two methylenes and two methines bearing an oxygen function [δ 3.25, 3.58 (1H each, both m, 9-H_2), 4.18, 4.42 (1H each, both br dd, *J=ca.* 6, 13 Hz, 9′-H_2), 4.28 (1H, br q, *J=ca.* 5 Hz, 8-H), 4.71 (1H, br s, 7-H)], two methoxyl protons [δ 3.72, 3.80 (3H each, both s, 3, 3′-OCH_3)], two *trans*-olefinic protons [δ 6.24 (1H, ddd, *J*=5.6, 6.1, 15.9 Hz, 8′-H), 6.56 (1H, br d, *J=ca.* 16 Hz, 7′-H)], and six aromatic protons [δ 6.68 (1H, d, *J*=8.1 Hz, 5-H), 6.76 (1H, dd, *J*=1.2, 8.1 Hz, 6-H), 6.89 (1H, br d, *J=ca.* 8 Hz, 6′-H), 6.98 (1H, d, *J*=8.2 Hz, 5′-H), 6.97 (1H, br s, 2-H), 7.06 (1H, d, *J*=1.2 Hz, 2′-H)], and an β-D-glucopyra-

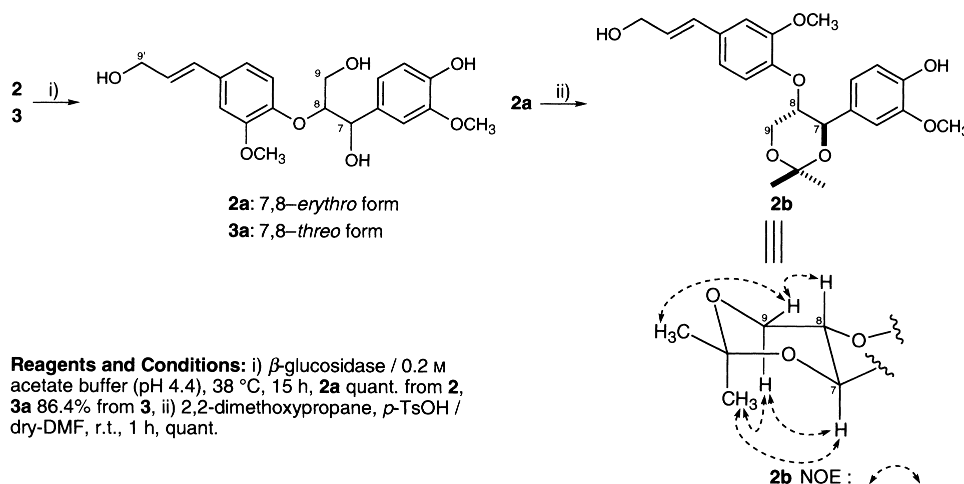

Fig. 2

nosyl part [δ 4.21 (1H, d, J=7.6 Hz, Glc-1-H)]}. By various 2D-NMR experiments, the planar structure of **3** was elucidated to be the same structure of **2**. Treatment of **3** with β-glucosidase furnished its aglycon (**3a**),[11] and the stereostructure of **3** was determined to be the 7,8-*threo*-form.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; EI-MS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JNM-LA500 (500 MHz) spectrometer; ^{13}C-NMR spectra, JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index, Shimadzu SPD-10A*vp* UV-VIS, and Shodex OR-2 optical rotation detectors.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150—350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); TLC, precoated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reverse phase); reverse-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4, followed by heating.

Extraction and Isolation The extraction and isolation from the fresh roots of *A. furcijuga* KITAGAWA (cultivated in Miyazaki prefecture, Japan) were described in our previous paper.[1,2] The methanolic extract from the fresh roots of *A. furcijuga* was subjected to Diaion HP-20 column chromatography to afford H_2O-eluted fraction and three fractions (Fr. 1—3). Fraction 2 was separated by ordinary- and reversed-phase column chromatographies to give five fractions (Fr. 2-7-1—2-7-5).[1,2] Fraction 2-7-3 (554 mg) was purified by HPLC [detection: RI, column: YMC-pack R&D-ODS-5-A, 20×250 mm i.d. (YMC Co., Ltd., Japan), mobile phase: MeOH–H_2O (30 : 70, v/v) and 2-PrOH–H_2O (10 : 90, v/v)] to give hyuganosides II (**1**, 93 mg, 0.003% from the fresh roots), IIIa (**2**, 25 mg, 0.0008%), and IIIb (**3**, 44 mg, 0.001%) together with 4-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyloxy]-3-methoxypropiophenone (**4**, 30 mg, 0.0009%), hymexelsin (**5**, 18 mg, 0.0006%), and (*R*)-peucedanol 7-*O*-β-D-glucopyranoside (**6**, 120 mg, 0.004%).

Compounds **4**—**6** were identified by comparison of its physical data ([α]$_D$, MS, ^{1}H-, and ^{13}C-NMR) with reported values.[1,7]

Hyuganoside II (**1**): A white powder, $[\alpha]_D^{25}$ +8.7° (c=1.11, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{20}H_{28}O_{10}Na$ (M+Na)$^+$: 451.1580. Found: 451.1576. UV (MeOH, nm, log ε): 230 (sh, 3.82), 285 (3.43), 323 (3.24). IR (KBr): 3410, 1719, 1708, 1613, 1560, 1509, 1075, 812 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) δ: 1.22, 1.24 (3H each, both s, 3′-*gem*-CH_3), [2.55 (1H, br dd, J=*ca.* 7, 16 Hz), 2.60 (1H, br dd, J=*ca.* 8, 16 Hz), 8-H_2], [2.89 (1H, ddd, J=7.6, 7.9, 14.4 Hz), 2.97 (1H, ddd, J=7.0, 7.3, 14.4 Hz), 7-H_2], [3.29 (1H, dd, J=8.8, 16.2 Hz), 3.35 (1H, dd, J=9.5, 16.2 Hz), 1′-H_2], [3.70 (1H, dd, J=5.2, 11.9 Hz), 3.85 (1H, dd, J=2.4, 11.9 Hz), Glc-6-H_2], 4.55 (1H, dd, J=8.8, 9.5 Hz, 2′-H), 4.86 (1H, d, J=7.3 Hz, Glc-1-H), 6.43 (1H, d, J=8.1 Hz, 6-H), 6.91 (1H, d, J=8.1 Hz, 5-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 1. Positive-ion FAB-MS: m/z 857 (2M+H)$^+$, 451 (M+Na)$^+$. Negative-ion FAB-MS m/z: 855 (2M−H)$^-$, 427 (M−H)$^-$, 265 (M−$C_6H_{11}O_5$)$^-$.

Hyuganoside IIIa (**2**): A white powder, $[\alpha]_D^{25}$ −6.1° (c=0.22, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{26}H_{34}O_{12}Na$ (M+Na)$^+$: 561.1948. Found: 561.1953. UV (MeOH, nm, log ε): 267 (4.16). IR (KBr): 3410, 1607, 1508, 1076, 1032 cm^{-1}. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 3.46, 3.69 (1H each, both br d, J=*ca.* 12 Hz, Glc-6-H_2), 3.60 (2H, br s, 9-H_2), 3.72, 3.73 (3H each, both s, 3, 3′-OCH_3), 4.18, 4.40 (1H each, both br dd, J=*ca.* 6, 13 Hz, 9′-H_2), 4.21 (1H, d, J=7.6 Hz, Glc-1-H), 4.31 (1H, br q, J=*ca.* 5 Hz, 8-H), 4.70 (1H, br s, 7-H), 6.21 (1H, ddd, J=5.8, 6.1, 15.9 Hz, 8′-H), 6.54 (1H, br d, J=*ca.* 16 Hz, 7′-H), 6.67 (1H, d, J=7.9 Hz, 5-H), 6.77 (1H, dd, J=1.5, 7.9 Hz, 6-H), 6.86 (1H, dd, J=1.5, 8.5 Hz, 6′-H), 6.92 (1H, d, J=8.5 Hz, 5′-H), 6.99 (1H, d, J=1.5 Hz, 2-H), 7.00 (1H, d, J=1.5 Hz, 2′-H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C: given in Table 1. Positive-ion FAB-MS: m/z 561 (M+Na)$^+$ Negative-ion FAB-MS m/z: 537 (M−H)$^-$.

Hyuganoside IIIb (**3**): A white powder, $[\alpha]_D^{23}$ −16.9° (c=0.51, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{26}H_{34}O_{12}Na$ (M+Na)$^+$: 561.1948. Found: 561.1961. UV (MeOH, nm, log ε): 270 (3.96). IR (KBr): 3432, 1603, 1508, 1076, 1030 cm^{-1}. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 3.25, 3.58 (1H each, both m, 9-H_2), [3.45 (1H, dd, J=6.3, 10.6 Hz), 3.69 (1H, br d, J=*ca.* 11 Hz), Glc-6-H_2], 3.72, 3.80 (3H each, both s, 3,3′-OCH_3), 4.18, 4.42 (1H each, both br dd, J=*ca.* 6, 13 Hz, 9′-H_2), 4.21 (1H, d, J=7.6 Hz, Glc-1-H), 4.28 (1H, br q, J=*ca.* 5 Hz, 8-H), 4.71 (1H, br s, 7-H), 6.24 (1H, ddd, J=5.6, 6.1, 15.9 Hz, 8′-H), 6.56 (1H, br d, J=*ca.* 16 Hz, 7′-H), 6.68 (1H, d, J=8.1 Hz, 5-H), 6.76 (1H, dd, J=1.2, 8.1 Hz, 6-H), 6.89 (1H, br d, J=*ca.* 8 Hz, 6′-H), 6.98 (1H, d, J=8.2 Hz, 5′-H), 6.97 (1H, br s, 2-H), 7.06 (1H, d, J=1.2 Hz, 2′-H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C: given in Table 1. Positive-ion FAB-MS: m/z 561 (M+Na)$^+$. Negative-ion FAB-MS m/z: 537 (M−H)$^-$.

Acid Hydrolysis of 1—3 A solution of **1**—**3** (2.0 mg each) in 1.0 M HCl (0.1 ml) were heated under reflux for 1 h. After cooling, the reaction mixture was extracted with AcOEt (0.1 ml). The H_2O layer was analyzed by HPLC under the following conditions [detection: optical rotation, column: Kaseisorb LC NH_2-60-5, 4.6×250 mm i.d., 5 μm (Tokyo Kasei Kogyo Co., Ltd., Japan), mobile-phase: CH_3CN–H_2O (3 : 1, v/v), flow rate: 0.8 ml/min, injection volume: 10 μl, column temperature: room temperature]. Identification of D-glucose present in the H_2O layer was carried out by comparison of its retention time and optical rotation with that of authentic sample. t_R: 12.3 min (D-glucose, positive optical rotation).

Enzymatic Hydrolysis of 1 A solution of **1** (12.6 mg, 0.029 mmol) in 0.2 M acetate buffer (pH 3.8, 1.5 ml) was treated with naringinase (15.0 mg, Sigma), and the solution was stood at 38 °C for 24 h. After treatment of the reaction mixture with EtOH, the mixture was evaporated to dryness under reduced pressure and the residue was purified by ordinary-phase silica gel column chromatography [1.0 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, v/v/v, lower layer)] to give hyuganol II (**1a**, 7.8 mg, quant.).

Hyuganol II (**1a**): A white powder, $[\alpha]_D^{25}$ +47.3° (c=0.41, MeOH). High-resolution EI-MS: Calcd for $C_{14}H_{18}O_5$ (M$^+$): 266.1154. Found: 266.1156.

UV (MeOH, nm, log ε): 215 (3.94), 227 (sh, 3.77), 281 (3.11). IR (KBr): 3400, 1718, 1701, 1611, 1560, 1508, 1061, 798 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) δ: 1.21, 1.23 (3H each, both s, 3′-*gem*-CH_3), 2.51 (2H, t, J=7.2 Hz, 8-H_2), 2.78 (2H, t, J=7.2 Hz, 7-H_2), [3.03 (1H, dd, J=8.5, 15.6 Hz), 3.07 (1H, dd, J=9.2, 15.6 Hz), 1′-H_2], 4.54 (1H, dd, J=8.5, 9.2 Hz, 2′-H), 6.19 (1H, d, J=7.9 Hz, 6-H), 6.79 (1H, d, J=7.9 Hz, 5-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 1. EI-MS *m/z* (%): 266 (M^+, 11), 248 (M^+-H_2O, 30), 190 (100).

Preparation of Acetonide Derivative (2b) A solution of **2** (5.4 mg, 0.010 mmol) in 0.2 M acetate buffer (pH 4.4, 1.0 ml) was treated with β-glucosidase (5.0 mg, Oriental Yeast Co., Ltd., Japan), and the solution was stood at 38 °C for 15 h. After treatment of the reaction mixture with EtOH, the mixture was evaporated to dryness under reduced pressure, and the residue was purified by ordinary-phase silica gel column chromatography [0.5 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, v/v/v, lower layer)] to give an aglycon (**2a**, 3.7 mg, quant.).[11] **2a**: $[\alpha]_D^{24}$ −2.3° (c=0.19, MeOH). High-resolution EI-MS: Calcd for $C_{20}H_{24}O_7$ (M^+): 376.1522. Found: 376.1514. UV (MeOH, nm, log ε): 270 (4.60). IR (KBr): 3450, 1654, 1564, 1509, 1270, 1135, 1030 cm^{-1}. EI-MS *m/z* (%): 376 (M^+, 9), 358 (M^+-H_2O, 43), 206 (65), 180 (53), 163 (22), 153 (22), 137 (100), 124 (53), 91 (67), 77 (48). A solution of **2a** (2.0 mg, 0.005 mmol) in dry-DMF (0.3 ml) was treated with 2,2-dimethoxypropane (0.1 ml) and *p*-toluensulfonic acid (*ca.* 2 mg), and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into H_2O and the whole was extracted with AcOEt. The AcOEt extract was treated in the usual manner to give a residue, which was purified by ordinary-phase silica gel column chromatography [0.5 g, $CHCl_3$–MeOH–H_2O (30 : 3 : 1, v/v/v, lower layer)] to give an acetonide derivative (**2b**, 2.2 mg, quant.).

2b: Colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.51, 1.63 (3H each, both s, –CH_{3eq}, –CH_{3ax}), 3.77, 3.84 (3H each, both s, 3, 3′-OCH_3), 4.00 (1H, dd, J=8.7, 10.8 Hz, 9-H_{ax}), 4.15 (1H, ddd, J=3.7, 8.7, 8.8 Hz, 8-H), 4.16 (1H, dd, J=3.7, 10.8 Hz, 9-H_{eq}), 4.28 (2H, br d, J=*ca.* 6 Hz, 9′-H_2), 4.88 (1H, d, J=8.8 Hz, 7-H), 6.19 (1H, td, J=5.7, 15.9 Hz, 8′-H), 6.44 (1H, d, J=8.2 Hz, 5′-H), 6.47 (1H, br d, J=*ca.* 16 Hz, 7′-H), 6.72 (1H, dd, J=2.1, 8.2 Hz, 6′-H), 6.83 (1H, d, J=2.1 Hz, 2′-H), 6.86 (1H, d, J=8.1 Hz, 5-H), 6.99 (1H, d, J=1.8 Hz, 2-H), 7.02 (1H, dd, J=1.8, 8.1 Hz, 6-H).

Enzymatic Hydrolysis of 3 A solution of **3** (6.0 mg, 0.022 mmol) in 0.2 M acetate buffer (pH 4.4, 1.0 ml) was treated with β-glucosidase (5.0 mg, Oriental Yeast Co., Ltd., Japan), and the solution was stood at 38 °C for 15 h. After treatment of the reaction mixture with EtOH, the mixture was evaporated to dryness under reduced pressure, and the residue was purified by ordinary-phase silica gel column chromatography [0.5 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, v/v/v, lower layer)] to give an aglycon (**3a**, 7.2 mg, 86.4%).[11] **3a**: $[\alpha]_D^{24}$ −7.8° (c=0.20, MeOH). High-resolution EI-MS: Calcd for $C_{20}H_{24}O_7$ (M^+): 376.1522. Found: 376.1528. UV (MeOH, nm, log ε): 272 (4.73). IR (KBr): 3453, 1655, 1561, 1509, 1271, 1136, 1032 cm^{-1}. EI-MS *m/z* (%): 376 (M^+, 6), 358 (M^+-H_2O, 9.2), 206 (45), 180 (56), 163 (23), 153 (51), 137 (100), 124 (57), 91 (43), 77 (51). The aglycon (**3a**) was identified by comparison of the ^{1}H- and ^{13}C-NMR data with reported values.[11]

References and Notes

1) Matsuda H., Murakami T., Nishida N., Kageura T., Yoshikawa M., *Chem. Pharm. Bull.*, **48**, 1429—1435 (2000).
2) Matsuda H., Murakami T., Kageura T., Ninomiya K., Toguchida I., Nishida N., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **8**, 2191—2196 (1998).
3) Matsuda H., Morikawa T., Tao J., Ueda K., Yoshikawa M., *Chem. Pharm. Bull.*, **50**, 208—215 (2002).
4) Tao J., Morikawa T., Toguchida I., Ando S., Matsuda H., Yoshikawa M., *Bioorg. Med. Chem.*, **10**, 4005—4012 (2002).
5) Morikawa T., Tao J., Toguchida I., Matsuda H., Yoshikawa M., *J. Nat. Prod.*, **66**, 86—91 (2003).
6) Morikawa T., Tao J., Ueda K., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **51**, 62—67 (2003).
7) Yuan Z., Tezuka Y., Kadota S., Li X., *Chem. Pharm. Bull.*, **50**, 73—77 (2002).
8) Yoshikawa M., Morikawa T., Kashima Y., Ninomiya K., Matsuda H., *J. Nat. Prod.*, **66**, 922—927 (2003).
9) Morikawa T., Sun B., Matsuda H., Wu L. J., Harima S., Yoshikawa M., *Chem. Pharm. Bull.*, **52**, 1194—1199 (2004).
10) The ^{1}H- and ^{13}C-NMR spectra of **1**—**3**, and **1a** were assigned with the aid of distortionless enhancement by polarization transfer (DEPT), homo- and hetero-correlation spectroscopy (^{1}H–^{1}H, ^{13}C–^{1}H COSY), and HMBC experiments.
11) Li S., Lundquist K., Wallis A. F. A., *Phytochemistry*, **49**, 2125—2128 (1998).