Chemical Constituents from the Peels of *Citrus sudachi*

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A methanol extract of the peels of *Citrus sudachi* gave five new compounds (**1**−**5**) and 27 known compounds. The structures were elucidated on the basis of spectroscopic evidence. Several of these compounds were assayed for antimicrobial activity against methicillin-resistant *Staphylococcus aureus* and *Helicobacter pylori*, and sudachitin (**6**) and 3′-demethoxysudachitin (**7**) were the most active.

Citrus sudachi Hort. ex Shirai (Rutaceae), called "sudachi", is an evergreen tree that is found mainly in Tokushima Prefecture in Japan.[1] Sudachi is a very well-known citrus fruit in the south part of Japan. Annual production of the fruit is 8000 tons. Half is sold as an acidulant for seasoning, and the remaining half is used for juice.[2] The residue of sudachi juice remains as food industrial waste.[3] We are seeking uses for industrial waste.

There are some reports on the constituents of the fruits of *C. sudachi*.[4−7] Previously we reported the isolation of four limonoid derivatives from the seeds of *C. sudachi*.[8] In this paper, we report the isolation and structural elucidation of five new compounds, including two limonoids (**1**, **2**), one ferulic acid derivative (**3**), two flavonoid glycosides (**4**, **5**), and 27 known compounds from the peels of *C. sudachi*.

Results and Discussion

The methanolic extract of freeze-dried peels of *C. sudachi* was partitioned between ethyl acetate and water. The aqueous layer was sequentially partitioned with *n*-butanol. The ethyl acetate and *n*-butanol phases were concentrated in vacuo, respectively, and then subjected to repeated column chromatography over silica gel, Toyo pearl HW-40, Sephadex LH-20, and high-performance liquid chromatography (HPLC) to yield compounds **1**−**5**.

Compound **1** was assigned the molecular formula $C_{26}H_{32}O_{10}$ on the basis of HRFABMS. The IR spectrum revealed absorption bands for −OH, lactone carbonyl, and ketone carbonyl groups. The ^{1}H NMR spectrum showed five methyl groups [δ_H 1.60, 1.44, 1.26 × 2, 1.19 (each 3H, s)] and three methine protons [δ_H 5.40 (s), 3.87 (m), 3.86 (s)] adjacent to oxygen functions. The ^{13}C NMR and DEPT spectra showed 26 carbon signals for five methyl, four methylene, seven methine (one acetal, one olefinic, and three oxygen bearing), and 10 quaternary carbons (one olefinic, two oxygen bearing, and four carbonyl). From the above data, **1** was considered as a limonoid derivative, and its NMR spectroscopic data were compared to those of limonoids reported previously. The ^{1}H and ^{13}C NMR data of **1** were very similar to those of deacetylnomilin,[9] except for signals of a furan ring moiety in deacetylnomilin. Instead of the signals of a furan ring moiety at C-17 in deacetylnomilin, the signals of an α,β-unsaturated butyrolactol function [δ_H 7.42 (1H, brs), 6.23 (1H, brs); δ_C 172.0, 153.3, 133.8, 99.1] were observed in **1**. The connection between C-17 and C-20 was confirmed on the basis of the long-range correlation between the proton signal of H-17 and the carbon signal of C-20. The relative configuration of **1** was deduced to be the same as that of deacetylnomilin by the analysis of its NOESY spectrum. Thus, the structure of **1** is determined to be 21,23-dihydro-23-hydroxy-21-oxodeacetylnomilin, as shown.

Compound **2** was assigned the molecular formula $C_{29}H_{38}O_{12}$ (HRFABMS). Comparison of the NMR data of **2** and **1** showed good agreement except for the signals of the A-ring moiety, suggesting that **1** and **2** have the same structure in rings B−E. The signals for the A ring in **2** resembled those of nomilinic acid[10] except for the signal of a methoxyl group in **2**. The presence of a methyl ester moiety was indicated by the long-range correlation between the methoxyl group and C-3 in its HMBC spectrum. Accordingly, **2** was established to be 3-*O*-methyl-21,23-dihydro-23-hydroxy-21-oxonomilinic acid. Compound **2** seems to be an artifact from the process of extraction and isolation.

Compound **3** was assigned the molecular formula $C_{17}H_{18}O_{10}$ (HRFABMS). On comparing the ^{1}H and ^{13}C NMR data of **3** with the literature, they were found to be similar to those of mucic acid 1,4-lactone 3-*O*-gallate.[11] The signals of an (*E*)-feruloyl and a methoxyl group were present in the spectra of **3**, instead of the signals of a galloyl group in mucic acid 1,4-lactone 3-*O*-gallate. The locations of (*E*)-feruloyl and methoxyl groups at C-3′ and C-6′, respectively, were confirmed by the following long-range correlations in its HMBC spectrum: H-5′ (δ_H 4.60), OCH_3 (δ_H 3.62) with C-6′ (δ_C 172.4); H-3′ (δ_H 4.93) with C-9 (δ_C 167.5). The relative configuration of **3** was determined by comparison of coupling systems in its ^{1}H NMR spectrum with mucic acid 1,4-lactone 3-*O*-gallate and was supported by analysis of the NOESY spectrum. However, the absolute configuration of **3** could not be assigned.

Compound **4** had the molecular formula $C_{29}H_{32}O_{16}$ (HRFABMS). The ^{1}H NMR spectrum showed the presence of one methyl group, two methoxyl groups, two methylene protons, one olefinic proton, one 1,4-disubstituted aromatic ring, and one sugar moiety. The ^{13}C NMR data of **4** were very similar to those of sudachiin C,[12] except for the signals arising from the B ring and the number of methoxyl groups (two methoxyl groups in **4**; three in sudachiin C). These data indicated that **4** had a 1,4-disubstituted benzene as a B ring instead of a 1,3,4-trisubstituted benzene as in sudachiin C. Consequently, the structure of **4** was determined to be 3′-demethoxysudachiin C.

Compound **5** had the molecular formula $C_{28}H_{32}O_{15}$ (HRFABMS). Comparison of the ^{1}H and ^{13}C NMR spectra of **5** with those of **4** indicated the presence of a β-glucose-6-*O*-(3-hydroxy-3-methylglutanyl)glucosyl moiety in **5**. The remaining ^{1}H and ^{13}C NMR signals of **5** revealed the presence of a 5,7,4′-trihydroxy-3′-*O*-methylflavanone (homoeriodictyol) moiety.[13,14] Long-range correlation between H-1″ (δ_H 4.96) and C-4′ (δ_C 147.9) indicated the β-glucose-6-*O*-(3-hydroxy-3-methylglutanyl)glucosyl moiety was connected to C-4′. Acid hydrosis of **5** gave 2*S*-homoeriodictyol. Therefore, compound **5** was determined to be 4′-β-D-glucosyl-2*S*-homoeriodictyol 6″-*O*-3-hydroxy-3-methylglutarate.

The absolute configuration of the 3-hydroxy-3-methylglutaric acid moiety of **4** and **5** could not determined due to insufficient materials.

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Chart 1

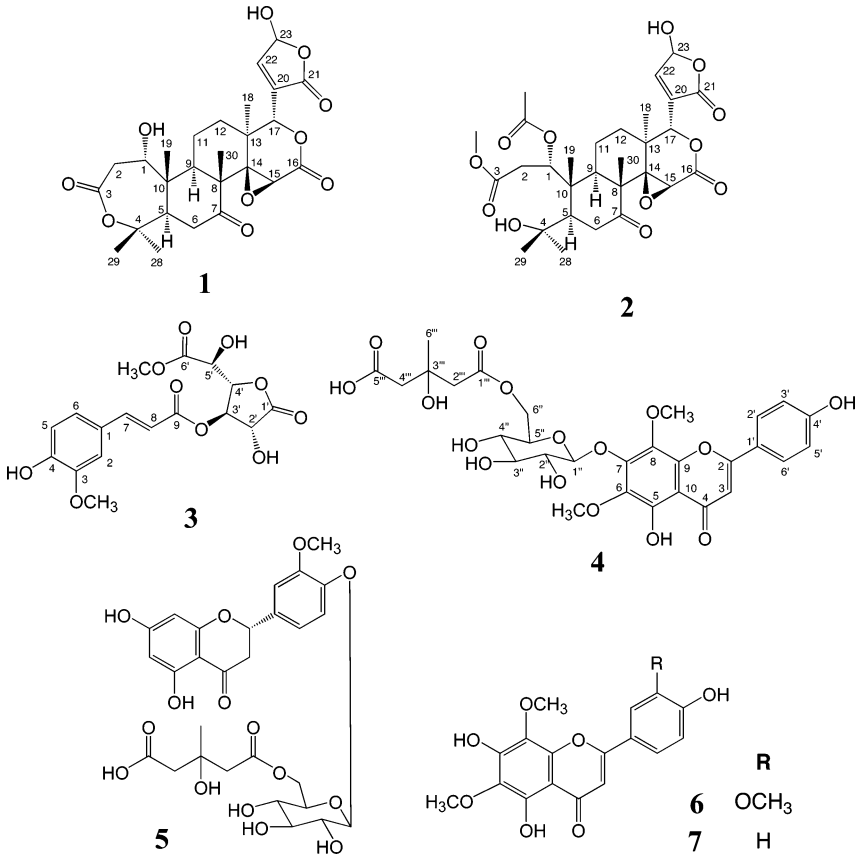

Table 1. Antibacterial Activity of Compounds **6** and **7** against Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Helicobacter pylori*.

		inhibition zone in diameter (mm)[a]		
		MRSA		
compound	amount (μg/disk)	COL	no. 5	*H. pylori* ATCC43504
6	100	12.0	25.4	14.0
7	100	12.5	23.8	10.5
amoxicillin	0.25	NT[b]	NT[b]	33.0
cephapirin	20	8.2	9.4	NT[b]

[a] Disk size: 6 mm (diameter). [b] Not tested.

Known compounds were also isolated as follows: isoobacunoic acid, limonin, methyl deacetylnomilinate, nomilinic acid, vanillic acid, 1*S*,2*S*,4*R*-limonene-1,2-diol, (+)-4*S*-7-hydroxypiperitone, methyl ferulate, ferulic acid, citrusin III, citrusin IX, sudachitin (**6**), 3′-demethoxysudachitin (**7**), 7-methylsudachitin, xanthomicrol, jaceosidin, sudachiin B, sudachiin C, prunin, narirutin, naringin, hesperidin, neohesperidin, eriocitrin, poncirin, hesperetin 7-*O*-(2″,6″-di-*O*-α-rhamnopyranosyl)-*β*-glucopyranoside, naringenin 7-*O*-(2″,6″-di-*O*-α-rhamnopyranosyl)-*β*-glucopyranoside. The known compounds were identified from spectral data comparison with the literature (see Supporting Information).

The isolated compounds were screened for antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Helicobacter pylori* (*H. pylori*) using the disc-diffusion test. Among the compounds tested, sudachitin (**6**) and 3′-demethoxysudachitin (**7**) showed significant antibacterial activity (Table 1) at a concentration of 100 μg/disk. Further investigation for anti-MRSA activity of **6** and **7** was carried out, and both of them showed weak MIC_{50} values of 125 μg/mL against 20 strains of MRSA and 250 μg/mL against seven strains of MSSA.

Experimental Section

General Experimental Procedures. NMR spectra (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR, both using TMS as internal standard) were measured on a Bruker AVANCE-400 instrument. MS were obtained on a JEOL JMSD-300 instrument. CC: Silica gel 60 N (Merck), Toyopearl HW-40 (Tosoh), Sephadex LH-20 (Pharmacia). HPLC: GPC (gel-permeation chromatography: Shodex H-2001, 2002, $CHCl_3$; Asahipak, GS-310 2G, MeOH), silica gel HPLC (Kanto Chemical, Mightysil Si 60, 250 × 20 mm). ODS: Mightysil RP 18 GP, 250 × 20 mm. IR spectra were recorded on a 1720 infrared Fourier transform spectrometer (Perkin-Elmer). Optical rotations were measured with a JASCO DIP-370 digital polarimeter.

Plant Material. The peel of *Citrus sudachi* was collected from Tokushima Prefecture in Japan in September 2003. A voucher specimen (SFB0001) is deposited in the Graduate School of Pharmaceutical Sciences, University of Tokushima, in Japan.

Extraction and Isolation. The freeze-dried peel (3.3 kg) of *C. sudachi* was extracted with MeOH. The MeOH extracts were concentrated in vacuo to give a residue (1.28 kg), which was suspended in H_2O and partitioned sequentially with *n*-hexane, EtOAc, and *n*-BuOH. The EtOAc layer was concentrated to give a residue (54 g), which was subjected to silica gel column chromatography (1 kg, 11 × 100 cm). The column was eluted with solvents of increasing polarity (*n*-hexane−EtOAc, EtOAc, EtOAc−MeOH, MeOH) to give 15 major fractions (1−15). Fraction 4 (3.7 g) was chromatographed on a silica gel column ($CHCl_3$−MeOH) to give three fractions (2.1−2.3) and **7** (300 mg). Fraction 2.3 was separated using a Toyopearl HW-40 column ($CHCl_3$−MeOH, 1:1), Si HPLC ($CHCl_3$−MeOH), and GPC (MeOH) to give **2** (7 mg). Fraction 5 (1.1 g) was subjected to Si HPLC ($CHCl_3$−MeOH) to give **6** (360 mg). Fraction 6 (2.4 g) was chromatographed on a Toyopearl HW-40 column ($CHCl_3$−MeOH) to give four fractions (6.1−6.4). Fraction 6.4 was separated on GPC (MeOH) to give **1** (18 mg). Fraction 7 (19.0 g) was chromatographed on a silica gel column ($CHCl_3$−MeOH) to give seven fractions (7.1−7.7). Fraction 7.7 was subjected to GPC (MeOH) to give **3** (35 mg). Fraction 11 (3.0 g) was separated by a Sephadex LH-20 (MeOH) column, GPC (MeOH), and ODS (MeOH−H_2O) to give **4** (41 mg) and **5** (20 mg). The isolation processes of the 32 known compounds are described in the Supporting Information.

Antibacterial Activity. The disc-diffusion method was used to screen compounds against *S. aureus* COL (a reference strain of MRSA)

and #5 (a clinical isolate of MRSA) and *H. pylori* ATCC43504 as described previously.[15,16] The compounds were dissolved in DMSO (Kanto Chemical Co., Inc., Tokyo, Japan), 10 mg/mL.

For the anti-MRSA assays, an overnight culture of the test strain of *S. aureus* at 37 °C in Mueller Hinton (MH) broth was diluted with MH agar (concentration of agar, 0.8%) supplemented with 25 mg/L Ca^{2+}, 50 mg/L Mg^{2+}, and 2% NaCl (cation-adjusted MH agar) to prepare a seed agar at a final concentration of 1×10^5 cfu/mL. Then, the seed agar (4 mL/64 cm^2) was spread uniformly onto the cation-adjusted MH agar (concentration of agar, 1.5%). Sterile Whatmann AA disks (6 mm) were placed on the solidified agar surface. Finally, 10 μL of each sample solution was transfused onto the disks. Cephapirin (20 μg/disk) was used as the reference drug. After 24 h of incubation at 37 °C, the plates were examined for growth inhibition zones.

For the anti *H. pylori* assays, the bacteria were cultured for 4 days at 37 °C in Brucella broth containing 5% horse serum (Bio Whittaker, Walkersville, MD) under microaerophilic conditions using a disposable O_2-absorbing and CO_2-generating agent (AnaeroPack Helico, Mitsubishi Gas Chemical Co., Inc., Tokyo, Japan), with humidity. The culture was diluted and adjusted to 10^7 cfu/mL and then inoculated evenly onto the Iso-Sensi test agar (Oxoid Ltd., Basingstoke, Hampshire, U.K.) containing 10% horse blood (Nippon Biotest Laboratories Inc., Tokyo, Japan). The sterile blank disks were placed on the agar surface. Then, 10 μL of the sample solutions was transfused onto the disks, for 4 days at 37 °C under microaerophilic conditions with humidity. After the incubation, the plates were examined for growth inhibition zones.

The MIC determination of oxacillin and the isolated compounds against 27 strains of *S. aureus* including 20 strains of MRSA was performed by the 2-fold plate-dilution method with the cation-adjusted MH agar.[44] Test strains, grown overnight at 37 °C in MH broth, were diluted with 0.85% NaCl, and the bacteria (about 10^6 cfu/mL) were applied onto the surface of 10 mL agar layers containing one of the test compounds. The plates were incubated for 24 h at 37 °C, and the MIC was determined as being the lowest concentration of the test compound that completely inhibited the growth.

Compound 1: white, amorphous powder; $[\alpha]_D$ −87.9 (*c* 0.3, MeOH); IR (KBr) ν_{max} 3473, 2360, 1747, 1708, 1274, 1116 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.42 (1H, brs, H-22), 6.23 (1H, brs, H-23), 5.40 (1H, s, H-17), 3.87 (1H, m, H-1), 3.86 (1H, s, H-15), 3.43 (1H, d, J = 15.4 Hz, H-2a), 3.03 (1H, t, J = 14.2 Hz, H-6β), 2.90 (1H, m, H-2b), 2.86 (1H, m, H-9), 2.61 (1H, dd, J = 3.2, 14.2 Hz, H-5), 2.50 (1H, dd, J = 3.2, 14.2 Hz, H-6α), 2.12 (1H, dd, J = 7.3, 13.5 Hz, H-12β), 1.84 (1H, m, H-11a), 1.66 (1H, m, H-11b), 1.60 (3H, s, H_3-29), 1.44 (3H, s, H_3-28), 1.40 (1H, m, H-12α), 1.26 (6H, s, H_3-19, 30) 1.19 (3H, s, H_3-18); ^{13}C NMR (CD_3OD, 100 MHz) δ 210.3 (C-7), 174.3 (C-3), 172.0 (C-21), 168.8 (C-16), 153.3 (C-22), 133.8 (C-20), 99.1 (C-23), 86.3 (C-4), 77.1 (C-17), 70.0 (C-1), 67.1 (C-14), 54.6 (C-15), 54.0 (C-8), 51.2 (C-5), 46.1 (C-10), 45.5 (C-9), 40.1 × 2 (C-2, 6), 39.3 (C-13), 33.7 (C-28), 30.9 (C-12), 23.6 (C-29), 20.3 (C-18), 18.1 (C-11), 17.3 (C-30), 16.9 (C-19); HRFABMS *m/z* 527.1848 $[M + Na]^+$ (calcd for $C_{26}H_{32}O_{10}Na$, 527.1893).

Compound 2: white, amorphous powder; $[\alpha]_D$ −80.0 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3440, 2954, 2360, 1743, 1719, 1376, 1272, 1024 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.30 (1H, brs, H-22), 6.48 (1H, brd, J = 8.3 Hz, H-1), 6.15 (1H, brs, H-23), 5.40 (1H, s, H-17), 3.66 (1H, s, H-15), 3.65 (3H, s, 3-OMe), 2.77 (1H, m, H-6β), 2.74 (1H, m, H-2a), 2.44 (1H, dd, J = 5.2, 14.6 Hz, H-6α), 2.37 (1H, m, H-11a), 2.34 (1H, m, H-2b), 2.20 (1H, dd, J = 7.2, 14.1 Hz, H-12β), 2.15 (1H, brd, J = 11.4 Hz, H-9), 2.05 (3H, s, 1-Ac), 1.99 (1H, dd, J = 5.2, 12.4 Hz, H-5), 1.69 (1H, s, H-11b), 1.35 (3H, s, H_3-28), 1.34 (3H, s, H_3-29), 1.30 (3H, s, H_3-19), 1.30 (1H, m, H-12α), 1.16 (3H, s, H_3-30), 1.12 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 209.5 (C-7), 171.8 (C-3), 170.6 × 3 (C-16, 21, 1-Ac), 152.1 (C-22), 133.1 (C-20), 97.0 (C-23), 76.0 (C-1), 75.6 (C-17), 74.3 (C-4), 65.1 (C-14), 52.9 (C-5), 52.8 (C-15), 52.2 × 2 (C-8, 3-OMe), 46.1 (C-10), 44.5 (C-9), 38.9 (C-6), 37.5 (C-13), 35.3 (C-2), 33.8 (C-28), 29.7 (C-12), 27.4 (C-29), 21.0 × 2 (C-18, 1-Ac), 18.7 (C-11), 16.5 × 2 (C-19, 30); HRFABMS *m/z* 601.2211 $[M + Na]^+$ (calcd for $C_{29}H_{38}O_{12}Na$, 601.2261).

Compound 3: yellow, amorphous powder; $[\alpha]_D$ +4.5 (*c* 1.1, MeOH); IR (KBr) ν_{max} 3411, 1773, 1718, 1629, 1600, 1515, 1272, 1159, 1029, 821 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.68 (1H, d, J = 15.9 Hz, H-7), 7.21 (1H, d, J = 1.4 Hz, H-2), 7.13 (1H, dd, J = 1.4, 8.2 Hz, H-6), 6.84 (1H, d, J = 8.2 Hz, H-5), 6.32 (1H, d, J = 15.9 Hz, H-8), 5.76 (1H, dd, J = 7.9, 8.7 Hz, H-3′), 5.14 (1H, dd, J = 2.7, 7.9 Hz, H-4′), 4.93 (1H, d, J = 8.7 Hz, H-2′), 4.60 (1H, d, J = 2.7 Hz, H-5′), 3.90 (3H, s, 3-OMe), 3.62 (3H, s, 6′-OMe); ^{13}C NMR (CD_3OD, 100 MHz) δ 175.4 (C-1′), 172.4 (C-6′), 167.5 (C-9), 151.0 (C-4), 149.4 (C-3), 148.6 (C-7), 127.4 (C-1), 124.4 (C-6), 116.6 (C-5), 113.8 (C-8), 111.9 (C-2), 77.9 (C-4′), 75.7 (C-2′), 71.5 (C-3′), 71.0 (C-5′), 56.5 (3-OMe), 52.9 (6′-OMe); HRFABMS *m/z* 381.0826 $[M - H]^-$ (calcd for $C_{17}H_{17}O_{10}$, 381.0822).

Compound 4: yellow, amorphous powder; $[\alpha]_D$ +49.3 (*c* 0.7, MeOH); IR (KBr) ν_{max} 3417, 2906, 1729, 1650, 1573, 1509, 1375, 1106, 837 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.86 (2H, brd, J = 7.9 Hz, H-2′, 6′), 6.93 (2H, brd, J = 7.9 Hz, H-3′, 5′), 6.64 (1H, s, H-3), 5.43 (1H, d, J = 7.2 Hz, H-1″), 4.43 (1H, brd, J = 11.9 Hz, H-6″a), 4.26 (1H, dd, J = 5.8, 11.9 Hz, H-6″b), 4.04 (3H, s, 6-OMe), 3.95 (3H, s, 8-OMe), 3.47−3.58 (4H, m, H-2″-5″), 2.66 (1H, d, J = 14.6 Hz, H-2‴a), 2.57 (1H, d, J = 14.6 Hz, H-4‴a), 2.54 (2H, d, J = 5.1 Hz, H-2‴b, 4‴b), 1.25 (3H, s, H_3-6‴); ^{13}C NMR (CD_3OD, 100 MHz) δ 184.4 (C-4), 174.7 (C-5‴), 172.2 (C-1‴), 166.5 (C-2), 162.9 (C-4′), 150.2 (C-7), 150.1 (C-9), 147.1 (C-5), 137.8 (C-8), 134.8 (C-6), 129.5 × 2 (C-2′, 6′), 123.0 (C-1′), 117.1 × 2 (C-3′, 5′), 108.5 (C-10), 104.8 (C-1″), 103.8 (C-3), 77.6 (C-3″), 75.8 (C-5″), 75.5 (C-2″), 71.5 (C-4″), 70.5 (C-3‴), 64.4 (C-6″), 62.8 (6-OMe), 61.6 (8-OMe), 46.2 (C-2‴), 45.7 (C-4‴), 27.6 (C-6‴); HRFABMS *m/z* 637.1765 $[M + H]^+$ (calcd for $C_{29}H_{33}O_{16}$, 637.1769).

Compound 5: yellow, amorphous powder; $[\alpha]_D$ −49.7 (*c* 0.7, MeOH); IR (KBr) ν_{max} 3399, 1725, 1643, 1516, 1463, 1268, 1162, 1024 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.19 (1H, brs, H-2′), 7.18 (1H, brd, J = 8.1 Hz, H-5′), 7.08 (1H, brd, J = 8.1 Hz, H-6′), 5.97 (1H, brd, J = 1.7 Hz, H-6), 5.93 (1H, brd, J = 1.7 Hz, H-8), 5.43 (1H, brd, J = 11.4 Hz, H-2), 4.96 (1H, d, J = 7.2 Hz, H-1″), 4.50 (1H, dd, J = 1.6, 11.8 Hz, H-6″a), 4.26 (1H, dd, J = 6.3, 11.8 Hz, H-6″b), 3.95 (3H, s, 3′-OMe), 3.66 (1H, m, H-5″), 3.54 (1H, m, H-2″), 3.52 (1H, m, H-3″), 3.43 (1H, m, H-4″), 3.17 (1H, m, H-3a), 2.79 (1H, d, J = 14.7 Hz, H-2‴a), 2.78 (1H, m, H-3b), 2.70 (1H, d, J = 14.7 Hz, H-4‴a), 2.66 (2H, m, H-2‴b, 4‴b), 1.37 (3H, s, H_3-6‴); ^{13}C NMR (CD_3OD, 100 MHz) δ 197.5 (C-4), 174.9 (C-5‴), 172.4 (C-1‴), 168.3 (C-9), 165.4 (C-7), 164.6 (C-5), 151.1 (C-3′), 147.9 (C-4′), 135.3 (C-1′), 120.2 (C-6′), 118.2 (C-5′), 112.1 (C-2′), 103.4 (C-10), 102.5 (C-1″), 97.2 (C-8), 96.3 (C-6), 80.3 (C-2), 77.6 (C-3″), 75.4 (C-5″), 74.8 (C-2″), 71.5 (C-4″), 70.7 (C-3‴), 64.6 (C-6″), 56.8 (3″-OMe), 46.3 (C-2‴), 45.8 (C-4‴), 44.1 (C-3), 27.7 (C-6‴); HRFABMS *m/z* 609.1791 $[M + H]^+$ (calcd for $C_{28}H_{33}O_{15}$, 609.1819).

Supporting Information Available: The isolation process for the known compounds is available free of charge via the Internet at http://pubs.acs.org.

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