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Chemical Synthesis of Citrus Flavanone Glucuronides

Muhammad Kamran Khan, Njara Rakotomanomana, Michele Loonis, and Olivier Dangles*

University of Avignon, INRA, UMR408, F-84000 Avignon, France

Flavanone glucuronides are the major phenolic metabolites detected in human plasma after consumption of citrus fruits. As such, they might display significant cardioprotective effects. In this work, glucuronides of naringenin (4′- and 7-*O*-*β*-D-glucuronides) and hesperetin (3′- and 7-*O*-*β*-D-glucuronides), the major flavanone aglycones in grapefruit and orange, respectively, have been chemically synthesized. On the one hand, the most reactive hydroxyl group, C7-OH, was protected by selective benzoylation to allow subsequent glucuronidation of C4′-OH (naringenin) or C3′-OH (hesperetin) (B-ring). On the other hand, the selective debenzoylation at C7-OH of the perbenzoylated flavanone aglycones allowed glucuronidation at the same position (A-ring). After careful deprotection, the target compounds were purified and characterized by nuclear magnetic resonance and mass spectrometry.

KEYWORDS: Flavanone; glucuronide; bioavailability; conjugation; chemical synthesis

INTRODUCTION

Because of the large and increasing worldwide consumption of citrus fruits and juices, potentially bioactive citrus polyphenols, which mainly belong to the flavanone class, are receiving increasing attention from nutrition biologists. Among flavanones, the naringenin and hesperetin glycosides are of particular interest because of their high prevalence in grapefruit and orange, respectively (*1*, *2*). Citrus flavanones may play a beneficial role in the prevention of cardiovascular diseases and cancers due to their anti-inflammatory, antioxidant, antimutagenic, and antitumor activities (*2*−*4*). After ingestion, the flavanone glycosides typically reach the colon, where they are hydrolyzed by microflora glycosidases into aglycones (naringenin, hesperetin), which are partially absorbed through the intestinal barrier (*5*, *6*). In contrast, the partial absorption of flavanone aglycones and *O*-*β*-D-glucosides (e.g., naringenin 7-*O*-*β*-D-glucoside) can occur earlier in the small intestine through passive diffusion for the former and with preliminary hydrolysis of the latter by endothelial *β*-D-glucosidases (*6*−*8*). In the intestinal and hepatic cells, flavanone aglycones are extensively converted into glucuronides and sulfates so that the entire fraction of dietary flavanone that crosses the intestinal barrier finally enters the general blood circulation as conjugates, mainly glucuronides (*5*, *9*−*11*). In particular, naringenin 4′- and 7-*O*-*β*-D-glucuronides and hesperetin 3′- and 7-*O*-*β*-D-glucuronides were all detected in the plasma and urine of human volunteers having consumed a single portion of orange fruit or juice (*11*).

Despite the detailed information now available on the bioavailability of flavanones, there is still a great need of purified metabolites for accurate titration and identification in biological fluids. In addition, a better knowledge of the biochemical mechanisms by which dietary flavanones exert their potential health effects requires investigations on appropriate cell models (e.g., endothelial or smooth muscle cells) with the authentic circulating metabolites instead of the commercially available glycosides and aglycones that are frequently used as a first approach despite the limited biological significance. As an alternative to the expensive, inconvenient, and low-yielding extraction of conjugates from biological fluids, chemical synthesis appears as the most direct strategy to obtain substantial amounts of these metabolites for bioavailability and in vitro cell studies. In this paper, we report the synthesis of the four circulating glucuronides of hesperetin and naringenin.

*Author to whom correspondence should be addressed [phone (33) 490 14 44 46; fax (33) 490 14 44 41; e-mail Olivier.Dangles@univ-avignon.fr].

MATERIALS AND METHODS

All starting materials were obtained from commercial suppliers, mainly Sigma-Aldrich (Steinheim, Germany), and were used without purification. Solvents were distilled over CaH_2. Analytical thin layer chromatography (TLC) was performed on silica gel 60 F254 obtained from Merck KGaA (Darmstadt, Germany). Detection was achieved by UV light (254 nm) and by charring after exposure to a 5% H_2SO_4 solution in EtOH. Purifications were performed by column chromatography on silica gel 60 (40−63 μm) (Merck KGaA). Dowex 50WX4-50 ion-exchange resin was used for acidification. Melting points were measured on a Barnstead Electrothermal 9100 apparatus and are uncorrected. Glucuronyl donor (**1**) (methyl 2,3,4-tri-*O*-acetyl-1-*O*-(trichloroacetimidoyl)-α-D-glucuronate) was synthesized from glucurono-3,6-lactone according to a three-step procedure already described in the literature (*12*, *13*).

NMR. 1D ^{1}H and ^{13}C NMR spectra of synthetic intermediates were recorded at 300 MHz on a Bruker Advance DPX-300 apparatus. 1D ^{1}H and ^{13}C NMR spectra and 2D ^{1}H−^{1}H (COSY) and ^{1}H−^{13}C (HMBC, HSQC) spectra of the final products were recorded at 500 MHz on a Bruker Advance DRX-500 apparatus. NMR chemical shifts (δ) are in parts per million (ppm) relative to tetramethylsilane using the deuterium signal of the solvent ($CDCl_3$, CD_3OD) for calibration. ^{1}H−^{1}H coupling constants (J) are in hertz (Hz).

HR-MS. High-resolution mass analyses were carried out on a Qstar Elite instrument (Applied Biosystems SCIEX, Foster City, CA) equipped with an atmospheric pressure ionization (API) interface. Mass detection was performed in the positive electrospray ionization mode in the

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following conditions: ion spray voltage, 5.5 kV; orifice voltage, 40 V; nebulizing gas (air) pressure, 20 psi. The mass spectra were obtained with a time-of-flight (TOF) analyzer. The accurate mass measurement was performed in triplicate along with internal calibration. The ions chosen for internal references were the ammonium adducts $[M+NH_4]^+$ of two oligomers of polypropylene glycol (PPG425) (m/z 442.3374 and 500.3792).

Analytical HPLC. HPLC analyses were performed on a Waters 600 Controller HPLC system equipped with a Waters 2996 photodiode array detector and monitored by the Waters Empower 2 chromatography data software. The wavelength used for the HPLC analyses was 280 nm. The chromatographic separation was carried out on a LiChrospher 100 RP-18 end-capped column (250 × 4 mm; 5 μm particle size) held at 37 °C. The flow rate was set at 1 mL min^{-1}. A mixture of 0.05% aqueous HCO_2H and MeCN (7:3) was used for the elution.

Semipreparative HPLC. Purifications were carried out on a Waters 600 chromatograph coupled to a UV−vis Waters 486 detector and equipped with a Waters Atlantis PrepT3OBD 5 μm (19 mm × 150 mm) column. The solvent was 0.05% aqueous HCO_2H/MeCN (7:3). The flow rate was 18 mL min^{-1}. Detection was carried out at 280 nm. Fractions obtained were concentrated under vacuum and freeze-dried. Their purity was checked by UPLC-MS analysis.

UPLC-MS. UPLC-MS analyses were performed on the Acquity Ultra Performance LC (UPLC) apparatus from Waters, equipped with an UV−visible diode array detector (DAD) and coupled with a Bruker Daltonics HCT ultra ion trap mass spectrometer with a negative electrospray ionization (ESI) mode. The separation was conducted on a 1.7 μm (2.1−50 mm) Acquity UPLC BEH C18 column thermostated at 30 °C with an isocratic elution (0.05% aqueous HCO_2H/MeCN (7:3)) at a flow rate of 0.2 mL min^{-1}. The mass spectra were generated in the Ultrascan mode in the m/z range of 100−900. The ion source parameters were as follows: nebulizer pressure, 40 psi; drying gas flow, 9 L min^{-1}; drying gas temperature, 350 °C.

Molecular Modeling. Semiempirical quantum mechanics calculations were performed in vacuum with Hyperchem software (Autodesk, Sausalito, CA) using the PM3 program.

Synthesis. *Naringenin*/*Hesperetin* (***2a***/***2b***). **2a**/**2b** are commercially available compounds. **2a:** mol wt, 272.25 g mol^{-1}; light brown powder; mp, 252−253 °C; R_f (cyclohexane (cHex)/EtOAc, 6:4) 0.36; ^{1}H NMR (CD_3OD), δ 7.33 (2H, d, J = 8.5, H2′, H6′), 6.82 (2H, d, J = 8.5, H3′, H5′), 5.90 (1H, d, J = 2.2, H6), 5.89 (1H, d, J = 2.2, H8), 5.33 (1H, dd, J = 13.0, 3.0, H2), 3.12 (1H, dd, J = 13.0, 17.0, H3a), 2.69 (1H, dd, J = 3.0, 17.0, H3b); ^{13}C NMR (CD_3OD), δ 198.19 (C4), 168.76 (C7), 165.88 (C5 or C9), 165.30 (C5 or C9), 159.43 (C4′), 131.50 (C1′), 129.45 (2C, C2′, C6′), 116.74 (2C, C3′, C5′), 103.77 (C10), 97.47 (C6 or C8), 96.58 (C6 or C8), 80.89 (C2), 44.44 (C3). **2b:** mol wt, 302.28 g mol^{-1}; light yellow powder; mp, 235−236 °C; R_f (cHex/EtOAc, 6:4) 0.27; ^{1}H NMR (CD_3OD), δ 6.93−6.92 (3H, m, H2′, H5′, H6′), 5.92 (1H, d, J = 2.2, H6), 5.89 (1H, d, J = 2.2, H8), 5.32 (1H, dd, J = 12.6, 3.1, H2), 3.87 (3H, s, OCH_3), 3.07 (1H, dd, J = 12.6, 17.1, H3a), 2.72 (1H, dd, J = 3.1, 17.1, H3b); ^{13}C NMR (CD_3OD), δ 198.01 (C4), 168.77 (C7), 165.87 (C5 or C9), 165.17 (C5 or C9), 149.76 (C3′ or C4′), 148.19 (C3′ or C4′), 133.55 (C1′), 119.40 (C6′), 114.94 (C5′), 112.97 (C2′), 103.78 (C10), 97.47 (C6 or C8), 96.59 (C6 or C8), 80.69 (C2), 56.83 (OCH3), 44.49 (C3).

7-O-Benzoylnaringenin (***3a***). Benzoyl chloride (2.15 mL, 18.5 mmol) was added dropwise to an ice-cold solution of **2a** (1 equiv) in pyridine (15 mL), and the reaction mixture was stirred overnight at room temperature. The mixture was extracted with EtOAc (300 mL) and then washed with water (200 mL), 2 M HCl (3 × 100 mL), saturated aqueous $NaHCO_3$ (100 mL), and saturated aqueous NaCl (100 mL). The combined organic extract was dried over Na_2SO_4, filtered, and concentrated. The product was purified by precipitation from EtOAc/*n*-hexane to give **3a** with a yield of about 90%: light brown powder; mp, 138−139 °C; R_f (cHex/EtOAc, 6:4) 0.58; ^{1}H NMR (CD_3OD), δ 8.18 (2H, d, J = 7.2, H2Bz, H6Bz), 7.71 (1H, t, J = 7.2, H4Bz), 7.56 (2H, t, J = 7.2, H3Bz, H5Bz), 7.36 (2H, d, J = 8.6, H2′, H6′), 6.85 (2H, d, J = 8.6, H3′, H5′), 6.47−6.45 (2H, br s, H6, H8), 5.50 (1H, dd, J = 13.0, 2.8, H2), 3.2 (1H, dd, J = 13.0, 17.2, H3a), 2.90 (1H, dd, J = 2.8, 17.2, H3b); ^{13}C NMR (CD_3OD), δ 197.24 (C4), 164.10, 163.41, 162.46 (O═C—Ph, C5, C9), 158.75 (C4′ or C7), 156.17 (C4′ or C7), 134.01 (C1′, C4Bz), 130.32, 128.69 (C1Bz, C2Bz, C3Bz, C5Bz, C6Bz), 127.97 (C2′, C6′), 115.73 (C3′, C5′), 106.31 (C10), 103.41 (C6 or C8), 101.91 (C6 or C8), 79.06 (C2), 43.45 (C3).

7-O-Benzoylhesperetin (***3b***). The same procedure as for **3a** was used by starting from **2b**: yield, 90%; light brown powder; mp, 146−147 °C; R_f (cHex/EtOAc, 6:4) 0.50; ^{1}H NMR (CD_3OD), δ 8.20 (2H, d, J = 7.2, H2Bz, H6Bz), 7.70 (1H, t, J = 7.2, H4Bz), 7.55 (2H, t, J = 7.2, H3Bz, H5Bz), 6.93−6.92 (3H, m, H2′, H5′, H6′), 6.46 (1H, d, J = 2.1, H6), 6.44 (1H, d, J = 2.1, H8), 5.49 (1H, dd, J = 12.7, 3.0, H2), 3.87 (3H, s, OMe), 3.07 (1H, dd, J = 12.7, 17.0, H3a), 2.82 (1H, dd, J = 3.0, 17.0, H3b); ^{13}C NMR (CD_3OD), δ 197.22 (C4), 164.05, 163.37, 162.42 (O═C—Ph, C5, C9), 158.74 (C7), 147.08 (C3′ or C4′), 145.97 (C3′ or C4′), 133.98 (C1′ or C4Bz), 131.15 (C1′ or C4Bz), 130.32, 128.68 (C1Bz, C2Bz, C3Bz, C5Bz, C6Bz), 118.20 (C6′), 112.64 (C2′ or C5′), 110.70 (C2′ or C5′), 106.32 (C10), 103.39 (C6 or C8), 101.91 (C6 or C8), 79.07 (C2), 56.06 (OCH3), 43.43 (C3).

4′,5,7-Tri-O-benzoylnaringenin (***4a***). Compound **2a** (10 g, 37 mmol) and benzoyl chloride (12 mL, 3 equiv) were dissolved in 700 mL of THF, and the mixture was cooled in ice−water. Then, 50 mL of triethylamine was added dropwise. The mixture was allowed to warm to room temperature and stirred overnight. Then it was diluted with EtOAc (500 mL), washed with saturated aqueous $NaHCO_3$ (100 mL) and NaCl (100 mL), dried over Na_2SO_4, filtered, and evaporated. The crude solid was triturated in *n*-hexane several times to give **4a**: yield, 85%; light brown powder; mp, 112−113 °C; R_f (cHex/EtOAc, 6:4) 0.76; ^{1}H NMR ($CDCl_3$), δ 8.27 − 8.19 (6H, m, 3H2bz, 3H6bz), 7.71−7.66 (3H, m, 3H4Bz), 7.66−7.48 (8H, m, 3H3Bz, 3H5Bz, H2′, H6′), 7.31 (2H, d, J = 8.6, H3′, H5′), 7.02 (1H, d, J = 2.2, H6), 6.86 (1H, d, J = 2.2, H8), 5.61 (1H, dd, J = 13.5, 2.7, H2), 3.12 (1H, dd, J = 13.5, 16.7, H3a), 2.84 (1H, dd, J = 2.7, 16.7, H3b); ^{13}C NMR ($CDCl_3$), δ 188.85 (C4), 165.05, 164.99, 163.91, 163.29 (3O═C—Ph, C5, C9), 156.33 (C7), 151.30 (C4′), 135.78, 134.14, 133.76, 133.59 (3C4Bz, C1′), 130.36, 128.64 (3C1Bz, 3C2Bz, 3C3Bz, 3C5Bz, 3C6Bz), 127.44 (C2′, C6′), 122.26 (C3′, C5′), 112.10, 111.13, 109.45 (C6, C8, C10), 79.18 (C2), 45.24 (C3).

3′,5,7-Tri-O-benzoylhesperetin (***4b***). The same procedure as for **4a** was used by starting from **2b** rather than from **2a**: yield, 85%; light brown powder; mp, 101−102 °C; R_f (cHex/EtOAc, 6:4) 0.68; ^{1}H NMR ($CDCl_3$), δ 8.21−8.17 (6H, m, 3H2Bz, 3H6Bz), 7.80−7.75 (3H, m, 3H4Bz), 7.75−7.50 (6H, m, 3H3Bz, 3H5Bz), 7.43 (1H, dd, J = 8.5, 2.1, H6′), 7.37 (1H, d, J = 2.1, H2′), 7.19 (1H, d, J = 8.5, H5′), 7.07 (1H, d, J = 2.2, H6), 6.90 (1H, d, J = 2.2, H8), 5.66 (1H, dd, J = 13.0, 2.6, H2), 3.87 (3H, s, OMe), 3.07 (1H, dd, J = 13.0, 17.1, H3a), 2.82 (1H, dd, J = 2.6, 17.1, H3b); ^{13}C NMR ($CDCl_3$), δ 188.98 (C4), 164.98, 164.58, 163.89, 163.32 (3O═C—Ph, C5, C7, C9), 151.57 (C4′), 140.19 (C3′), 134.11, 133.59 (3C4Bz, C1′), 130.70, 128.56 (3C1Bz, 3C2Bz, 3C3Bz, 3C5Bz, 3C6Bz), 124.94 (C2′ or C6′), 121.21 (C2′ or C6′), 112.64, 112.07, 111.03, 109.42 (C5′, C6, C8, C10), 78.97 (C2), 56.12 (OCH3), 44.98 (C3).

4′,5-Di-O-benzoylnaringenin (***5a***). Compound **4a** (5 g, 8.5 mmol) and imidazole (0.465 g, 0.7 equiv) were dissolved in *N*-methyl-2-pyrrolidinone (NMP, 5 mL). Then, thiophenol (0.87 mL, 1 equiv) was slowly added at 0 °C. The resulting mixture was stirred for 1 h at room temperature and then diluted with EtOAc (50 mL) and washed with saturated aqueous NaCl (3 × 100 mL) and with 1 M HCl (100 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude solid was triturated with cyclohexane and precipitated from a *n*-hexane/AcOEt mixture to give **5a** (4 g): yield, 95%; white powder; mp, 218−219 °C; R_f (cHex/EtOAc, 6:4) 0.50; ^{1}H NMR (CD_3OD), δ 8.30−8.16 (4H, m, 2H2Bz, 2H6Bz), 7.70−7.66 (2H, m, 2H4Bz), 7.64−7.50 (6H, m, 2H3Bz, 2H5Bz, H2′, H6′), 7.32 (2H, d, J = 8.6, H3′, H5′), 6.42 (1H, d, J = 2.3, H6), 6.32 (1H, d, J = 2.3, H8), 5.59 (1H, dd, J = 12.9, 2.9, H2), 3.06 (1H, dd, J = 12.9, 16.7, H3a), 2.70 (1H, dd, J = 2.9, 16.7, H3b); ^{13}C NMR (CD_3OD), δ 192.63 (C4), 178.79, 174.58 (2O═C—Ph, C5 or C7, C9), 155.13 (C5 or C7), 151.82 (C4′), 133.28, 132.28 (2C4Bz, C1′), 130.61, 129.22 (2C1Bz, 2C2Bz, 2C3Bz, 2C5Bz, 2C6Bz), 128.74 (C2′, C6′), 122.50 (C3′, C5′), 103.61, 100.13, 96.92 (C6, C8, C10), 84.34 (C2), 39.62 (C3).

3′,5-Di-O-benzoylhesperetin (***5b***). The same procedure as for **5a** was used by starting from **4b**: yield, 95%; white powder; mp, 197−198 °C; R_f (cHex/EtOAc, 6:4) 0.35; ^{1}H NMR (CD_3OD), δ 8.21−8.17 (4H, m, 2H2Bz, 2H6Bz), 7.80−7.75 (2H, m, 2H4Bz), 7.75−7.50 (4H, m, 2H3Bz, 2H5Bz), 7.43 (1H, dd, J = 8.5, 2.1, H6′), 7.37 (1H, d, J = 2.1, H2′), 7.19 (1H, d, J = 8.5, H5′), 6.41 (1H, d, J = 2.4, H6), 6.32 (1H, d, J = 2.4, H8), 5.51 (1H, dd, J = 13.0, 2.8, H2), 3.87 (3H, s, OMe), 3.07 (1H, dd, J = 13.0, 17.0, H3a), 2.82 (1H, dd, J = 2.8, 17.0, H3b); ^{13}C NMR ($CDCl_3$), δ 188.79 (C4), 165.56, 164.92, 164.12, 163.79 (2O═C—Ph, C5, C7, C9), 151.65

(C4′), 140.04 (C3′), 133.67, 133.44, 131.29 (2C4Bz, C1′), 130.38, 128.53 (2C1Bz, 2C2Bz, 2C3Bz, 2C5Bz, 2C6Bz), 125.08 (C2′ or C6′), 121.18 (C2′ or C6′), 112.59 (C5′), 107.48, 105.92, 101.96 (C6, C8, C10), 78.65 (C2), 56.09 (OCH3), 44.86 (C3).

7-O-Benzoylnaringenin 4′-O-[*Methyl (2″,3″,4″-tri-O-acetyl)-β-D-glucopyranosyl uronate*] (***6a***). Compound **3a** (0.5 g, 1.3 mmol) was added to a solution of glucuronyl donor (**1**) (0.45 g, 1 equiv) in dry CH_2Cl_2 (15 mL) containing 4 Å molecular sieves. The reaction mixture was cooled in an ice−water bath for 15 min under N_2 atmosphere. Then, $BF_3 \cdot Et_2O$ (170 μL, 1 equiv) was added, and the mixture was stirred at room temperature for about 30 min. Then, the mixture was diluted with EtOAc, filtered on Celite, concentrated, and purified by column chromatography (cHex/EtOAc; 6:4) to provide **6a**: yield, 50%; white solid; mp, 149−150 °C; R_f (cHex/EtOAc, 6:4) 0.31; ^{1}H NMR ($CDCl_3$), δ 11.89 (1H, s, C5-OH), 8.18 (2H, d, J = 7.3, H2Bz, H6Bz), 7.67 (1H, t, J = 7.3, H4Bz), 7.53 (2H, t, J = 7.3, H3Bz, H5Bz), 7.43 (2H, d, J = 8.7, H2′, H6′), 7.08 (2H, d, J = 8.7, H3′, H5′), 6.47 (1H, br d J = 2.0, H6 or H8), 6.45 (1H, m, H6 or H8) 5.48 (1H, dd, J = 13.0, 2.8, H2), 5.39 − 5.30 (3H, m, H2″, H3″, H4″), 5.20 (1H, d, J = 7.1, H1″), 4.23 (d, J = 7.0) plus 4.22 (d, J = 7.0, H5″) (1H, H5″), 3.75 (3H, s, OMe), 3.15 (1H, dd, J = 13.0, 17.0, H3a), 2.9 (1H, dd, J = 2.8, 17.0, H3b), 2.09, 2.08, 2.07, 2.06 (9H, 4bs, 3Ac); ^{13}C NMR ($CDCl_3$), δ 196.90 (C4), 170.10, 169.34, 169.22 (3O$\underline{C}$OCH$_3$, C6″), 166.80, 164.04, 163.40, 162.25 (O═C—Ph, C5, C9), 158.79 (C4′ or C7), 157.04 (C4′ or C7), 134.02, 133.08 (C4Bz, C1′), 130.31, 128.70 (C1Bz, C2Bz, C3Bz, C5Bz, C6Bz), 127.75 (C2′, C6′), 117.38 (C3′, C5′), 106.29, 103.53, 101.91, 98.97 (C1″, C6, C8, C10), 78.76 (C2), 72.71, 71.82, 71.05, 69.07 (C2″, C3″, C4″, C5″), 53.02 (OCH$_3$ GlcU), 43.45 (C3), 20.62 (3OCO$\underline{C}$H$_3$).

7-O-Benzoylhesperetin 3′-O-[*Methyl (2″,3″,4″-tri-O-acetyl)-β-D-glucopyranosyl uronate*] (***6b***). The same procedure as for **6a** was used by starting from **3b**: yield, 50%; light yellow powder; mp, 115−116 °C; R_f (cHex/EtOAc, 6:4) 0.19; ^{1}H NMR ($CDCl_3$), δ 11.89 (s) plus 11.88 (s) (1H, C5-OH), 8.18 (2H, d, J = 7.3, H2Bz, H6Bz), 7.67 (1H, t, J = 7.3, H4Bz), 7.53 (2H, t, J = 7.3, H3Bz, H5Bz), 7.28 (1H, br s, H2′), 7.19 (dd, J = 8.5, 2.3) plus 7.18 (dd, J = 8.5, 2.3) (1H, H6′), 6.96 (d, J = 8.5) plus 6.95 (d, J = 8.5) (1H, H5′), 6.44−643 (2H, m, H6, H8) 5.51−5.20 (4H, m, H4″, H2″, H3″, H2), 5.10 (d, J = 6.5) plus 5.08 (d, J = 6.5) (1H, H1″), 4.15 (d, J = 9.2) plus 4.12 (d, J = 9.2) (1H, H5″), 3.87 (3H, s, OMe hesperetin), 3.74 (3H, s, OMe GlcU), 3.07 (1H, dd, J = 12.6, 17.0, H3a), 2.82 (1H, dd, J = 2.9, 17.0, H3b), 2.1 (3H, s, Ac), 2.06 (3H, s, Ac), 2.04 (s) plus 2.01 (s) (3H, Ac); ^{13}C NMR ($CDCl_3$), δ 196.99 (C4), 170.16, 169.37, 169.29 (3O$\underline{C}$OCH$_3$, C6″), 166.87, 163.40, 162.21 (O═C—Ph, C5, C9), 158.76 (C7), 151.24 (C4′), 145.75 (C3′), 134.02, 130.50, 130.37, 130.32, 128.70 (C1′, C1Bz, C2Bz, C3Bz, C4Bz, C5Bz, C6Bz), 123.07 (C5′ or C6′), 118.81 (C5′ or C6′), 112.76 (C2′), 106.32, 103.50, 101.93, 100.58 (C1″, C6, C8, C10), 78.62 (C2), 72.56, 71.87, 71.12, 69.27 (C2″, C3″, C4″, C5″), 56.14 (OCH$_3$ hesperetin), 52.98 (OCH$_3$ GlcU), 43.03 (C3), 20.66 (3OCO$\underline{C}$H$_3$).

4′,5-Di-O-benzoylnaringenin 7-O-[*Methyl (2″,3″,4″-tri-O-acetyl)-β-D-glucopyranosyl uronate*] (***7a***). The same procedure as for **6a** was used by starting from **5a**: yield, 50%; white solid; mp, 165−166 °C; R_f (cHex/EtOAc, 6:4) 0.32; ^{1}H NMR ($CDCl_3$), δ 8.14 (2H, br d, J = 7.0, H2Bz, H6Bz), 8.06 (2H, m, H2Bz, H6Bz), 7.60−7.50 (2H, m, H4Bz), 7.46−7.40 (4H, m, H3Bz, H5Bz, H2′, H6′), 7.22 (2H, d, J = 8.6, H3′, H5′), 6.10 (d, J = 2.0, H6 or H8) plus 6.09 (d, J = 2.0) (1H, H6 or H8), 6.07 (d, J = 2.0, H6 or H8) plus 6.07 (d, J = 2.0) (1H, H6 or H8), 5.40 (dd, J = 13.0, 2.9, H2), 5.30−5.13 (4H, m, H1″, H2″, H3″, H4″), 4.14 (d, J = 8, H5″) plus 4.13 (d, J = 8, H5″), 3.75 (3H, s, OMe GlcU), 3.0 (1H, dd, J = 13.0, 18.0, H3a), 2.8 (1H, dd, J = 2.9, 18.0, H3b), 1.18 (3 × 3H, bs, 3Ac); ^{13}C NMR ($CDCl_3$), δ 196.07 (C4), 170.04, 169.35, 169.10 (3O$\underline{C}$OCH$_3$, C6″), 166.61, 165.06, 163.96 (2O═C—Ph, C5 or C7, C9), 154.72 (C5 or C7), 151.33 (C4′), 133.80, 133.70 (2C4Bz, C1′), 130.24, 129.20 (2C1Bz, 2C2Bz, 2C3Bz, 2C5Bz, 2C6Bz), 127.43 (C2′, C6′), 122.30 (C3′, C5′), 110.55, 104.53, 97.58, 96.32 (C1″, C6, C8, C10), 78.80 (C2), 72.74, 71.55, 70.70, 68.91 (C2″, C3″, C4″, C5″), 53.09 (OCH$_3$ GlcU), 43.40 (C3), 20.50 (3OCO$\underline{C}$H$_3$).

3′,5-Di-O-benzoylhesperetin 7-O-[*Methyl (2″,3″,4″-tri-O-acetyl)-β-D-glucopyranosyl uronate*] (***7b***). The same procedure as for **6a** was used by starting from **5b**: yield, 50%; white powder; mp, 119−120 °C; R_f (cHex/EtOAc, 6:4) 0.25; ^{1}H NMR ($CDCl_3$), δ 8.21−8.17 (4H, m, H2Bz, H6Bz), 7.78 (2H, m, H4Bz), 7.75−7.50 (4H, m, H3Bz, H5Bz), 7.33 (1H, dd, J = 2.1, 9.0, H6′), 7.37 (1H, d, J = 2.1, H2′), 7.19 (1H, d, J = 9.0, H5′), 6.59 (1H, d, J = 2.4, H8), 6.50 (1H, d, J = 2.4, H6), 5.51 (1H, dd, J = 13.0, 2.9, H2), 5.40−5.20 (4H, m, H1″, H2″, H3″, H4″), 4.23 (1H, br d, J = 7.6, H5″), 3.87 (3H, s, OMe hesperetin), 3.74 (3H, s, OMe GlcU), 3.07 (1H, dd, J = 13.0, 17.0, H3a), 2.82 (1H, dd, J = 2.9, 17.0, H3b), 2.08, 2.08, 2.07 (3 × 3H, br s, 3Ac); ^{13}C NMR ($CDCl_3$), δ 188.61 (C4), 169.97, 169.34, 169.11 (3O$\underline{C}$OCH$_3$, C6″), 166.62, 164.60, 163.85, 161.69 (2O═C—Ph, C5, C7, C9), 152.07 (C4′), 140.21 (C3′), 133.63, 130.67 (2C4Bz, C1′), 130.36, 128.58 (2C1Bz, 2C2Bz, 2C3Bz, 2C5Bz, 2C6Bz), 124.98 (C2′ or C6′), 121.21 (C2′ or C6′), 112.63 (C5′), 109.98, 106.39, 102.57, 97.71 (C1″, C6, C8, C10), 79.02 (C2), 72.73, 71.48, 70.67, 68.82 (C2″, C3″, C4″, C5″), 56.12 (OCH$_3$ hesperetin), 53.06 (OCH$_3$ GlcU), 43.73 (C3), 20.59 (3OCO$\underline{C}$H$_3$).

Naringenin 4′-O-β-D-Glucuronide (***8a***). To a solution of **6a** (0.85 mmol) in MeOH (30 mL) under N_2 atmosphere at 0 °C was slowly added a 0.5 M aqueous solution of Na_2CO_3 (13 mL). The mixture was stirred at room temperature with HPLC monitoring at 280 nm every hour. After 5 h, **8a** was detected as a single product (estimated analytical yield ca. 60%). Then, Dowex 50WX4-50 ion-exchange resin (H^+ form) was added under stirring to lower the pH to ca. 6. The resin was filtered off and the filtrate concentrated at 50 °C under vacuum. Purification was carried out by semipreparative HPLC: overall yield, 30%; white amorphous powder; R_f (*n*-BuOH/CH_3CO_2H/H_2O, 10:1:1) 0.18; HPLC (0.05% aq HCO_2H/MeCN (7:3)), t_R = 2.41 min, λ_{max} = 290, 330 nm; ^{1}H NMR (D_2O), δ 7.37 (2H, d, J = 7.9, H2′, H6′), 7.07 (2H, d, J = 7.9, H3′, H5′), 5.91 (2H, br s, H6, H8), 5.53 (1H, br d, J = 11.2, H2), 5.05 (1H, br d, J = 6.9, H1″), 3.90 (1H, d, J = 8.4, H5″), 3.69−3.61 (3H, m, H2″, H3″, H4″), 3.26 (dd, J = 17.0, 11.2) plus 3.23 (dd, J = 17.0, 11.2) (1H, H3a), 2.86 (1H, br d, J = 17.0, H3b); DEPTQ ^{13}C NMR (D_2O), δ 196.56 (C4), 174.03 (C6″), 170.22, 167.13, 164.22 (C5, C7, C9), 134.05 (C1′), 129.65 (C2′, C6′), 118.30 (C3′, C5′), 103.74 (C10), 101.47 (C1″), 97.76, 96.97 (C6, C8), 79.96 (C2), 76.45, 76.24, 73.93, 72.64 (C2″, C3″, C4″, C5″), 42.98 (C3); HRMS-ESI, m/z $[M + H]^+$ calcd for $C_{21}H_{21}O_{11}$, 449.1078; found, 449.1082; molar ratio of epimers ca. 1:1 (based on H_{3a} integration) (see the Supporting Information).

Hesperetin 3′-O-β-D-Glucuronide (***8b***). The same procedure as for **8a** was used by starting from **6b**: overall yield, 30%; white amorphous powder; R_f (*n*-BuOH/CH_3CO_2H/H_2O, 10:1:1) 0.21; HPLC (0.05% aq HCO_2H/MeCN (7:3)), t_R = 4.67 min, λ_{max} = 288, 333 nm; ^{1}H NMR (D_2O), δ 7.30 (d, J = 8.4) plus 7.28 (d, J = 8.4) (1H, H6′), 7.23 (1H, br s, H2′), 7.19 (d, J = 8.4) plus 7.16 (d, J = 8.4) (1H, H5′), 6.02 (1H, br s, H6 or H8), 6.00 (1H, br s, H6 or H8), [5.55 (1H, dd, J = 3.1, 12.0) plus 5.47 (dd, J = 3.1, 12.0)] (1H, H2), 4.7 (br d, J = 5.5, partly masked by the water peak), 3.78 (3H, s, OMe), 3.88 (d, J = 9.8) plus 3.83 (d, J = 9.8) (1H, H5″), 3.48−3.23 (3H, m, H2″, H3″, H4″), 3.27 (dd, J = 12.0, 17.0) plus 3.19 (dd, J = 12.0, 17.0) (1H, H3a), 2.85 (dd, J = 3.1, 17.0) plus 2.84 (dd, J = 3.1, 17.0) (1H, H3b); DEPT ^{13}C NMR (D_2O), δ 200.48 (C4), 167.08, 164.16 (C5, C7, C9, C6″), 146.59 (C3′, C4′), 131.93 (C1′), 123.22 (C6′), 117.08 (C2′), 114.38 (C5′), 103.63 (C10), 101.72 (C1″), 97.92, 97.00 (C6, C8), 80.34 (C2), 76.67, 76.56, 73.78, 72.76 (C2″, C3″, C4″, C5″), 57.20 (OCH$_3$), 42.86 (C3); molar ratio of epimers ca. 7:3 (based on H_2 integration); HRMS-ESI, m/z $[M + H]^+$ calcd for $C_{22}H_{23}O_{12}$, 479.1184; found, 479.1185.

Naringenin 7-O-β-D-Glucuronide (***9a***). The same procedure as for **8a** was used by starting from **7a**: overall yield, 30%; white amorphous powder; R_f (*n*-BuOH/CH_3CO_2H/H_2O, 10:1:1) 0.18; HPLC (0.05% aq HCO_2H/MeCN (7:3)), t_R = 2.38 min, λ_{max} = 283, 329 nm; ^{1}H NMR (D_2O), δ 7.43 (2H, d, J = 8.0, H2′, H6′), 6.96 (2H, d, J = 8.0, H3′, H5′), 6.27 (1H, br s, H6 or H8), 6.24 (1H, br s, H6 or H8),5.49 (1H, br d, J = 14.1, H2), 5.12 (1H, m, H1″), 3.77 (1H, br d, J = 9.3, H5″), 3.67−3.54 (3H, m, H2″, H3″, H4″), 3.27 (1H, br dd, J = 16.7, 14.1, H3a), 2.85 (1H, br d, J = 16.7, H3b); ^{13}C NMR (D_2O), δ 198.32 (C4), 172.15 (C6″), 164.63, 163.27, 162.59, 162.59 (C5, C7, C9), 156.69 (C4′), 134.13 (C1′), 129.98 plus 129.06 (C2′, C6′, 2 epimers), 115.91 (C3′, C5′), 104.13 (C10), 99.02 (C1″), 97.47, 96.27 (C6, C8), 79.20 (C2), 75.28, 74.87, 72.53, 71.37 (C2″, C3″, C4″, C5″), 42.63 plus 41.59 (C3, 2 epimers); HRMS-ESI, m/z $[M + H]^+$ calcd for $C_{21}H_{21}O_{11}$, 449.1078; found, 449.1078.

Hesperetin 7-O-β-D-Glucuronide (***9b***). The same procedure as for **8a** was used by starting from **7b**: overall yield, 30%; light brown amorphous powder; R_f (*n*-BuOH/CH_3CO_2H/H_2O, 10:1:1) 0.17; HPLC (0.05% aq HCO_2H/MeCN (7:3)), t_R = 4.51 min, λ_{max} = 284, 328 nm; ^{1}H NMR (D_2O), δ 6.83−6.77 (3H, m, H5′, H6′, H2′), 6.07 (1H, br s, H8), 6.03 (1H, br s, H6), 5.04 (1H, br d, J = 14.8, H2), 4.80 (1H, d, J = 7.5, H1″), 3.70 (3H, s, OMe), 3.59−3.34 (4H, m, H2″,H3″, H4″, H5″), 2.83 (1H, br t (dd), J = 16.0, 14.8, H3a), 2.54 (1H, br d, J = 16.0, H3b); ^{13}C NMR (D_2O), δ 199.31 (C4), 165.78, 165.62, 164.22, 163.62 (C5, C7, C9, C6″), 146.35

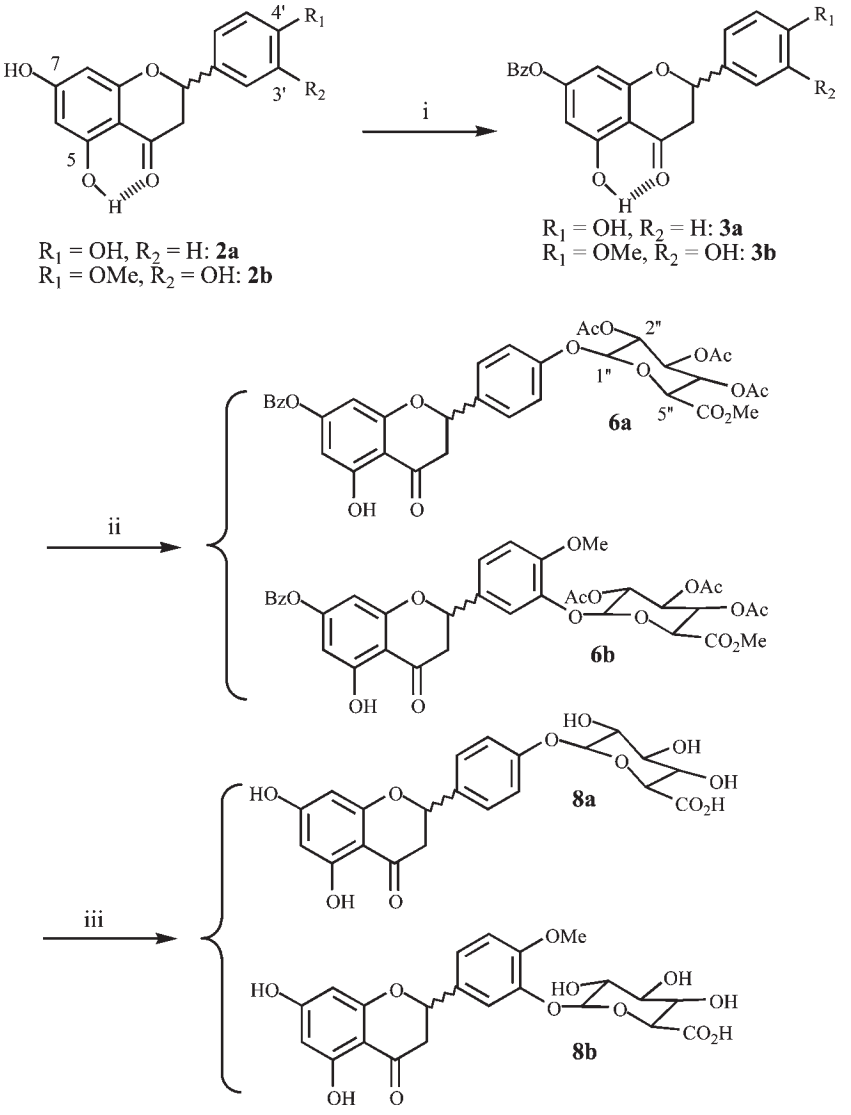

Figure 1. Glucuronidation of citrus flavanones on the B-ring: (i) BzCl (1.1−1.5 equiv), pyridine; (ii) methyl 2,3,4-tri-*O*-acetyl-1-*O*-(trichloroacetimidoyl)-α-D-glucuronate (**1**), BF_3−OEt_2 (2 equiv), CH_2Cl_2; (iii) Na_2CO_3 (1.2 equiv per ester group), H_2O/MeOH (2:5), then acidification to pH 6 by Dowex resin (H^+ form).

(C3′ or C4′), 144.07 (C3′ or C4′), 132.37 (C1′), 120.21 (C6′), 114.94 (C2′ or C5′), 113.50 (C2′ or C5′), 105.17 (C10), 100.11 (C1″), 98.46, 97.17 (C6, C8), 80.24 (C2), 76.52, 76.36, 73.59, 72.51 (C2″, C3″, C4″, C5″), 57.18 (OCH_3 hesperetin), 43.59 (C3); HRMS-ESI, *m*/*z* $[M + H]^+$ calcd for $C_{22}H_{23}O_{12}$, 479.1184; found, 479.1185.

RESULTS AND DISCUSSION

Investigations over the past three decades have shown that dietary polyphenols are only moderately bioavailable and that the fraction crossing the intestinal barrier is typically extensively metabolized in the intestinal and hepatic cells. Thus, the potential cell effects of dietary polyphenols must be mainly mediated by their metabolites, of which glucuronides make the largest contribution. Hence, there is a growing interest for polyphenol glucuronides as standards for identification and titration of in vivo metabolites and as biologically pertinent compounds for cell studies aiming at elucidating the potential health effects of polyphenols. Several works have been published about the chemical synthesis of polyphenol glucuronides. For instance, the popular procedure, based on the Lewis acid-activated coupling of methyl-2,3,4-tri-*O*-acetyl-1-*O*-(trichloroacetimidoyl)-α-D-glucuronate (**1**) with partially protected polyphenols, was applied to the synthesis of isoflavone 7-*O*-β-D-glucuronides (*14*), quercetin 3′-*O*-β-D-glucuronide (*15*), and a series of hydroxycinnamic acid *O*-β-D-glucuronides (*16*). Catechin *O*-β-D-glucuronides were also prepared with methyl-2,3,4-tri-*O*-acetyl-1-*O*-bromo-α-D-glucuronate as the glucuronyl donor (*17*). Recently, the synthesis of a flavanone glucuronide (persicogenin 3′-*O*-β-D-glucuronide) was carried out with methyl-2,3,4-tri-*O*-acetyl-1-*O*-(trifluoroacetimidoyl)-α-D-glucuronate, followed by a final deprotection step involving pig liver esterase (PLE) for the hydrolysis of the methyl ester of the glucuronyl residue (*18*). A synthesis of quercetin 3-*O*-β-D-glucuronide was also performed by regioselective oxidation of the corresponding 3-*O*-β-D-glucoside (phenolic OH groups protected as benzyl ethers) using TEMPO/NaOCl/NaBr under phase transfer conditions (*19*).

In this particular work, an efficient chemical synthesis of the four major metabolites of citrus flavanones (hesperetin 7- and 3′-*O*-β-D-glucuronides, naringenin 7- and 4′-*O*-β-D-glucuronides) is reported. The glucuronides were completely characterized by NMR, HRMS, and UPLC-MS.

In a preliminary study, partial protection of the flavanone OH groups was achieved by acetylation (*20*). However, the low stability of the arylacetate groups in the glucuronidation step resulted in low yields and tedious purifications. By contrast, partial protection by benzoylation was found to be satisfactory.

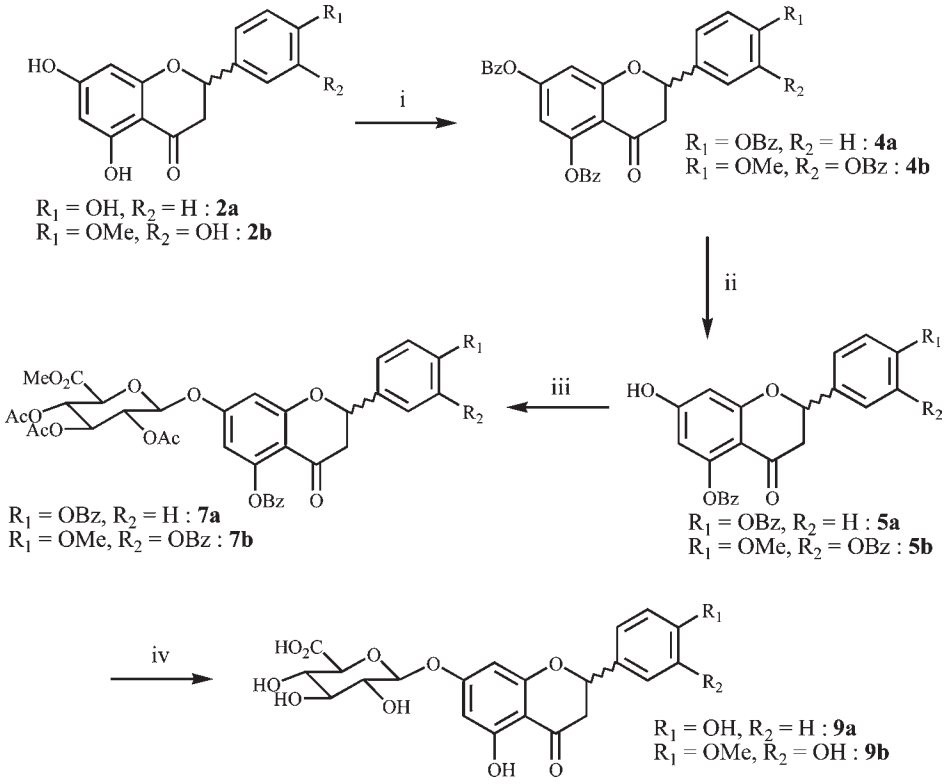

Figure 2. Glucuronidation of citrus flavanones on the A-ring: (i) BzCl (excess), NEt_3, THF, 0 °C; (ii) PhSH (1 equiv), imidazole, NMP; (iii) methyl 2,3,4-tri-*O*-acetyl-1-*O*-(trichloroacetimidoyl)-α-D-glucuronate (**1**), BF_3-OEt_2 (2 equiv), CH_2Cl_2; (iv) Na_2CO_3 (1.2 equiv per ester group), H_2O/MeOH (2:5), then acidification to pH 6 by Dowex resin (H^+ form).

Among the three phenolic OH groups of hesperetin and naringenin, C7-OH is the most acidic because of the stabilization of the corresponding phenolate with the keto group. Such an activation is absent for the B-ring OH groups and is canceled out for C5-OH by the strong hydrogen bond it forms with the keto group. Hence, both hesperetin and naringenin can be selectively benzoylated at C7-OH in the presence of one equivalent of benzoyl chloride at 0 °C (**Figure 1**). As expected, benzoylation at C7-OH induced a deshielding of the C6, C8, and C10 NMR signals. A strong shielding of the C7 signal was also noted. This first step allowed us to carry out the regioselective glucuronidation of the B-ring phenolic OH by compound **1** in the presence of the boron trifluoride–diethyl ether complex as the Lewis acid. In the ^1H NMR spectra of the protected glucuronides **6a** and **6b**, most B-ring protons (H3′ and H5′ in **6a**, H2′ and H5′ in **6b**) appeared to be strongly deshielded with respect to the corresponding protons in **3a** and **3b** as a consequence of the weakening of the electron-donating effect of the O atom by the attached glucuronyl group. In the glucuronidation step, the unprotected strongly H-bonded C5-OH group remained inactive.

For A-ring conjugation, hesperetin and naringenin were first converted into the corresponding tri-*O*-benzoates (**Figure 2**). Then, the selective thiolysis of the most reactive 7-*O*-benzoate group was successfully achieved using thiophenol as the nucleophile. Displacements of NMR signals opposite those recorded upon selective benzoylation at C7-OH were observed. The flavanone dibenzoates thus formed are convenient substrates for glucuronidation at C7-OH, which was performed under the same conditions as for B-ring conjugation.

In this work, the final deprotection step consisted of the alkaline hydrolysis of one or two benzoates (flavanone nucleus) along with three acetates and one methyl ester (sugar moiety). It was found to be particularly difficult to complete without degradation of the flavanone nuclei (probably starting by a retro-Michael reaction leading to opening of the pyrane ring and concomitant formation of chalcones). In particular, strongly alkaline conditions (NaOH/EtOH or KOH/EtOH) and even the milder methanolysis of the acetate and benzoate groups catalyzed by MeONa led to extensive degradation. Among the deprotection reagents tested, the most satisfactory one was a solution of sodium carbonate (1.2 equiv per ester group) in water/methanol (2:5). Such conditions were successfully used for the synthesis of 7-hydroxycoumarin *O*-β-D-glucuronide (*22*). The ^1H and ^{13}C NMR signals of the deprotected glucuronides were assigned from 2D COSY, HMBC, and HSQC experiments. Our results are consistent with the literature (*9*, *21*).

The major citrus flavanones are naringin (7-*O*-β-D-(L-rhamnosyl-α-1,2-D-glucosyl)-(2*S*)-naringenin) and hesperidin (7-*O*-β-D-(L-rhamnosyl-α-1,6-D-glucosyl)-(2*S*)-hesperetin). In both compounds, the configuration of C2 is *S* (*1*) as a consequence of the enantioselectivity of the chalcone isomerase enzyme catalyzing the formation of the flavanone nucleus by intramolecular Michael addition within the chalcone precursor (*23*). However, mixtures of (2*R*)- and (2*S*)-hesperidin epimers in an approximate molar ratio of 1:6 can be detected in orange juice (*25*), possibly because of the propensity of flavanones to undergo epimerization at C2 via the chalcone form. Interestingly, the minor 2*R* epimer seems about twice as bioavailable as the 2*S* epimer based on the excreted rates in urine of 2*R* and 2*S* hesperetin conjugates (*25*). In this work, the commercially available racemic naringenin and hesperetin were used. After the glucuronidation step, the ^1H NMR spectra of the glucuronides (except **7b**) are complicated by the splitting of several signals in two signals of nearly equal intensity, thus confirming that the compounds are equimolar mixtures of epimers. For instance, the NMR spectrum of **6b** in $CDCl_3$ displays two strongly deshielded singlets at ca. 11.9 ppm for the C5-OH proton, which is

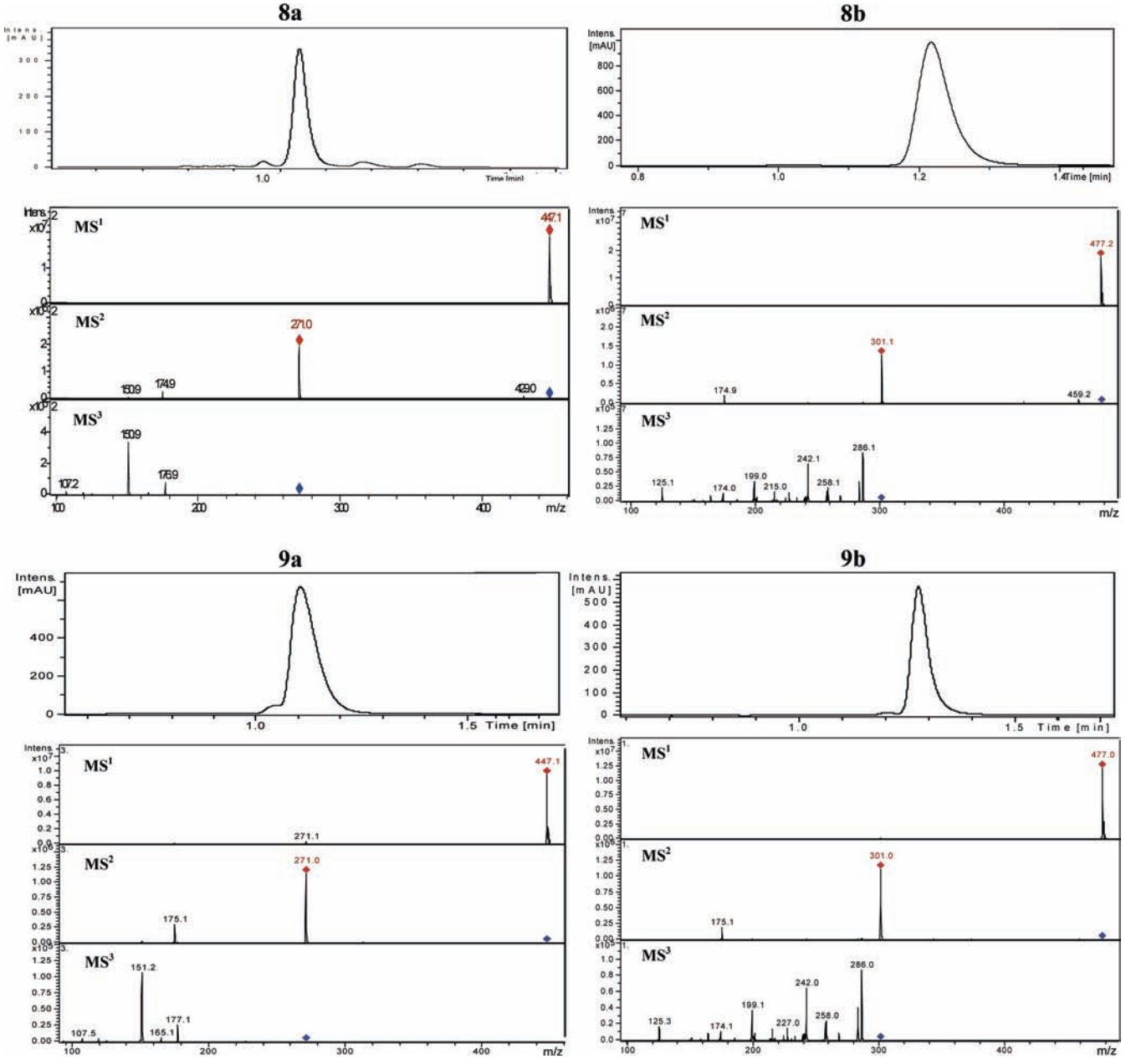

Figure 3. UPLC-MS analyses of flavanone glucuronides.

strongly hydrogen-bonded to the neighboring O4 atom. This splitting is also frequently observed not only for other protons of the flavanone nucleus but also for some benzoyl, acetyl, and GlcU protons, in particular H5″. After deprotection, only the B-ring glucuronides displayed some split signals confirming the presence of the two epimers. The fraction of hesperetin 3′-*O*-*β*-D-glucuronide (**8b**) purified by semipreparative HPLC was enriched in one of them (7:3 molar ratio) during this step. The UPLC-MS analysis did not permit the two epimers to be distinguished. The molecular ion of each pure glucuronide (MS^1) was sequentially fragmented into aglycone and D-glucuronic acid (MS^2) and, finally, into the aglycone fragments (MS^3) (**Figure 3**). No significant differences were observed between regioisomers, thus indicating that they possess the same fragmentation patterns. Furthermore, the aglycone fragmentation patterns were consistent with the literature (*24*−*28*). Unfortunately, it is impossible to differentiate between the A-ring and B-ring glucuronides on the basis of their mass fragmentation patterns as both MS^2 spectra give the aglycone and D-glucuronic acid as the main fragments. By contrast, the A-ring and B-ring glucuronides can be distinguished from their UV spectra. Indeed, C7-OH is conjugated with the keto group through the A-ring and as such contributes to the main UV absorption band. Hence, glucuronidation of the A-ring causes a hypsochromic shift of 4−5 nm in comparison to the parent aglycones ($\lambda_{max} \approx$ 288 nm), whereas glucuronidation of the B-ring leaves the spectra unchanged. Molecular modeling experiments with the deprotected glucuronides yielded low-energy conformations showing a pseudo-equatorial position for the B-ring (vs C-ring) and an *anti* arrangement of H2 and one of the H3 protons, which is consistent with the observed coupling constants in the NMR spectra.

In conclusion, simple and reasonably efficient procedures (10−15% overall yield from the starting aglycones) have been developed for the chemical synthesis of four major citrus flavanone metabolites. After a one- or two-step partial protection of the flavanone moiety by benzoyl groups, the glucuronidation step was carried out either on the A- or B-ring and followed by the removal of all protecting groups in optimized mild alkaline conditions, thereby avoiding significant degradation of the glucuronides.

The affinity of the flavanone glucuronides for serum albumin, their likely carrier in the blood plasma, and their cell effects in relation with the protection against cardiovascular diseases are currently investigated.

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Supporting Information Available: Optimized conformations of the naringenin glucuronides and high-resolution mass spectra of the citrus flavanone glucuronides. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Tomas-Barberan, F. A; Clifford, M. N. Flavanones, chalcones and dihydrochalcones − nature, occurrence and dietary burden. *J. Sci. Food Agric*. **2000**, *80*, 1073–1080.

(2) Erlund, I. Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology. *Nutr. Res.* (*N.Y.*) **2004**, *24*, 851–874.

(3) Tripoli, E.; Guardia, M. L.; Giammanco, S.; Majo, D. D.; Giammanco, M. Citrus flavonoids: molecular structure, biological activity and nutritional properties: a review. *Food Chem*. **2007**, *104*, 466–479.

(4) Benavente-Garcia, O.; Castillo, J. Update on uses and properties of citrus flavonoids: new findings in anticancer, cardiovascular, and anti-inflammatory activity. *J. Agric. Food Chem*. **2008**, *56*, 6185–6205.

(5) Manach, C.; Morand, C.; Gil-Izquierdo, A.; Bouteloup-Demange, C.; Remesy, C. Bioavailability in humans of the flavanones hesperidin and narirutin after the ingestion of two doses of orange juice. *Eur. J. Clin. Nutr*. **2003**, *57*, 235–242.

(6) Felgines, C.; Texier, O.; Morand, C.; Manach, C.; Scalbert, A.; Regerat, F.; Remesy, C. Bioavailability of the flavanone naringenin and its glycosides in rats. *Am. J. Physiol. Gastrointest. Liver Physiol*. **2000**, *279*, G1148–G1154.

(7) Choudhury, R.; Chowrimootoo, G.; Srai, K.; Debnam, E.; Rice-Evans, C. A. Interaction of the flavonoid naringenin in the gastrointestinal tract and the influence of glycosylation. *Biochem. Biophys. Res. Commun*. **1999**, *265*, 410–415.

(8) Serra, H.; Mendes, T.; Bronze, M. R.; Simplício, A. L. Prediction of intestinal absorption and metabolism of pharmacologically active flavones and flavanones. *Bioorg. Med. Chem*. **2008**, *16*, 4009–4018.

(9) Matsumoto, H.; Ikoma, Y.; Sugiura, M.; Yano, M.; Hasegawa, Y. Identification and quantification of the conjugated metabolites derived from orally administered hesperidin in rat plasma. *J. Agric. Food Chem*. **2004**, *52*, 6653–6659.

(10) Mullen, W.; Archeveque, M. A.; Edwards, C. A.; Matsumoto, H.; Crozier, A. Bioavailability and metabolism of orange juice flavanones in humans: impact of a full-fat yogurt. *J. Agric. Food Chem*. **2008**, *56*, 11157–11164.

(11) Brett, G. M.; Hollands, W.; Needs, P. W.; Teucher, B.; Dainty, J. R.; Davis, B. D.; Brodbelt, J. S.; Kroon, P. A. Absorption, metabolism and excretion of flavanones from single portions of orange fruit and juice and effects of anthropometric variables and contraceptive pill use on flavanone excretion. *Br. J. Nutr*. **2009**, *101*, 664–675.

(12) Fischer, B.; Nudelman, A.; Ruse, M.; Herzig, J.; Gottlieb, H. E.; Keinan, E. A novel method for stereoselective glucuronidation. *J. Org. Chem*. **1984**, *49*, 4988–4993.

(13) Pearson, A. G.; Kiefel, M. J.; Ferro, V.; Itzstein, M. V. Towards the synthesis of aryl glucuronides as potential heparanase probes. An interesting outcome in the glycosidation of glucuronic acid with 4-hydroxycinnamic acid. *Carbohydr. Res*. **2005**, *340*, 2077–2085.

(14) Al-Maharik, N.; Botting, N. P. A facile synthesis of isoflavone 7-*O*-glucuronides. *Tetrahedron Lett*. **2006**, *47*, 8703–8706.

(15) Needs, P. W.; Kroon, P. A. Convenient syntheses of metabolically important quercetin glucuronides and sulfates. *Tetrahedron* **2006**, *62*, 6862–6868.

(16) Galland, S.; Rakotomanomana, N.; Dufour, C.; Mora, N.; Dangles, O. Synthesis of hydroxycinnamic acid glucuronides and investigation of their affinity for human serum albumin. *Org. Biomol. Chem*. **2008**, *6*, 4253–4260.

(17) González-Manzano, S.; González-Paramás, A.; Santos-Buelga, C.; Duenãs, M. Preparation and characterization of catechin sulfates, glucuronides, and methylethers with metabolic interest. *J. Agric. Food Chem*. **2009**, *57*, 1231–1238.

(18) Boumendjel, A.; Blanc, M.; Williamson, G.; Barron, D. Efficient synthesis of flavanone glucuronides. *J. Agric. Food Chem*. **2009**, *57*, 7264–7267.

(19) Bouktaib, M.; Atmanib, A.; Rolando, C. Regio- and stereo-selective synthesis of the major metabolite of quercetin, quercetin-3-*O*-β-D-glucuronide. *Tetrahedron Lett*. **2002**, *43*, 6263–6266.

(20) Learmonth, D. A. A concise synthesis of the 3-*O*-β-D- and 4′-*O*-β-D-glucuronide conjugates of *trans*-resveratrol. *Bioconjugate Chem*. **2003**, *14*, 262–267.

(21) Chakraborti, A. K.; Nayak, M. K.; Sharma, L. Selective deprotection of aryl acetates, benzoates, pivalates, and tosylates under nonhydrolytic and virtually neutral conditions. *J. Org. Chem*. **1999**, *64*, 8027–8030.

(22) Brown, R. T.; Scheinmann, F.; Stachulski, A. V. Intermediates for glucuronide synthesis: 7-hydroxycoumarin glucuronide. *J. Chem. Res*. (*S*) **1997**, 370–371.

(23) Yoshimura, M.; Sano, A.; Kamei, J. I.; Obata, A. Identification and quantification of metabolites of orally administered naringenin chalcone in rats. *J. Agric. Food Chem*. **2009**, *57*, 6432–6437.

(24) Jez, J. M.; Bowman, M. E.; Noel, J. P. Role of hydrogen bonds in the reaction mechanism of chalcone isomerase. *Biochemistry* **2002**, *41*, 5168–5176.

(25) Si-Ahmed, K.; Tazerouti, F.; Badjah-Hadj-Ahmed, A. Y.; Aturki, Z.; Orazio, G. D.; Rocco, A.; Fanali, S. Analysis of hesperetin enantiomers in human urine after ingestion of blood orange juice by using nano-liquid chromatography. *J. Pharm. Biomed. Anal*. **2010**, *51*, 225–229.

(26) Weintraub, R. A.; Ameer, B.; Johnson, J. V.; Yost, R. A. Trace determination of naringenin and hesperitin by tandem mass spectrometry. *J. Agric. Food Chem*. **1995**, *43*, 1966–1968.

(27) Zhou, D.; Xu, Q.; Xue, X.; Zhang, F.; Liang, X. Identification of *O*-diglycosyl flavanones in *Fructus aurantii* by liquid chromatography with electrospray ionization and collision-induced dissociation mass spectrometry. *J. Pharm. Biomed. Anal*. **2006**, *42*, 441–448.

(28) Duenas, M.; Mingo-Chornet, H.; Pérez-Alonso, J. J.; Paola-Naranjo, R. D.; González-Paramás, A. M.; Santos-Buelga, C. Preparation of quercetin glucuronides and characterization by HPLC−DAD−ESI/MS. *Eur. Food Res. Technol*. **2008**, *227*, 1069–1076.

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