

Efficient Synthesis of Flavanone Glucuronides

AHCENE BOUMENDJEL,[†] MADELEINE BLANC,[†] GARY WILLIAMSON,[‡] AND
DENIS BARRON^{*,‡}

[†]Département de Pharmacochimie Moléculaire, UMR 5063, ICMG-FR 2607, Grenoble University,
B.P. 53, 38041 Grenoble Cedex 9, France, and [‡]Nestlé Research Center, P.O. Box 44,
CH-1000 Lausanne 26, Switzerland

The first efficient synthesis of flavanone glucuronides as potential human metabolites is described. The synthetic strategy is based on acetyl protection, followed by a combination of chemical and enzymatic deprotection steps. As an example, the method is applied to a synthesis of 7,4'-di-*O*-methylesteriodictyol 3'-*O*- β -D-glucuronide. The aglycone is a flavanone naturally present in tarragon spice (*Artemisia dracunculus*) as well as in various Chinese, Brazilian, and Malaysian medicinal plants.

KEYWORDS: Tarragon; *Artemisia dracunculus*; flavanone glucuronides; human metabolites; synthesis; enzymatic deprotection

INTRODUCTION

Flavanones and their glycosides are phenolic compounds that are common constituents of the diet, especially of citrus fruits (1). The most classically encountered forms are the rhamnoglucosides of naringenin 1, eriodictyol 2, and hesperetin 3, namely, narirutin 6, eriocitrin 7, and hesperidin 8 (Figure 1). Other naringenin or eriodictyol derivatives are less common in edible plants. However, glycosides of isosakuranetin 4 are present in honeybush tea (2), and persicogenin 5 has been reported in tarragon (3). On the other hand, a number of biological activities have been described for hesperetin and naringenin (4), as well as citrus flavonoids (5). Consequently, the animal and human metabolism of flavanones has received a lot of scientific interest. In rats (6) and humans (7), flavanones do not circulate as their aglycone forms. Instead, they have been shown to be present in both plasma and urine mainly in the form of glucuronide and/or sulfate conjugates. For example, among the biliary metabolites of orally administered hesperetin in rats, hesperetin 7-*O*- β -D-glucuronide 9, 3'-*O*- β -D-glucuronide 10, and 3'-*O*- β -D-glucuronide-7-*O*-sulfate 11 have been identified (8). Metabolites 9 and 10 have been detected in rat plasma after oral administration of hesperidin (9). On the other hand, only very recently have human circulating flavanone conjugates been documented in more detail (10, 11). This must be related to the fact that, both in rats (6, 12) and in humans (13–15), most of the studies have been carried out after treatment of the plasma by a mixture of glucuronidase and sulfatase. The main interest of this enzymatic treatment is that the extraction from plasma and the quantification of the aglycone are straightforward. In contrast, due to their polar nature, difficulties may be encountered in the extraction of the glucuronide and sulfate

conjugates. Furthermore, in the absence of suitable chromatographic standards, the quantification of conjugates is difficult. Thus, the design of efficient methods for the preparation of analytical standards of polyphenol conjugates is of the most interest. Since the beginning of the century, a number of reports on the synthesis of flavonoid conjugates have been made (for reviews, see refs 16–18). In the case of glucuronide conjugates, various glycosylation agents have been used, including acetobromo- α -D-glucuronic acid methyl ester (19), more efficient glucuronyl trichloroacetimidates (20), and even more efficient glucuronyl trifluoroacetimidates (21). All of these methods share in common the fact that, in the final step of the synthesis, strong aqueous alkaline conditions are necessary to remove the protective acetate and methyl ester groups of the glucuronic acid residue. Such an approach was not expected to be applicable to the preparation of flavanone glucuronides, because upon alkaline treatment, the flavanone ring has been shown to open to its corresponding chalcone form (22). Surprisingly, strong alkaline deprotection conditions (NaOMe/MeOH/H₂O) have been used in a previous attempt to chemically prepare hesperetin 7-glucuronide (9). However, the yield of hesperetin 7-glucuronide was only ~20%, and the nature of the other products of the reaction (which could have included appreciable amounts of hesperetin chalcone glucuronide) was not specified. Thus, a more appropriate approach for the synthesis of flavanone glucuronides was urgently required. In the present paper, we report a general and efficient method for the preparation of flavanone glucuronides. The synthetic route is based on (i) the selective deprotection of the acetyl groups of the glucuronyl residue using zinc acetate and (ii) the enzymatic hydrolysis of the methyl ester of the glucuronyl residue under the catalysis of pig liver esterase (PLE). We describe here the preparation of a potential human metabolite of 7,4'-di-*O*-methylesteriodictyol (persicogenin), a flavanone naturally present in tarragon spice (*Artemisia dracunculus*) (3), as well as in various Chinese, Brazilian, and Malaysian medicinal plants (23–26).

*Author to whom correspondence should be addressed (telephone + 41 21 785 9497; fax + 41 21 785 8554; e-mail Denis.Barron@rdls.nestle.com).

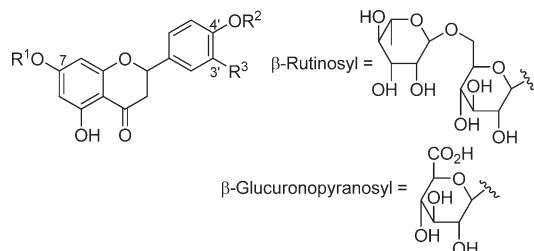


Figure 1. Structures of flavanones present in edible plants and their metabolites: **1**, $R^1 = R^2 = R^3 = H$, naringenin; **2**, $R^1 = R^2 = H$, $R^3 = OH$, eriodictyol; **3**, $R^1 = H$, $R^2 = Me$, $R^3 = OH$, hesperetin; **4**, $R^1 = R^3 = H$, $R^2 = Me$, isosakuranetin; **5**, $R^1 = R^2 = Me$, $R^3 = OH$, 7,4-di-*O*-methyletheriodictyol (persicogenin); **6**, $R^1 = \beta$ -rutinosyl, $R^2 = R^3 = H$, narirutin; **7**, $R^1 = \beta$ -rutinosyl, $R^2 = H$, $R^3 = OH$, eriocitrin; **8**, $R^1 = \beta$ -rutinosyl, $R^2 = Me$, $R^3 = OH$, hesperidin; **9**, $R^1 = \beta$ -D-glucuronopyranosyl, $R^2 = Me$, $R^3 = OH$, hesperetin 7-*O*- β -D-glucuronide; **10**, $R^1 = H$, $R^2 = Me$, $R^3 = O$ - β -D-glucuronopyranosyl, hesperetin 3'-*O*- β -D-glucuronide; **11**, $R^1 = SO_3^-$, $R^2 = Me$, $R^3 = O$ - β -D-glucuronopyranosyl, hesperetin 3'-*O*- β -D-glucuronide-7-*O*-sulfate; **12**, $R^1 = R^2 = Me$, $R^3 = O$ - β -D-glucuronopyranosyl, persicogenin 3'-*O*- β -D-glucuronide.

MATERIALS AND METHODS

Chemicals and Instruments. Thin layer chromatography (TLC) was carried out on RP-18 F_{254s} (Merck). Analytical HPLC was performed on a C₁₈ reverse-phase 2 μ m \times 250 mm column. The mobile phase was a mixture of (A) methanol and (B) 0.1% aqueous trifluoroacetic acid. The gradient program was as follows: 35% A + 65% B for 10 min, followed by a linear gradient to 95% A + 5% B in 60 min. Column chromatography was carried out using Merck silica gel 60, 200–400 mesh. Vacuum liquid chromatography (VLC) was performed on bonded phase octadecyl (C₁₈, Chromabond) from Macherey-Nagel. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker Avance 400 operating at 400.13 and 100.03 MHz for ¹H and ¹³C, respectively. The chemical shifts (in ppm) were expressed with respect to tetramethylsilane (TMS) as an internal reference. Mass spectra (ESI⁺) were recorded on either a JEOL HX-110 spectrometer or a Varian MAT 311. Liquid chromatography–high resolution mass spectra (ESI⁺) were recorded using an Agilent-1200 Series Rapid Resolution LC System including a binary pump SL, a high-performance autosampler, a diode array detector SL, and a thermostated column compartment SL. The HPLC system was coupled to an Agilent 6210 time-of-flight mass spectrometer. Elemental analysis was performed at the University of Geneva, Switzerland.

7-*O*-Methylhesperetin 3'-*O*-[Methyl- β -D-glucopyranosyluronate] (15**).** To a solution of 7,4'-di-*O*-methyletheriodictyol **5** (273 mg, 0.86 mmol) in dry CH₂Cl₂ (25 mL in the presence of mol sieve) was added the glucuronic acid donor **13** (27) (1.08 g, 2.15 mmol) followed by BF₃·(OEt)₂ (32 μ L). The mixture was stirred overnight under nitrogen and at room temperature. The solution was diluted with a mixture of CH₂Cl₂/H₂O 1:1 (50 mL). The organic layer was separated, washed with H₂O, dried over MgSO₄, and evaporated. The crude product **14** was directly submitted to the subsequent deacetylation reaction. However, an aliquot of **14** was isolated and characterized. ¹H NMR (400 MHz, CDCl₃) δ 2.03 (s, 3, OAc), 2.05 (s, 3, OAc), 2.09 (s, 3, OAc), 2.17 (s, 3, OAc), [2.78 (dd, $J = 17.2$ and 3.1 Hz) plus 3.04 (dd, $J = 17.2$ and 12.8 Hz), plus 3.06 (dd, $J = 17.2$ and 12.8 Hz)] (2, H3), 3.73 (s, 1, H7''), 3.81 (s, 3, 7-OMe), 3.84 (s, 3, 4'-OMe), [4.10 (d, $J = 9.4$ Hz) plus 4.12 (d, $J = 9.6$ Hz)] (1, H5''), [5.06 (d, $J = 6.8$ Hz) plus 5.08 (d, $J = 6.8$ Hz)] (1, H1''), ca. 5.30–5.40 (m, 4, H2 + H2'' + H3'' + H4''), 6.03 (br d, $J = 2.3$ Hz, H8), 6.06 (d, $J = 2.2$ Hz, H6), [6.94 (d, $J = 8.4$ Hz) plus 6.95 (d, $J = 8.4$ Hz)] (1, H5'), ca. 7.15–7.19 (m, 1, H6'), 7.28 (m, 1, H2'), 12.00 (s, 1, 5-OH); ¹³C NMR (100 MHz, CDCl₃) δ 20.50 (OAc), 20.63 (OAc), 42.89 (C3), 43.04 (C3), 52.95 (C7''), 55.70 (7-OMe), 56.10 (4'-OMe), 69.20 (C4''), 71.08 (C2''), 71.82 (C3''), 72.53 (C5''), 78.47 (C2), 94.29 (C8), 95.08 (C6), 100.56 (C1''), 100.65 (C1''), 103.09 (C10), 112.69 (C5'), 118.92 (C2'), 119.34 (C2'), 122.86 (C6'), 123.02 (C6'), 130.88 (C1'), 130.97 (C1'), 145.58 (C3'), 145.65 (C3'), 151.11 (C4'), 162.65 (C9), 164.10 (C5), 166.89 (C6'), 167.98 (C7), 169.32 (OAc), 169.39 (OAc), 170.18 (OAc), 195.78 (C4). To the solution of crude **14** in dry methanol (20 mL) was added zinc acetate (787 mg, 4.3 mmol). The solution was

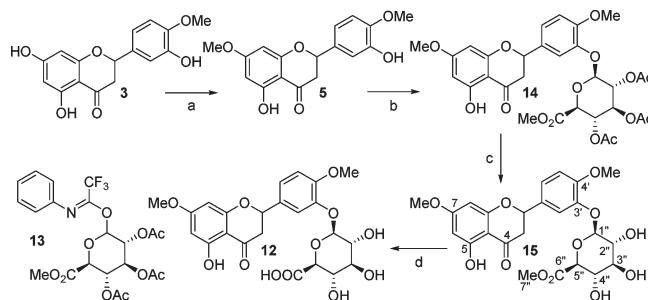


Figure 2. Synthesis of persicogenin 3'-*O*- β -D-glucuronide **12**. a) MeI, iPr₂NEt, DMF, 24 h at rt, 55%; b) Compound **13**, BF₃ etherate, dry CH₂Cl₂; c) Zn(OAc)₂, MeOH, 75 °C, 41% on two steps b + c; d) PLE, aq. phosphate buffer pH 7.2, 37 °C, 73%.

heated at 75 °C for 24 h, then MeOH was evaporated, and the crude was purified by column chromatography on silica gel (60 g) eluted with CH₂Cl₂/MeOH (9:1), to provide compound **15** (177 mg, 0.35 mmol). Yield = 41% from **5** (two steps). ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.78 (dd, $J = 17.1$ and 2.9 Hz, H3), ca. 3.29–3.52 (m, 4, H3 + H2'' + H-3'' + H4''), [3.64 (s), plus 3.65 (s)] (1, H7''), 3.78 (s, 3, 4'-OMe), 3.80 (s, 3, 7-OMe), 4.05 (d, $J = 9.6$ Hz, H5''), [5.20 (d, $J = 7.8$ Hz) plus 5.22 (d, $J = 8.2$ Hz)] (1, H1''), ca. 5.47–5.55 (m, 1, H2), 6.10 (d, $J = 2.3$ Hz, H6 or H8), 6.13 (d, $J = 2.3$ Hz, H6 or H8), 7.03 (d, $J = 8.5$ Hz, H5'), 7.11 (m, 1, H6'), [7.26 (d, $J = 2.0$ Hz) plus 7.29 (d, $J = 2.0$ Hz)] (1, H2'), 12.11 (s, 1, 5-OH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 42.35 (C3), 42.40 (C3), 52.37 (C7''), 56.18 (4'-OMe), 56.39 (7-OMe), 71.83 (C4''), 73.28 (C2''), 75.62 (C5''), 76.36 (C3''), 78.75 (C2), 78.84 (C2), 94.32 (C6 or C8), 95.15 (C6 or C8), 99.80 (C1''), 99.91 (C1''), 103.06 (C10), 112.82 (C5'), 114.35 (C2'), 114.44 (C2'), 121.30 (C6'), 121.39 (C6'), 131.10 (C1'), 146.18 (C3'), 146.26 (C3'), 149.73 (C4'), 149.81 (C4'), 163.16 (C5 or C9), 163.66 (C5 or C9), 167.94 (C7), 169.70 (C6''), 197.26 (C4); MS (ESI⁺) 529 (M + Na).

7-*O*-Methylhesperetin-3'-*O*- β -D-glucuronide (12**).** A solution of **15** (116 mg, 0.23 mmol) was made in 1.5 mL of DMSO, and 35 mL of 20 mM NaH₂PO₄/Na₂HPO₄ buffer (pH 7.2) was added. The mixture was warmed in a water bath at 37 °C for 5 min, and then 80 mg of PLE (2149 units) was added. The solution was stirred at 37 °C for 8 h. Purification of the medium was carried out by VLC on RP-18 (30 g), using a gradient of MeOH in H₂O (from 10 to 100%), to give 83 mg (0.16 mmol) of previously unreported 7-*O*-methylhesperetin-3'-*O*- β -D-glucuronide (persicogenin 3'-*O*- β -D-glucuronide) **12**, as a white powder (73%). *R*_f, 0.5 (RP-18, H₂O/MeOH 1:1); HPLC retention time, 26.16 min; ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.76 (m, 1, H3), ca. 3.22–3.48 (m, 4, H3 + H2'' + H3'' + H4''), 3.51 (m, 1, H5''), 3.78 (s, 6, 7-OMe + 4'-OMe), 5.07 (br s, 1, H1''), 5.49 (br d, $J = 10.0$ Hz, H2), 6.08 (br s, 1, H6 or H8), 6.14 (br s, 1, H6 or H8), 7.02 (d, $J = 8.4$ Hz, H5'), 7.10 (d, $J = 8.1$ Hz, H6'), 7.29 (br s, 1, H2'); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 42.59 (C3), 56.19 (7-OMe + 4'-OMe), 72.42 (C4''), 73.50 (C2''), 74.82 (C5'), 77.12 (C3''), 79.03 (C2), 94.30 (C6 or C8), 95.41 (C6 or C8), 100.03 (C1''), 112.79 (C5'), 114.62 (C2'), 121.09 (C6'), 131.31 (C1'), 146.69 (C3'), 149.79 (C4'), 163.19 (C5), 167.83 (C6'), 173.63 (C7); MS (ESI⁺) 492 (M + Na); HRMS (ESI⁺), calcd for C₂₃H₂₃O₁₂ 491.1189 [M - H]⁻, found 491.1193.

RESULTS AND DISCUSSION

The objective of the work was the optimization of a glucuronidation method, compatible with the stability of the flavanone ring. Thus, we chose 7,4'-di-*O*-methyletheriodictyol (persicogenin) **5** as substrate for glucuronidation, because it did not involve a complex protection/deprotection strategy of the aglycone moiety. The preparation of persicogenin 3'-*O*- β -D-glucuronide **12** is displayed in **Figure 2**. The structures of the compounds have been established on the basis of their 1D NMR (¹H, ¹³C), and 2D NMR (direct and long-distance heteronuclear correlations) data. 7,4'-Di-*O*-methyletheriodictyol **5** has been previously prepared either from its corresponding protected chalcone (**28**) or by methylation of hesperetin derivatives (**29**, **30**). In the present study, compound **5** was synthesized in 55% yield (**Figure 2**) by

selective methylation of a commercial mixture of (*S*)- and (*R*)-hesperetin **3**. The ^1H and ^{13}C NMR data of our synthetic persicogenin **5** (see the Supporting Information) were in accordance with the published ones (31, 32). Because in 7,4'-di-*O*-methylerydiol a strong hydrogen bond is established between the 5-hydroxyl and the 4-carbonyl group, the glucuronidation of **5** was expected to be directed to position 3'. To achieve this, we chose the recently introduced 2,3,4-triacetyl-*D*-methyl glucuronopyranosyl-(*N*-phenyl)-2,2,2-trifluoroacetimidate **13** (Figure 2) (27). The latter was treated with **5** in the presence of $\text{BF}_3 \cdot (\text{OEt})_2$ to yield the protected glucuronide **14**. Because the resulting glucuronide **14** was a mixture of two diastereoisomers (which unfortunately did not separate on usual silica or RP-18 chromatographic phases), some of the ^1H and ^{13}C NMR signals of **14** were split (see Materials and Methods), which rendered the interpretation of the spectra more difficult. Nevertheless, the regioselective glucuronidation at position 3' in **14** was unequivocally confirmed by the presence, on its long-distance proton-carbon correlation spectrum, of a 3J correlation between the anomeric proton of the glucuronyl moiety ($\text{H}1''$) and the aromatic C3' carbon of the flavanone residue. Compound **14** was subsequently subjected to deprotection step c (Figure 2). The first challenge was to achieve the deacetylation of **14** while preventing the opening of the flavanone into its corresponding chalcone. Nudelman et al. (33) have reported the deprotection of podophyll glucuronides by zinc acetate in anhydrous conditions. When using these conditions, we were pleased to see that compound **14** yielded compound **15** with no opening to the chalcone form (41% yield over two steps from **5**). However, in accordance with the previous study (33), the methyl ester of the glucuronic acid moiety was not hydrolyzed under these conditions. Furthermore, the hydrolysis of the methyl ester function of **15** turned out to be difficult by chemical means. Fortunately, the methyl ester hydrolysis proceeded smoothly under the catalysis of pig liver esterase (PLE) (34). Thus, persicogenin 3'-*O*- β -*D*-glucuronide **12** was successfully obtained in 73% yield from **15**. In conclusion, this is the first efficient chemical synthesis of a flavanone glucuronide. The method can be applied to the preparation of a wide range of flavanone glucuronides. The resolution of the two enantiomers of the starting product hesperetin was out of the scope of this study. Nevertheless, none of the steps of this newly reported synthesis are susceptible to inducing flavanone-chalcone isomerization. Thus, the method can be potentially applied to the preparation of pure (*S*)- and (*R*)-flavanone glucuronides from the appropriate enantiomerically pure aglycone. Its application to the synthesis of the glucuronides of the dietarily relevant hesperetin is currently under investigation in our laboratory.

Supporting Information Available: Chemical synthesis, ^1H NMR, ^{13}C NMR, and MS data, and elemental analysis of 7,4'-di-*O*-methylerydiol **5**. 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) De Nysschen, A. M.; Van Wyk, B. E.; Van Heerden, F. R.; Schutte, A. L. The major phenolic compounds in the leaves of *Cyclopia* species (honeybush tea). *Biochem. Syst. Ecol.* **1996**, *24*, 243–246.
- (3) Balza, F.; Jamieson, L.; Towers, G. H. N. Chemical constituents of the aerial parts of *Artemisia dracunculul*. *J. Nat. Prod.* **1985**, *48*, 339–340.
- (4) Erlund, I. Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology. *Nutr. Res.* **2004**, *24*, 851–874.
- (5) 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.
- (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.* **2000**, *279*, G1148–G1154.
- (7) 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.
- (8) Abe, K.; Nakada, Y.; Suzuki, A.; Yumioka, E. Biliary metabolites of hesperetin in rats. *Shoyakugaku Zasshi* **1993**, *47*, 43–46.
- (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) Miyake, Y.; Shimoi, K.; Kumazawa, S.; Yamamoto, K.; Kinae, N.; Osawa, T. Identification and antioxidant activity of flavonoid metabolites in plasma and urine of eriocitrin-treated rats. *J. Agric. Food Chem.* **2000**, *48*, 3217–3224.
- (13) Erlund, I.; Meririnne, E.; Alfthan, G.; Aro, A. Plasma kinetics and urinary excretion of the flavanones naringenin and hesperetin in humans after ingestion of orange juice and grapefruit juice. *J. Nutr.* **2001**, *131*, 235–241.
- (14) Kanaze, F. I.; Bounartzi, M. I.; Georgarakis, M.; Niopas, I. Pharmacokinetics of the citrus flavanone aglycones hesperetin and naringenin after single oral administration in human subjects. *Eur. J. Clin. Nutr.* **2007**, *61*, 472–477.
- (15) Miyake, Y.; Sakurai, C.; Usuda, M.; Fukumoto, S.; Hiramitsu, M.; Sakaida, K.; Osawa, T.; Kondo, K. Difference in plasma metabolite concentration after ingestion of lemon flavonoids and their aglycones in humans. *J. Nutr. Sci. Vitaminol.* **2006**, *52*, 54–60.
- (16) Barron, D.; Cren-Olive, C.; Needs, P. W. Chemical synthesis of flavonoid conjugates. In *Methods in Polyphenol Analysis*; Santos-Buelga, C., Williamson, G., Eds.; Royal Society of Chemistry: Cambridge, U.K., 2003; Chapter 9, pp 187–213.
- (17) Williamson, G.; Barron, D.; Shimoi, K.; Terao, J. *In vitro* biological properties of flavonoid conjugates found *in vivo*. *Free Radical Res.* **2005**, *39*, 457–469.
- (18) Barron, D. Recent advances in the chemical synthesis and biological activity of phenolic metabolites. *Recent Adv. Polyphenol Res.* **2008**, *1*, 317–358.
- (19) Needs, P. W.; Kroon, P. A. Convenient syntheses of metabolically important quercetin glucuronides and sulfates. *Tetrahedron* **2006**, *62*, 6862–6868.
- (20) Pearson, A. G.; Kiefel, M. J.; Ferro, V.; von Itzstein, M. 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.
- (21) Al Maharik, N.; Botting, N. P. A facile synthesis of isoflavone 7-*O*-glucuronides. *Tetrahedron Lett.* **2006**, *47*, 8703–8706.
- (22) Davis, B. D.; Needs, P. W.; Kroon, P. A.; Brodbelt, J. S. Identification of isomeric flavonoid glucuronides in urine and plasma by metal complexation and LC-ESI-MS/MS. *J. Mass Spectrom.* **2006**, *41*, 911–920.
- (23) Miyazawa, M.; Okuno, Y.; Nakamura, S. I.; Kosaka, H. Antimutagenic activity of flavonoids from *Pogostemon cablin*. *J. Agric. Food Chem.* **2000**, *48*, 642–647.
- (24) Li, W. X.; Cui, C. B.; Cai, B.; Wang, H. Y.; Yao, X. S. Flavonoids from *Vitex trifolia* L. inhibit cell cycle progression at G2/M phase and induce apoptosis in mammalian cancer cells. *J. Asian Nat. Prod. Res.* **2005**, *7*, 615–626.

- (25) Kanashiro, A.; Kabeya, L. M.; Polizello, A. C. M.; Lopes, N. P.; Lopes, J. L. C.; Lucisano-Valim, Y. M. Inhibitory activity of flavonoids from *Lychnophora* sp. on generation of reactive oxygen species by neutrophils upon simulation by immune complexes. *Phytother. Res.* **2004**, *18*, 61–65.
- (26) Ling, S. K.; Pizar, M. M.; Man, S. Platelet-activating factor (PAF) receptor binding antagonist activity of the methanol extracts and isolated flavonoids from *Chromolaena odorata* (L.) King and Robinson. *Biol. Pharm. Bull.* **2007**, *30*, 1150–1152.
- (27) Wang, P.; Zhang, Z.; Yu, B. Total synthesis of CRM646-A and -B, two fungal glucuronides with potent heparinase inhibition activities. *J. Org. Chem.* **2005**, *70*, 8884–8889.
- (28) Jain, A. C.; Sharma, B. N. Synthesis of (±)-7,3'- and 7,4'-di-O-methyletheriodictyol and of velutin and pilloin. *Phytochemistry* **1973**, *12*, 1455–1458.
- (29) Sun, Z.; Wang, X. Synthesis of 5,7-dihydroxy-3',4'-dimethoxy and 3',5-dihydroxy-4',7-dimethoxyflavanones. *Zhongguo Yiyao Gongye Zazhi* **1999**, *30*, 179–180.
- (30) Shan, Y.; Li, G. Y.; Wang, Q. A.; Li, Z. H. Semisynthesis of five bioactive flavonoids from hesperidin. *Youji Huaxue* **2008**, *28*, 1024–1028.
- (31) Suksamrarn, A.; Chotipong, A.; Suavansri, T.; Boongird, S.; Timsuksai, P.; Vimutipong, S.; Chuaynugul, A. Antimycobacterial activity and cytotoxicity of flavonoids from the flowers of *Chromolaena odorata*. *Arch. Pharmacol. Res.* **2004**, *27*, 507–511.
- (32) Backheet, E. Y.; Farag, S. F.; Ahmed, A. S.; Sayed, H. M. Flavonoids and cyanogenic glycosides from the leaves and stem bark of *Prunus persica* (L.) Batsch (Meet Ghamr) peach local cultivar in Assiut region. *Bull. Pharm. Sci., Assiut Univ.* **2003**, *26*, 55–66.
- (33) Nudelman, A.; Ruse, M.; Gottlieb, H. E.; Fairchild, C. Studies in sugar chemistry. Part 8. Glucuronides of podophyllum derivatives. *Arch. Pharm. (Weinheim, Germany)* **1997**, *330*, 285–289.
- (34) Rawale, S.; Hrihorczuk, L. M.; Wei, W. Z.; Zemlicka, J. Synthesis and biological activity of the prodrug of class I major histocompatibility peptide GILGFVFTL activated by β -glucuronidase. *J. Med. Chem.* **2002**, *45*, 937–943.

Received April 6, 2009. Revised manuscript received June 15, 2009.
Accepted July 16, 2009.