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First Chemical Synthesis and in Vitro Characterization of the Potential Human Metabolites 5-*O*-Feruloylquinic Acid 4′-Sulfate and 4′-*O*-Glucuronide

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Supporting Information

ABSTRACT: Feruloylquinic acids are a major class of biologically active phenolic antioxidants in coffee beans, but their metabolic fate is poorly understood. The present study investigated the phase II metabolism of feruloylquinic acids with selected human sulfotransferases (SULT1A1 and SULT1E1) and uridine 5′-diphosphoglucuronosyltransferases (UGT1A1 and UGT1A9). For unequivocal metabolite identification, the chemical synthesis of two potential human metabolites of 5-*O*-feruloylquinic acid, the 4′-sulfated and 4′-*O*-glucuronidated conjugates, has been performed for the first time. Following incubation with human SULT1A1 or SULT1E1, formation of 5-*O*-feruloylquinic acid 4′-*O*-sulfate was confirmed by matching its HPLC and MS data with those of the authentic standard. On the other hand, no glucuronide conjugates were detected after incubation with human uridine 5′-diphosphoglucuronosyltransferases. These results suggest that sulfation can take place on the ferulic acid moiety of feruloylquinic acids and may be a major metabolic pathway for feruloylquinic acids in humans.

KEYWORDS: feruloylquinic acid glucuronide, feruloylquinic acid sulfate, human metabolites, coffee, synthesis, sulfotransferase

INTRODUCTION

Chlorogenic acids are naturally occurring polyphenolic compounds possessing a quinic acid unit and a *trans*-cinnamic acid unit. The most widespread subgroup is the 5-monoester of caffeic acid, present in coffee beans, potatoes, and many fruits and vegetables.[1,2] Chlorogenic acids possess many biological properties, such as antioxidant[3−6] and antibacterial.[7] However, the human metabolism of chlorogenic acids is far from fully understood. Chlorogenic acids are believed to be metabolized, mainly in the colon, into a range of hydroxycinnamic, hydroxyphenylpropionic, and hydroxyphenylacetic acids.[8−13] It has also been demonstrated that chlorogenic acids could be absorbed in their intact form in rats,[14] and a number of recent studies have indicated the presence of intact chlorogenic acid forms in human plasma[15−17] and human urine.[18,19] However, the exact nature of the human metabolites of feruloylquinic acids remained unclear from these studies since their potential sulfated or glucuronidated conjugates either were not investigated or were enzymatically hydrolyzed prior to analysis.

The phase II metabolism in humans is carried out by uridine 5′-diphosphoglucuronosyltransferases (UGTs) and sulfotransferases (SULTs). SULTs and UGTs are abundant in the human liver and intestine, where they catalyze conjugation of xenobiotics.[20] The UGT1A subfamily are in general active toward phenolic hydroxyl groups, with UGT1A1 and UGT1A9 being the most active isoforms.[21] A recent study by our group suggested that SULT1A1 and SULT1E1 are highly effective in the sulfation of phenolic acids.[22] Theoretically, conjugation of chlorogenic acids could affect the phenolic hydroxyls of the hydroxycinnamic acid moiety, the alcoholic hydroxyls of the quinic acid part, or both. Recently, the presence of feruloylquinic acid sulfates and glucuronides has been demonstrated in the ileal fluid of human volunteers with an ileostomy.[23] However, the position of conjugation was not determined. On the other hand, a previous study performed on rats [24] suggested that glucuronidation of 1,5-dicaffeoylquinic acid was directed to the quinic acid skeleton, although no clear scientific evidence was provided to support this hypothesis. In the absence of suitable chromatographic standards, the identification and quantification of these conjugates is difficult. Moreover, the availability of appropriate standards of chlorogenic acid conjugates is essential for studying their biological effects.

Herein, we describe the first synthesis of two potential human metabolites of 5-*O*-feruloylquinic acid, i.e., its 4′-sulfate and 4′-*O*-glucuronide metabolites. The synthesized authentic standards were used for unequivocal identification of 5-feruloylqinic acid metabolites from in vitro incubations using human recombinant UGTs and SULTs.

MATERIALS AND METHODS

Chemicals and Instruments. The authentic standards of the 3-*O*-, 4-*O*-, and 5-*O*-feruloylquinic acids have been chemically synthesized according to published methods.[25,26] Thin-layer chromatography (TLC) was carried out on silica 60 F_{254} (Merck) and RP-18 F_{254s} (Merck) plates. The TLC plates were visualized by short-wave UV light or ceric

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ammonium molybdate stain. Flash chromatography was performed using a Biotage SP1 high-performance flash chromatography system and FLASH cartridges (25+M, 40+M, and 65i, KP-SIL). ^{1}H NMR (360.13 MHz) and ^{13}C NMR (90.56 MHz) spectra were recorded on a Bruker DPX-360 spectrometer equipped with a broad-band multinuclear *z*-gradient probehead. The chemical shifts (ppm) are expressed with respect to an internal reference (TMS or TSP). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quatruplet, m = multiplet, br s = broad singlet. Melting points were recorded on a Buchi melting point B-545 apparatus and are uncorrected. Optical rotations were measured with a JASCO P2000 polarimeter. Elementary analyses were performed at the University of Geneva, Switzerland (Service de Microanalyse). HPLC/DAD/ESI-TOF-MS analyses were performed on 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. HPLC/DAD analyses were carried out using an Agilent 1200 series liquid chromatography system. Chromatograhy was performed with a Zorbax XDB-C18 column (4.6 × 50 mm, 1.8 μm) with 1% formic acid (A) and methanol (B) as the mobile phase. The gradient started at 17% (B), which was kept for 2 min, followed by an increase to 30% B in 13 min and to 60% in 2 min, and then was returned to 17% B for 5 min at 0.5 mL/min. A 25 μL volume of the supernatant was injected into the column. Isoferulic acid was used as the internal standard. UV detection was carried out at 310 nm using a photodiode array detector. Recombinant human UGT1A1 and UGT1A9 expressed in Sf9 insect cells (Supersomes) were obtained from BD Gentest (Woburn, MA). The total protein content (5 mg/mL) of Supersomes was specified on data sheets provided by the manufacturer. SULTs were expressed in *Salmonella typhimurium* and purified as previously described.[27] All activities were adjusted to the expression levels of SULTs measured by immunoblot analysis.

Chemical Synthesis of 5-*O*-Feruloylquinic Acid 4′-Sulfate (1). To a solution of vanillin (**3**; 400 mg, 2.62 mmol) in anhydrous THF (6 mL) were added triethylamine (0.546 mL, 3.93 mmol) and DMAP (320 mg, 2.62 mmol). After the resulting solution was stirred for 10 min, neopentyl chlorosulfate[28] (678 mg, 3.63 mmol) was added, and the mixture was stirred for 2 h at room temperature. The solution was diluted with ethyl acetate and water. The organic layer was washed with water and brine, dried over sodium sulfate (Na_2SO_4), filtered, concentrated, and purified by flash chromatography on silica gel using a gradient of ethyl acetate in petroleum ether. 4-Formyl-2-methoxyphenyl neopentyl sulfate (**4**) was isolated as a white solid (530 mg, 1.85 mmol, 71%). To a solution of **4** (214 mg, 1.41 mmol) and malonyl quinic fragment **8** (353 mg, 1.26 mmol) in anhydrous DMF (4 mL) were added DMAP (55 mg, 0.45 mmol) and piperidine (71 μL) at room temperature. The details of the synthesis of compound **8** are displayed in the Supporting Information. The mixture was stirred for 7 days at room temperature in a sealed tube. It was then concentrated and purified by flash chromatography on RP-18 using a gradient of methanol in water. After lyophilization, the white solid **9** (550 mg) was directly deprotected in anhydrous DMF (5 mL) using sodium azide (96 mg, 1.45 mmol). The solution was heated at 55 °C for 12 h, concentrated, purified by flash chromatography on RP-18 using a gradient of methanol in water, and lyophilized to give the sodium salt of **1** as a white solid (315 mg, 0.67 mmol, 53% from compounds **4** and **8** over the two steps).

Chemical Synthesis of 5-*O*-Feruloylquinic Acid 4′-*O*-Glucuronide (2). The synthesis of the glucuronic acid donor (2,3,4-tri-*O*-acetyl-D-methylglucuronopyranosyl)-*N*-phenyl-2,2,2-trifluoroacetimidate (**5**) is detailed in the Supporting Information. To compounds **5** (1.47 g, 2.91 mmol) and **3** (225 mg, 1.48 mmol) in 20 mL of anhydrous dichloromethane, BF_3 etherate (56 μL, 1.48 mmol), was added dropwise, and the mixture was stirred overnight at room temperature. The solution was then diluted with dichloromethane (50 mL), and the organic layer was washed with water (2 × 50 mL), dried over sodium sulfate (Na_2SO_4), filtered, concentrated, and purified by flash chromatography on silica gel using a gradient of ethyl acetate in petroleum ether to give a yellow foam. Recrystallization and trituration in diethyl ether gave a white solid of compound **6** (480 mg, 1.02 mmol, 69%). Alkaline hydrolysis of **6** was carried out in MeOH (11 mL) and aqueous NaOH (1 N, 10 mL). The mixture was stirred for 5 h at room temperature and then acidified to pH 6 by addition of Amberlite IRC 50 ion-exchange resin. The resulting suspension was filtered, concentrated under reduced pressure, and purified by flash chromatography on reversed-phase RP-18 using a gradient of methanol in water. Compound **7** was isolated as a white foam (183 mg, 0.56 mmol, 64%). To a solution of **7** (150 mg, 0.45 mmol) and malonyl quinic fragment **8** (115 mg, 0.41 mmol) in anhydrous DMF (1.3 mL) were added DMAP (20 mg, 0.16 mmol) and piperidine (22 μL). The mixture was stirred for 7 days at room temperature in a sealed tube. The solution was then concentrated under reduced pressure and purified by flash chromatography on RP-18 using a gradient of methanol in water to give, after freeze-drying, **2** as a white solid (20 mg, 0.037 mmol, 9%).

Data for 1. ^{1}H NMR (360 MHz, MeOD-d_4) δ 7.72 (d, J = 15.9 Hz, 1H), 7.54 (d, J = 8.3 Hz, 1H), 7.31 (d, J = 2.0 Hz, 1H), 7.19 (dd, J = 8.5, 1.9 Hz, 1H), 6.55 (d, J = 15.9 Hz, 1H), 5.42 (ddd, J = 11.8, 10.3, 5.4 Hz, 1H), 4.17 (dt, J = 6.2, 3.0 Hz, 1H), 3.91 (s, 3H), 3.72 (dd, J = 9.9, 3.1 Hz, 1H), 2.22−1.96 (m, 4H); ^{13}C NMR (90 MHz, MeOD-d_4) δ 182.44, 170.08, 154.72, 147.31, 146.34, 134.54, 125.96, 123.69, 120.16, 114.48, 79.22, 76.34, 74.40, 74.22, 58.08, 42.13, 40.40; LC/ESI-TOF-MS obsd m/z ($[M+1]^+$) 471.06 (M + Na), 391.11, 177.05; obsd m/z ($[M-1]^-$) 468.99 (M + Na − 2H), 447.10 (M − H), 367.16, 193.04. Anal. Calcd for $C_{17}H_{18}Na_2O_{12}S$: C, 37.99; H, 4.31. Found: C, 38.08; H, 4.20.

Data for 2. ^{1}H NMR (400 MHz, D_2O) δ 7.53 (d, J = 16.2 Hz, 1H), 7.08−7.02 (m, 3H), 6.35 (d, J = 16.1 Hz, 1H), 5.27 (br s, 1H, OH), 5.19 (td, J = 15.8, 4.7 Hz, 1H), 5.08 (d, J = 5.4 Hz, 1H), 4.19 (m, 1H, OH), 4.15 8 m, 1H), 3.87−3.70 (m, 5H), 3.61−3.55 (m, 3H), 3.28 (m, 1H, OH), 3.06 (m, 1H, OH), 2.10−1.86 (m, 4H); ^{13}C NMR (90 MHz, D_2O) δ 181.00, 170.87, 168.86, 148.54, 147.42, 145.61, 129.30, 122.88, 116.16, 115.49, 111.35, 99.78, 76.89, 76.43, 75.27, 72.78, 72.63, 71.72, 71.28, 70.78, 55.84, 38.52, 38.13; LC/ESI-TOF-MS obsd m/z ($[M+1]^+$) 471.06 (M + Na), 567.16, 177.05; obsd m/z ($[M-1]^-$) 543.06 (M − H), 193.04.

Data for 4. Mp 49−51 °C; ^{1}H NMR (360 MHz, $CDCl_3$, TMS as reference) δ 9.99 (s, 1H), 7.58−7.48 (m, 3H), 4.18 (s, 2H), 3.97 (s, 3H), 1.01 (s, 9H); ^{13}C NMR (90 MHz, $CDCl_3$, TMS as reference) δ 190.67, 152.03, 143.55, 135.87, 124.49, 123.24, 111.35, 83.93, 56.25, 31.88, 25.25; LC/ESI-TOF-MS obsd m/z 289.0000 (289.0000 calcd for $[M-H]^+$), obsd m/z 311.0000 (311.0000 calcd for $[M+Na]^+$).

Data for (3*R*,4*S*,5*S*,6*S*)-2-(4-Formyl-2-methoxyphenoxy)-6-(methoxycarbonyl)tetrahydro-2*H*-pyran-3,4,5-triyl Triacetate (6). Mp 137−139 °C; $[\alpha]_D^{25}$ = −47.17° (c = 1.06 g/L, MeOH); ^{1}H NMR (360 MHz, $CDCl_3$, TMS as reference) δ 9.89 (s, 1H), 7.47 (s, 1H), 7.43 (dd, J = 6.6, 1.9 Hz, 1H), 7.25 (d, J = 8.6 Hz, 1H), 5.38−5.29 (m, 3H), 5.18 (m, 1H), 4.17 (m, 1H), 3.89 (s, 3H), 3.73 (s, 3H), 2.06 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (90 MHz, $CDCl_3$, TMS as reference) δ 190.97, 170.11, 169.20, 166.81, 151.07, 133.02, 125.57, 118.81, 110.65, 99.66, 72.73, 71.52, 70.89, 69.01, 56.10, 53.00, 20.64, 20.61, 20.53; LC/ESI-TOF-MS obsd m/z ($[M+1]^+$) 491 (M + Na). Anal. Calcd for $C_{21}H_{24}O_{12}\cdot 0.4H_2O$: C, 53.03; H, 5.26. Found: C, 52.85; H, 5.19.

Data for (2*S*,3*S*,4*S*,5*R*)-Methyl 6-(4-Formyl-2-methoxyphenoxy)-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-carboxylate (7). $[\alpha]_D^{25}$ = −93.27° (c = 1.04 g/L, MeOH); ^{1}H NMR (360 MHz, D_2O, TSP as internal reference) δ 9.70 (s, 1H), 7.50 (d, J = 8.3 Hz, 1H), 7.42 (s, 1H), 7.23 (d, J = 8.6 Hz, 1H), 5.24 (d, J = 7.1 Hz, 1H), 3.93 (d, J = 8.6 Hz, 1H), 3.88 (s, 3H), 3.72−3.58 (m, 3H); ^{13}C NMR (90 MHz, D_2O, TSP as internal reference) δ 194.29, 174.70, 150.65, 148.32, 130.46, 126.37, 114.35, 110.63, 98.91, 76.00, 74.72, 72.02, 71.15, 55.33; LC/ESI-TOF-MS obsd m/z ($[M-1]^-$) 327.07 (M − H).

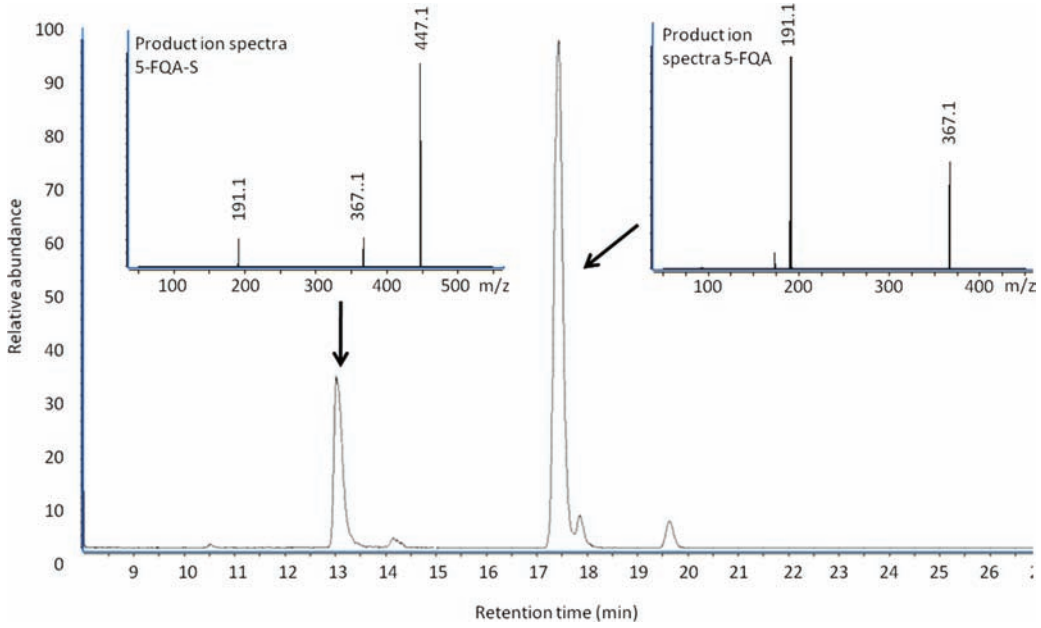

Figure 1. HPLC/DAD/ESI-MS/MS analysis of 5-*O*-feruloylquinic acid incubated with SULT1E1. The product ion spectra of 5-*O*-feruloylquinic acid (m/z 367.1) and 5-*O*-feruloylquinic acid 4′-sulfate (m/z 447.1) were obtained in product ion mode.

Glucuronidation of Feruloylquinic Acids by Recombinant Human UGTs. The contribution of individual isozymes to the glucuronidation of the feruloylquinic acids was assessed using human recombinant UGT1A1 and UGT1A9. The reaction was performed in 100 mM potassium phosphate buffer (pH 7.4) containing 100 μM vitamin C, 1 mM UDPGA, 5 mM saccharolactone, and 0.025 mg/mL alamethicin (final volume 50 μL). Control Supersomes and UGTs were added at a final concentration of 2 mg/mL. The reaction was started by the addition of 100 μM 5-*O*-feruloylquinic acid, allowed to proceed at 37 °C for 1 h, and finally stopped by the addition of 10 μL of ice-cold acetonitrile containing 500 mM HCl. After centrifugation at 13000*g* for 5 min, the supernatant was dried in vacuum and the residue redissolved in the initial mobile phase and analyzed by HPLC and HPLC/DAD/ESI-MS.

Sulfation of Feruloylquinic Acids by Recombinant Human SULTs. Sulfation of the feruloylquinic acids by SULT1A1 and SULT1E1 was performed in 100 mM potassium phosphate buffer (pH 7.4) containing 100 μM vitamin C, 100 μM 3′-phosphoadenosine 5′-phosphosulfate, and 1 mM DL-dithiothreitol (final volume 50 μL). The control cytosolic fraction and cytosolic preparations from SULT-expressing bacteria were added to a final concentration of 0.25 mg/mL total protein. The reaction was initiated by the addition of 100 μM feruloylquinic acid. The reaction was allowed to proceed at 37 °C for 1 h and stopped by adding 10 μL of ice-cold acetonitrile with 500 mM HCl. After centrifugation at 13000*g* for 5 min, the supernatant was dried in vacuum and the residue redissolved in the initial mobile phase and analyzed by HPLC/DAD/ESI-TOF-MS.

RESULTS AND DISCUSSION

To gain insight into the potential feruloylquinic acid metabolites that may be formed in vivo in man, we synthesized 4′-*O*-sulfated (**1**) and 4′-*O*-glucuronidated (**2**) conjugates of 5-feruloylquinic acid and studied the metabolism of feruloylquinic acids using human recombinant UGT and SULT enzymes. The feruloylquinic acid metabolites were analyzed by HPLC/DAD and mass spectrometry following incubations with UGT1A1, UGT1A9, SULT1A1, and SULT1E1, respectively. Besides the presence of intact 5-feruloylquinic acid, significant formation of 5-feruloylquinic acid sulfate was observed with SULT1A1 and SULT1E1 (Figure 1). In the spectrum of 5-feruloylquinic acid, two ions were present at m/z 367.1 ($[M - H]^-$) and 191.1 (corresponding to the loss of the feruloyl part; Figure 1). In the spectrum of the corresponding sulfated conjugate, three ions were detected at m/z 447.1 ($[M]^-$), 367.1 ([feruloyl quinic acid $- H]^-$), and 191.1 ([quinic acid $- H]^-$). The two latter ions originated after the loss of the sulfate group. Thus, the MS data did not allow determination of which of the two ferulic or quinic acid moieties of feruloylquinic acid was bearing the sulfate group. Similar incubation of authentic standards of 3-*O*- and 4-*O*-feruloyquinic acids with SULTs was carried out (Figure 2). Among the feruloylquinic acids, 4-*O*-feruloylquinic acid was the most highly sulfated isomer by both SULTs tested, followed by 5-*O*-feruloylquinic and 3-*O*-feruloylquinic acids. In addition, SULT1E1 was more active than SULT1A1 in the sulfation of all feruloylquinic acids tested. This is in agreement with our previous study, in which it was shown that SULT1E1 possessed a unique regioselectivity toward the 4-hydroxyl group of ferulic acid and catalyzed sulfation with high efficiency.[22] In humans, SULT1A1 and SULT1E1 are highly expressed in the intestine and liver;[29] hence, sulfation may be a major phase II metabolic pathway for feruloylquinic acids in vivo.

To unequivocally determine the structure of the product of SULT incubations, we embarked on the chemical synthesis of an authentic standard of 5-feruloylquinic acid 4′-sulfate (**1**), the preparation of which has not been reported before. Classical approaches for the synthesis of chlorogenic acids are based on the esterification of quinic acid (A1) by a hydroxycinnamoyl fragment (A2; Figure 3, pathway A).[30−33] However, we followed the synthetic strategy we recently designed for 5-*O*-feruloylquinic acid and its methyl ester derivative.[34] In this case the elaboration of the (hydroxycinnamoyl)quinic acid skeleton is based on a Knoevenagel condensation of a malonate ester of quinic acid (B1) with a hydroxybenzaldehyde fragment (B2; Figure 3, pathway B). This approach does not require any

protective groups. Consequently, the deprotection step can be carried out on the quinic and benzaldehyde fragments before the formation of the hydroxycinnamoyl core. In this way, any hydrogenating, basic or strongly acidic conditions, which could be detrimental to the sensitive feruloylquinic skeleton, were avoided. The sulfation of vanillin (**3**) with neopentyl chlorosulfate[28] afforded the desired sulfate monoester **4** after stirring for 2 h at room temperature (Figure 4). Vanillin derivative **4** and malonate ester **8** were treated with piperidine and 4-(dimethylamino)pyridine in anhydrous DMF and stirred for 7 days at room temperature to give the neopentyl sulfate conjugate **9**. Deprotection of the sulfate group in **9** afforded **1** in fair 53% yield from the malonylquinic acid derivative **8** (Figure 4).

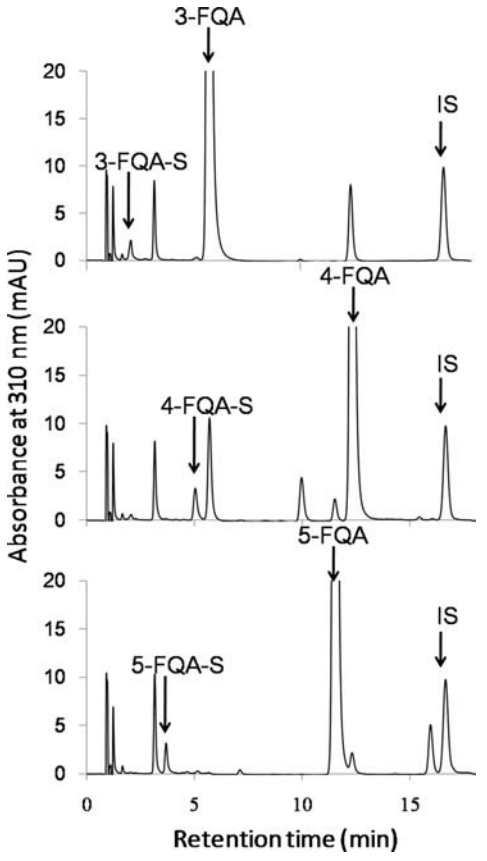

Figure 2. HPLC chromatograms of 3-*O*-feruloylquinic acid (top), 4-*O*-feruloylquinic acid (middle), and 5-*O*-feruloylquinic acid (bottom) incubated with SULT1A1: 3-FQA, 3-*O*-feruloylquinic acid; 4-FQA, 4-*O*-feruloylquinic acid; 5-FQA, 5-*O*-feruloylquinic acid; 3-FQA-S, 3-*O*-feruloylquinic acid 4′-sulfate; 4-FQA-S, 4-*O*-feruloylquinic acid 4′-sulfate; 5-FQA-S, 5-*O*-feruloylquinic acid 4′-sulfate; IS, internal standard. The two peaks around 2 min are the solvent front, and the peak at about 3.5 min is 3′-phosphoadenosine-5′-phosphosulfate (PAPS), the cofactor. Arrows indicate retention times of the standards.

The identity of the product of the incubation of 5-*O*-feruloylquinic acid with SULT1E1 was definitively established as 5-*O*-feruloylquinic acid 4′-sulfate by comparison with the authentic synthesized compound **1** using the HPLC retention time and the electrospray ionization mass spectrum. This demonstrated that sulfate conjugation was targeted to the ferulic acid moiety of feruloylquinic acids by human SULTs. The synthetic **1** was accompanied by minor amounts of isomers displaying the same molecular ion in ESI-MS. We suspected that these could be the 3-*O*- and 4-*O*-feruloylquinic acid sulfate isomers, probably formed during the last deprotection step of the sulfate group by sodium azide (step e of Figure 4). This was confirmed by matching their retention times with those of the corresponding sulfates obtained by sulfation of authentic standards of 3-*O*- and 4-*O*-feruloyquinic acids with SULTs (Figure 2). Having identified the three components of the synthetic feruloyl quinic acid sulfates, the 5-*O*-, 3-*O*-, and 4-*O*-feruloylquinic sulfate isomer ratio of the synthetic standard was established as 116:3:2 on the basis of HPLC/DAD/ESI-TOF-MS analysis.

Similar synthesis of glucuronidated 5-feruloylquinic acid 4′-*O*-glucuronide (**2**) was carried out by reaction of malonyl ester **8** with glucuronidated vanillin **7**, beforehand prepared by glucuronidation of **3** followed by deprotection of the intermediate glucuronide **6** (Figure 4, steps b, c, and f). However, no product matching the chromatographic behavior of **2** or corresponding to the mass of a glucuronidated ferulic acid was detected following incubation of 5-*O*-feruloylquinic acid with UGT1A1 or UGT1A9 (data not shown). Under the same conditions, isoferulic acid was in contrast well conjugated.[22] Since feruloylquinic acid glucuronides are present in the ileostomy fluid after consumption of coffee by ileostomists,[23] it is possible that isoforms other than UGT1A1 or UGT1A9 could be responsible for the production of these conjugates.

In vivo sulfation of feruloylquinic acids has important implications for their bioactivity in humans. The phenolic group of these compounds is critical for their biological properties, such as antioxidant activity and photoprotective activities.[35] In some cases, conjugation of polyphenols may lead to an enhancement of biological activities, such as the ability to interact with transporter proteins.[36] Thus, future research should emphasize the potential bioactivity of the conjugates, especially sulfated

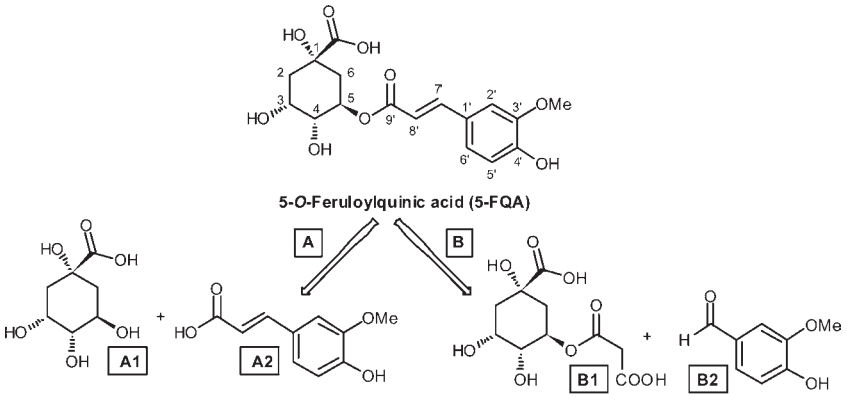

Figure 3. Retrosynthetic scheme for the elaboration of the 5-feruloylquinic acid skeleton.

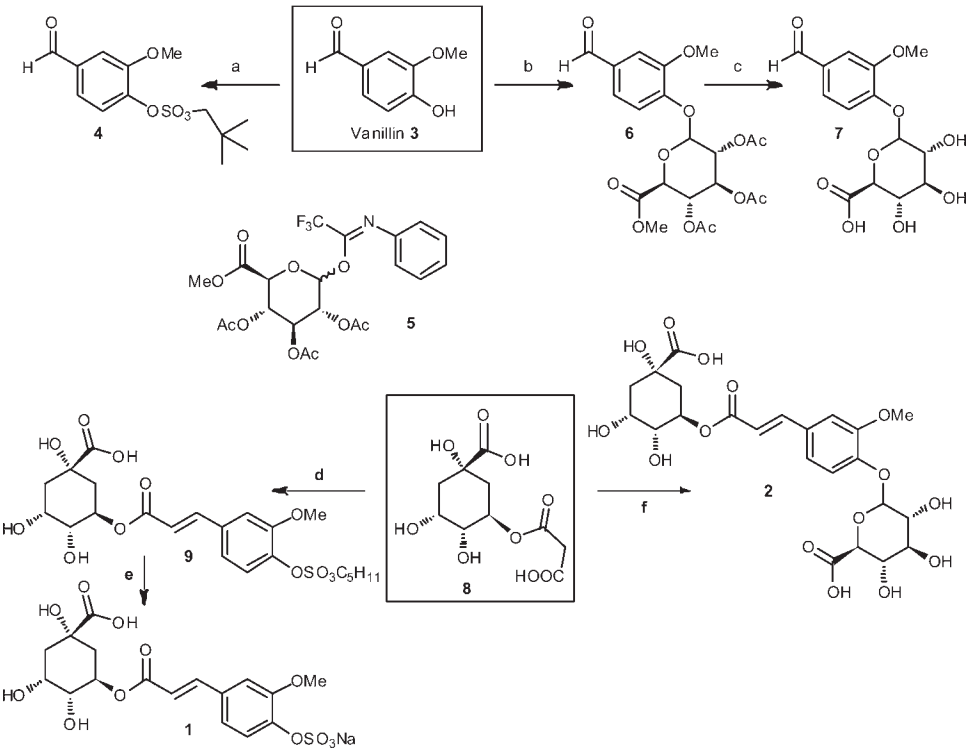

Figure 4. Preparation of 5-*O*-feruloylquinic acid metabolites **1** and **2**. Reagents and conditions: (a) DMAP, Et_3N, THF, room temperature (rt), 10 min, and then $ClSO_3C_5H_{11}$, THF, rt, 2 h, 71%; (b) compound **5**, BF_3 etherate, CH_2Cl_2, rt, 12 h, 69%; (c) NaOH (1 N), MeOH, rt, 5 h, 64%; (d) compound **4**, DMAP, piperidine, DMF, rt, 7 days; (e) NaN_3, DMF, 55 °C, overnight, (d) + (e) 53%; (f) compound **7**, DMAP, piperidine, DMF, rt, 7 days, 9%.

conjugates of feruloylquinic acids, which are the relevant metabolites in vivo. The current work also shows that the sulfate conjugation is on the ferulic acid moiety of 5-*O*-feruloylquinic acid. For 1,5-dicaffeoylquinic acid, it was suggested that glucuronidation might also occur on the quinic acid moiety.[24] In contrast, the data presented here support the ferulic acid moiety as a site for conjugation.

In conclusion, the first syntheses of sulfate and glucuronide conjugates of 5-*O*-feruloylquinic acid have been achieved. In vitro incubation with SULTs showed that sulfation at the ferulic acid moiety may be a major metabolic pathway for feruloylquinic acids in humans. These novel conjugates may prove valuable for further investigating the bioavailability and bioactivity of feruloylquinic acids.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Synthesis of **5** and **8** and the NMR data for the synthetic intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

BF_3, boron trifluoride; calcd, calculated; DMF, dimethylformamide; DMAP, (dimethylamino)pyridine; HPLC/DAD/ESI-TOF-MS, high-performance liquid chromatography/diode-array detection/electrospray ionization time-of-flight mass spectrometry; SULT, sulfotransferase; THF, tetrahydrofuran; TMS, tetramethylsilane; TSP, (trimethylsilyl)-propionic acid; UDPGA, uridine 5′-diphosphoglucuronic-acid; UGT, uridine 5′-diphosphoglucuronyltransferase.

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