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Synthesis and Structure Determination of Covalent Conjugates Formed from the Sulfury–Roasty-Smelling 2-Furfurylthiol and Di- or Trihydroxybenzenes and Their Identification in Coffee Brew

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Recent investigations demonstrated that the reaction of odor-active thiols such as 2-furfurylthiol with thermally generated chlorogenic acid degradation products is responsible for the rapid aroma staling of coffee beverages. To get a clear understanding of the molecular mechanisms underlying this aroma staling, the existence of putative phenol/thiol conjugates needs to be verified in coffee. The aim of the present study was therefore to synthesize such conjugates for use as reference substances for LC-MS screening of coffee. To achieve this, catechol, 3-methyl-, 4-methyl-, and 4-ethylcatechol, pyrogallol, hydroxyhydroquinone, 5-*O*-caffeoylquinic acid, and caffeic acid, respectively, were reacted with 2-furfurylthiol in the presence of iron(III) chloride and air oxygen. After purification, the structures of 25 phenol/thiol conjugates were identified by means of LC-MS/MS and 1D/2D NMR experiments. Using these compounds as reference materials, four conjugates, namely, 3-((2-furylmethyl)sulfanyl)-catechol, 3-((2-furylmethyl)sulfanyl)-5-ethylcatechol, 4-((2-furylmethyl)sulfanyl)hydroxyhydroquinone, and 3,4-bis((2-furylmethyl)sulfanyl) hydroxyhydroquinone, were identified for the first time in coffee brew by means of HPLC-MS/MS(MRM). These findings clearly demonstrate catechol, 4-ethylcatechol, and hydroxyhydroquinone as the primary thiol trapping agents involved in the aroma staling of coffee beverages.

KEYWORDS: Coffee; aroma staling; 2-furfurylthiol; chlorogenic acid; caffeic acid; catechol; hydroxyhydroquinone; quinone; phenol oxidation

INTRODUCTION

With an overall consumption of about 5 million tons in 2001, coffee is one of the most popular beverages in the world. The habitual consumer highly appreciates coffee beverages for their salubrious, desirable aroma and taste as well as their stimulating properties. Unfortunately, the alluring aroma of a freshly prepared coffee brew is not persistent and, in particular, the intensity of the roasty−sulfury odor quality decreases rather rapidly (*1*−*4*).

Recent investigations combining instrumental analyses with human olfactory perception, such as HRGC−olfactometry, have been applied to characterize undesirable changes of coffee aroma on a molecular level. These studies have revealed a strong decrease in the concentrations of odorous thiols when coffee brews were stored or processed. The manufacturing of instant coffee (*5*) and heat sterilization of coffee beverages (*6*), as well as the keeping warm of a freshly prepared coffee brew in a Thermos flask (*7*), drastically reduced the concentration of 2-furfurylthiol, which is well accepted as a key odorant imparting the sulfury−roasty odor quality of a coffee brew. The decrease of that compound together with a decrease in additional thiols such as 3-methyl-2-butene-1-thiol, 3-mercapto-3-methylbutyl formate, 2-methyl-3-furanthiol, and methane thiol was reported to be responsible for the aroma change (*1*−*4*).

Aimed at understanding the molecular mechanisms underlying the thiol degradation, various coffee ingredients were recently investigated for their influence on 2-furfurylthiol degradation (*2*, *3*). Reports on the influence of coffee melanoidins on the thiol stability pointed out that these polymers exhibiting molecular masses above 3000 Da are able to effectively bind 2-furfurylthiol (*2*, *3*). By comparing LC-MS experiments using nonlabeled and $^2H_2$-labeled 2-furfurythiol, pyrazinium dications, which have been identified as key intermediates in roasting-induced melanoidin genesis, were shown to covalently bind 2-furfurylthiol (*8*−*10*). In addition, reaction products derived from the Maillard reaction were shown to reduce the 2-furfurylthiol concentration during incubation in model systems (*2*).

Even though model studies did not demonstrate any pronounced effect of 5-*O*-caffeoylquinic acid on the decrease of 2-furfurylthiol, "in bean" model roast experiments have recently identified this phenol as well as its thermal degradation products,

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caffeic acid and quinic acid, as important precursors for low molecular weight thiol-binding sites (*11*). During the roasting process, a major part of the 5-*O*-caffeoylquinic acid is well-known to be thermally decomposed to produce pyrogallol, hydroxyhydroquinone, catechol, 4-ethylcatechol, and 4-methylcatechol as degradation products in the coffee bean (*12*). In a recent study, *o*-quinones derived from oxidation of these phenols were supposed to function as trapping agents for thiols (*13*). The addition of thiols to quinones derived from enzymic phenol oxidation has been described for various foodstuffs, for example, grape juices and wine (*14*−*16*), and ferric ions have been reported as an important chelating agent for the oxidation of dopamine to dopamine quinone (*17*, *18*). On the basis of the recent discovery that transition metals accelerate the thiol-binding activity of roasted, chlorogenic acid loaded coffee beans (*11*), oxidation of thermally generated di- and trihydroxybenzenes, followed by the nucleophilic attack of the thiols, might be a possible mechanism underlying the thiol binding observed for coffee beverages. To identify and quantify such thiol/phenol conjugates in roasted coffee beverages, synthetic reference compounds are required.

The purpose of this study was to prepare reaction products formed by enzymic or iron-mediated oxidative coupling of di- and trihydroxybenzenes with 2-furfurylthiol, to isolate and to determine the chemical structures of the conjugates produced, and, finally, to identify these conjugates in a roasted coffee beverage by means of LC-MS/MS.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially: pyrogallol, hydroxyhydroquinone, catechol, 4-methylcatechol, 3-methylcatechol, caffeic acid, 5-*O*-caffeoylquinic acid, 2-furfurylthiol, tyrosinase from mushroom (1000 unit/mg), iron(III) chloride, 1,4-dithioerythritol (Sigma-Aldrich, Steinheim, Germany); acetonitrile, ethyl acetate, formic acid, sodium hydroxide, sodium sulfate (Merck KGA, Darmstadt, Germany); and 4-ethylcatechol (Lancaster, Eastgate, U.K.). Solvents were of HPLC grade, and water was of Millipore grade. Roasted coffee (Arabica) was obtained from the food industry.

Synthesis and Preparative Separation of Phenol/Thiol Conjugates. 5-*O*-Caffeoylquinic acid, caffeic acid, catechol, 3-methylcatechol, 4-methylcatechol, 4-ethylcatechol, pyrogallol, or hydroxyhydroquinone (2 mmol each), respectively, was dissolved in water (200 mL) in an Erlenmeyer flask (500 mL), 2-furfurylthiol (1.8 mmol) was added, and the solution was stirred vigorously at 30 °C. A solution of iron(III) chloride (2 mmol; 50 mL) was dropped into the reaction mixture over a period of 30 min. After an additional 30 min of stirring, ethyl acetate was added and stirring was continued for another 10 min. The organic layer was separated and the aqueous phase was extracted with ethyl acetate (3 × 150 mL). The combined organic fractions were dried over anhydrous Na_2SO_4, filtered, and, finally, freed from solvent in vacuum to give a crude mixture of reaction products. This material was dissolved in a mixture (6 mL; 1:1, v/v) of acetonitrile and aqueous formic acid (1%) and separated by preparative HPLC on a 250 × 21.2 mm i.d., 5 μm, Phenyl-Hexyl Luna column (Phenomenex, Aschaffenburg, Germany). Monitoring the effluent at a wavelength of 280 nm, chromatography was performed by starting with a mixture (85:15, v/v) of aqueous formic acid (1%; A) and acetonitrile (B) for 2 min, then increasing B to 60% within 18 min, followed by an increase of B to 100% within 5 min, and, finally, maintaining B at 100% for 3 min. Separation of the phenol/thiol conjugates of pyrogallol and hydroxyhydroquinone was performed by extending the time to increase B from 15 to 60% to 23 min instead of 18 min. The effluent of the major reaction products was collected, the fractions were concentrated to about 20 mL in vacuum, and then water (100 mL) was added and, finally, extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with water (20 mL), dried over anhydrous sodium sulfate, and then freed from solvents in vacuum. After additional freeze-drying

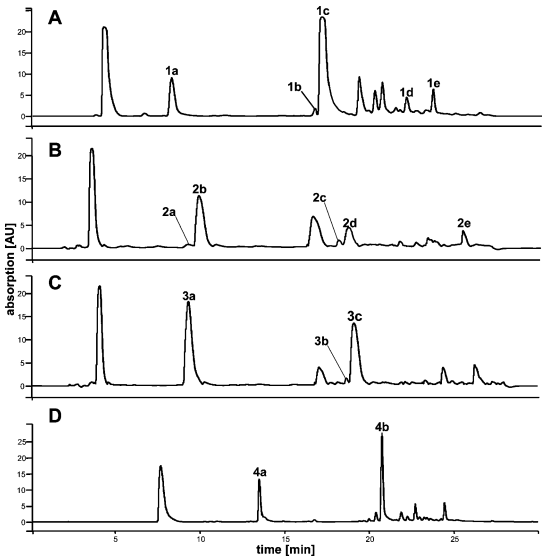

Figure 1. Preparative HPLC chromatograms of reaction products formed from dihydroxybenzenes with 2-furfurylthiol in the presence of iron(III) ions and air oxygen: (**A**) catechol; (**B**) 3-methylcatechol; (**C**) 4-methylcatechol; (**D**) 4-ethylcatechol.

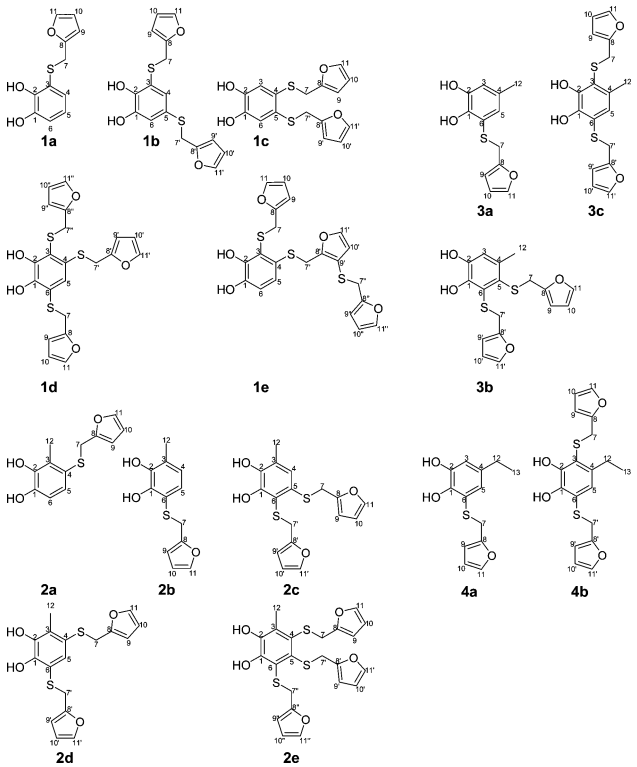

Figure 2. Structures of 2-furfurylthiol conjugates formed from catechol (**1a**–**1e**), 3-methylcatechol (**2a**–**2e**), 4-methylcatechol (**3a**–**3c**), and 4-ethylcatechol (**4a**, **4b**).

for 48 h, the main reaction products detected by HPLC-DAD (**Figures 1**, **3**, and **5**) were analyzed by means of LC-MS/MS and 1D/2D NMR experiments, and their chemical structures were determined as covalent conjugates of 2-furfurylthiol and the corresponding phenol (**Figures 2**, **4**, and **6**).

*3-((2-Furylmethyl)sulfanyl)catechol, **1a** (0.30 mmol, 17% yield) (**Figure 2**):* LC/TOF-MS, $C_{11}H_{10}O_3S$; MS-ESI$^-$, *m*/*z* 221 (100, [M − H]$^-$); MS/MS (−30 V), *m*/*z* 140 (*100*), 112 (*8*); ^{1}H NMR (400 MHz, $CDCl_3$), δ 4.01 [s, 2H, H−C(7)], 5.99 [d, 1H, J = 3.2 Hz, H−C(9)], 6.23 [dd, 1H, J = 1.8, 3.2 Hz, H−C(10)], 6.58 [∼t, 1H, J = 7.9 Hz, H−C(5)], 6.71 [dd, 1H, J = 1.5, 8.0 Hz, H−C(6)], 6.73 [dd, 1H, J = 1.6, 8.0 Hz, H−C(4)], 7.35 [dd, 1H, J = 0.9, 1.8 Hz, H−C(11)]; ^{13}C

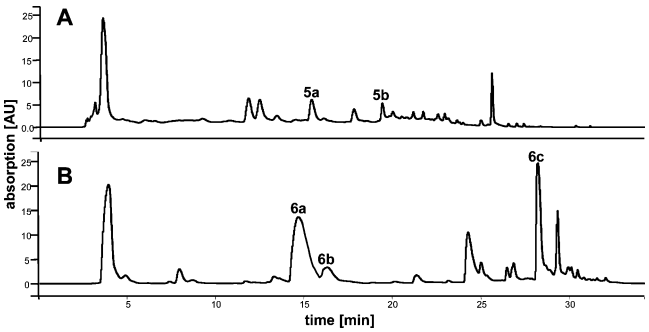

Figure 3. Preparative HPLC chromatograms of reaction products formed from the trihydroxybenzenes pyrogallol (**A**) and hydroxyhydroquinone (**B**), respectively, with 2-furfurylthiol in the presence of iron(III) ions and air oxygen.

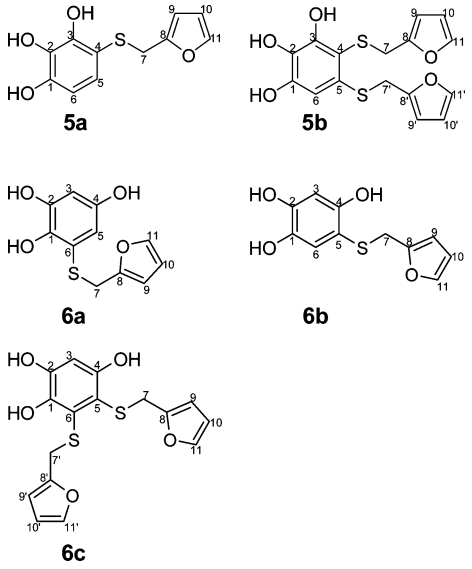

Figure 4. Structures of 2-furfurylthiol conjugates formed from pyrogallol (**5a**, **5b**) and hydroxyhydroquinone (**6a**, **6b**), respectively.

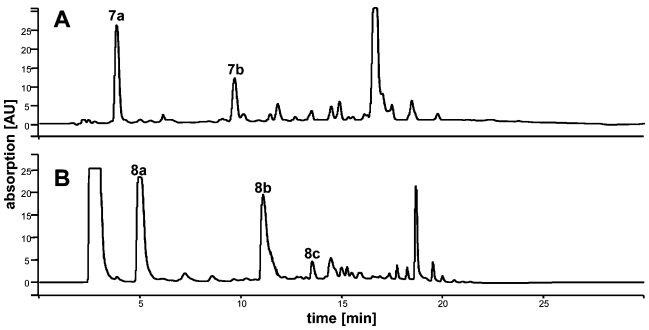

Figure 5. Preparative HPLC chromatograms of reaction products formed from caffeic acid (**A**) and 5-*O*-caffeoylquinic acid (**B**), respectively, with 2-furfurylthiol in the presence of iron(III) ions and air oxygen.

NMR (100 MHz, $CDCl_3$, HMQC, HMBC), δ 33.6 [C(7)], 107.2 [C(9)], 110.0 [C(10)], 115.0 [C(4) or C(6)], 119.2 [C(5)], 119.5 [C(3)], 124.5 [C(4) or C(6)], 141.5 [C(11)], 145.0/145.3 [C(1), C(2)], 151.3 [C(8)].

3,5-Bis((2-furylmethyl)sulfanyl)catechol, ***1b*** *(0.02 mmol, 1% yield) (**Figure 2**):* LC/TOF-MS, $C_{16}H_{14}O_4S_2$; MS-ESI$^-$, *m/z* 333 (100, [M − H]$^-$); MS/MS (−30 V), *m/z* 252 (*25*), 171 (*100*); ^{1}H NMR (400 MHz, $CDCl_3$), δ 3.85 [s, 2H, H−C(7,7′)], 3.97 [s, 2H, H−C(7,7′)], 5.89 [d, 1H, J = 3.2 Hz, H−C(9,9′)], 6.03 [d, 1H, J = 3.2 Hz, H−C(9,9′)], 6.24 [dd, 1H, J = 2.0, 3.2 Hz, H−C(10,10′)], 6.26 [dd, 1H, J = 2.0, 3.2 Hz, H−C(10,10′)], 6.92 [d, 1H, J = 2.1 Hz, H−C(4)/H−C(6)], 6.95 [d, 1H, J = 2.1 Hz, H−C(4)/H−C(6)], 7.35 [m, 2H, H−C(11,11′)].

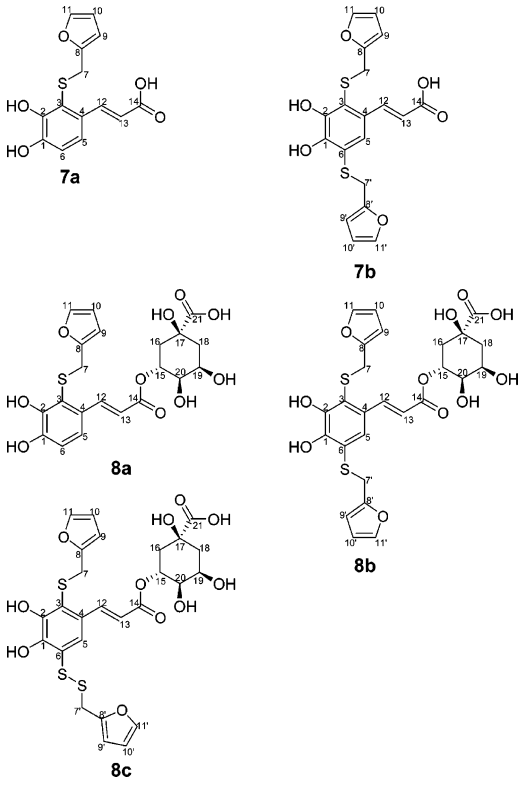

Figure 6. Structures of 2-furfurylthiol conjugates formed from caffeic acid (**7a**, **7b**) and 5-*O*-caffeoylquinic acid (**8a**–**8c**).

4,5-Bis((2-furylmethyl)sulfanyl)catechol, ***1c*** *(0.62 mmol, 34% yield) (**Figure 2**):* LC/TOF-MS, $C_{16}H_{14}O_4S_2$; MS-ESI$^-$, *m/z* 333 (100, [M − H]$^-$); MS/MS (−30 V), *m/z* 252 (*42*), 219 (*8*), 171 (*100*), 143 (*15*); ^{1}H NMR (400 MHz, $CDCl_3$), δ 3.96 [s, 4H, H−C(7,7′)], 5.95 [dd, 2H, J = 0.9, 3.2 Hz, H−C(9,9′)], 6.24 [dd, 2H, J = 1.8, 3.2 Hz, H−C(10,-10′)], 6.80 [s, 2H, H−C(3,6)], 7.35 [dd, 2H, J = 0.9, 1.8 Hz, H−C(11,-11′)]; ^{13}C NMR (100 MHz, $CDCl_3$, HMQC, HMBC), δ 32.2 [C(7), C(7′)], 108.3 [C(9), C(9′)], 110.5 [C(10), C(10′)], 120.0 [C(4), C(5)], 125.6 [C(3), C(6)], 142.5 [C(11), C(11′)], 144.2 [C(1), C(2)], 150.1 [C(8), C(8′)].

3,4,6-Tris((2-furylmethyl)sulfanyl)catechol, ***1d*** *(0.04 mmol, 2% yield) (**Figure 3**):* LC/TOF-MS, $C_{21}H_{18}O_5S_3$; MS-ESI$^-$, *m/z* 445 (100, [M − H]$^-$); MS/MS (−30 V), *m/z* 364 (*10*), 283 (*100*), 249 (*5*), 202 (*7*); ^{1}H NMR (400 MHz, $CDCl_3$), δ 3.95/3.98/4.04 [s, 3 × 1H, H−C(7, 7′, 7″)], 5.80/5.97/6.05 [d, 3 × 1H, J = 0.8, 3.2 Hz, H−C(9, 9′, 9″)], 6.19/6.25 [dd, 3 × 1H, J = 1.9, 3.2 Hz, H−C(10/10′/10″)], 6.87 [s, 1H, H−C(5)], 7.32/7.34/7.35 [dd, 3 × 1H, J = 0.8, 1.8 Hz, H−C(11/11′/ 11″)]; ^{13}C NMR (100 MHz, $CDCl_3$, HMQC, HMBC), δ 31.7 [C(7),C(7′), C(7″)], 108.1/108.5 [C(9), C(9′), C(9″)), 110.7 [C(10), C(10′), C(10″)], 119.2/121.4 [C(3), C(6)], 126.6 [C(5)], 131.5 [C(4)], 142.4 [C(11), C(11′), C(11″)], 143.0/146.1 [C(1), C(2)], 149.8/150.1/150.4 [C(8), C(8′), C(8″))].

3-((2-Furylmethyl)sulfanyl)-4-((2-(3-(2-furylmethyl)sulfanyl)furylmethyl)sulfanyl) catechol, ***1e*** *(0.06 mmol, 3% yield) (**Figure 2**):* LC/TOF-MS, $C_{21}H_{18}O_5S_3$; MS-ESI$^-$, *m/z* 445 (100, [M − H]$^-$); MS/MS (−30 V), *m/z* 364 (*40*), 333(2), 283 (*100*), 252 (*24*), 202 (*7*), 171 (*4*); ^{1}H NMR (400 MHz, $CDCl_3$), δ 3.90 [s, 2H, H−C(7)], 3.91 [s, 2H, H−C(7′)], 5.01 [s, 2H, H−C(7″)], 5.94/6.18 [d, 2 × 1H, J = 3.1 Hz, H−C(9, 9″)], 6.21/6.24 [dd, 2 × 1H, J = 1.9, 3.1 Hz, H−C(10, 10″)], 6.35 [d, 1H, J = 1.9 Hz, H−C(10′)], 6.99 [d, 1H, J = 8.4 Hz, H−C(6)], 7.05 [d, 1H, J = 8.4 Hz, H−C(5)], 7.30/7.36 [dd, 2 × 1H, J = 0.8, 1.9 Hz, H−C(11, 11″)], 7.45 [dd, 1H, J = 0.8, 1.9 Hz, H−C(11′)]; ^{13}C NMR (100 MHz, $CDCl_3$, HMQC, HMBC), δ 33.1 [C(7)], 35.5 [C(7′)], 65.9 [C(7″)], 108.5/109.2 [C(9), C(9″)], 110.7 [C(9′),C(10),C(10′),C-(10″)], 117.5 [C(6)], 117.7 [C(3)], 130.9 [C(5)], 133.7 [C(4)], 141.7 [C(1)], 142.6/143.2 [C(11), C(11″)], 149.5/150.4/150.6 [C(8), C(8′), C(8″)], 149.9 [C(2)].

4-((2-Furylmethyl)sulfanyl)-3-methylcatechol, ***2a*** *(0.04 mmol, 3% yield) (**Figure 2**):* LC/TOF-MS: $C_{12}H_{12}O_3S$; MS-ESI$^-$, *m/z* 235 (100,

[M − H]⁻); MS/MS (−30 V), *m/z* 154 (*100*), 139 (*4*); ^{1}H NMR (400 MHz, MeOD), δ 2.21 [s, 3H, H−C(12)], 3.84 [s, 2H, H−C(7)], 5.88 [d, 1H, *J* = 3.1 Hz, H−C(9)], 6.23 [dd, 1H, *J* = 1.9, 3.1 Hz, H−C(10)], 6.55 [d, 1H, *J* = 8.3 Hz, H−C(6)], 6.77 [d, 1H, *J* = 8.3 Hz, H−C(5)], 7.36 [dd, 1H, *J* = 0.7, 1.9 Hz, H−C(11)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 12.3 [C(12)], 32.5 [C(7)], 107.0 [C(9)], 109.9 [C(10)], 112.0 [C(6)], 123.9 [C(3)], 126.1 [C(5)], 128.4 [C(4)], 141.6 [C(11)], 143.3/145.3 [C(1), C(2)], 151.5 [C(8)].

3-((2-Furylmethyl)sulfanyl)-6-methylcatechol, ***2b*** *(0.48 mmol, 27% yield) (**Figure 2**):* LC/TOF-MS, $C_{12}H_{12}O_3S$; MS-ESI⁻, *m/z* 235 (100, [M − H]⁻); MS/MS (−30 V), *m/z* 154 (*100*); ^{1}H NMR (400 MHz, MeOD), δ 2.17 [s, 3H, H−C(12)], 3.92 [s, 2H, H−C(7)], 5.94 [d, 1H, *J* = 3.2 Hz, H−C(9)], 6.23 [dd, 1H, *J* = 1.9, 3.1 Hz, H−C(10)], 6.52 [d, 1H, *J* = 8.0 Hz, H−C(4)], 6.64 [d, 1H, *J* = 8.0 Hz, H−C(5)], 7.36 [dd, 1H, *J* = 0.7, 1.9 Hz, H−C(11)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 14.7 [C(12)], 31.3 [C(7)], 107.3 [C(9)], 109.9 [C(10)], 116.0 [C(6)], 121.2 [C(4)], 125.3 [C(5)], 125.9 [C(3)], 141.8 [C(11)], 142.7/145.3 [C(1), C(2)], 151.1 [C(8)].

3,4-Bis((2-furylmethyl)sulfanyl)-6-methylcatechol, ***2c*** *(0.04 mmol, 3% yield) (**Figure 2**):* LC/TOF-MS, $C_{17}H_{16}O_4S_2$; MS-ESI⁻, *m/z* 347 (100, [M − H]⁻); MS/MS (−30 V), *m/z* 266 (*65*), 185 (*100*); ^{1}H NMR (400 MHz, MeOD), δ 2.13 [s, 3H, H−C(12)], 3.95/4.02 [s, 2 × 2H, H−C(7, 7′)], 5.86/6.04 [d, 2 × 1H, *J* = 3.2 Hz, H−C(9, 9′)], 6.20/6.26 [dd, 2 × 1H, *J* = 2.0, 3.2 Hz, H−C(10, 10′)], 6.68 [s, 1H, H−C(4)], 7.34/7.37 [dd, 2 × 1H, *J* = 0.7, 2.0 Hz, H−C(11, 11′)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 14.5 [C(12)], 31.0 [C(7), C(7′)], 107.3/107.7 [C(9), C(9′)], 109.9 [C(10, C(10′)], 116.7 [C(6)], 124.3 [C(4)], 126.4 [C(3)], 129.9 [C(5)], 141.6 [C(11), C(11′)], 142.0/146.6 [C(1), C(2)], 150.9/151.3 [C(8), C(8′)].

3,5-Bis((2-furylmethyl)sulfanyl)-6-methylcatechol, ***2d*** *(0.24 mmol, 13% yield) (**Figure 2**):* LC/TOF-MS, $C_{17}H_{16}O_4S_2$; MS-ESI⁻, *m/z* 347 (100, [M − H]⁻); MS/MS (−30 V), *m/z* 266 (*100*), 235 (*35*), 185 (*75*); ^{1}H NMR (400 MHz, MeOD), δ 2.17 [s, 3H, H−C(12)], 3.81/3.91 [s, 2 × 2H, H−C(7, 7′)], 5.87/5.95 [d, 2 × 1H, *J* = 3.1 Hz, H−C(9, 9′)], 6.23 [m, 2H, H−C(10, 10′)], 6.84 [s, 1H, H−C(5)], 7.36/7.38 [dd, 2 × 1H, *J* = 0.7, 1.6 Hz, H−C(11, 11′)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 12.4 [C(12)], 31.3/32.5 [C(7, C(7′)], 107.1/107.5 [C(9), C(9′)], 109.9 [C(10, C(10′)], 116.2 [C(6)], 124.3 [C(4)], 131.8 [C(5)], 132.6 [C(3)], 141.7 [C(11), C(11′)], 143.3 [C(2)], 145.8 [C(1)], 151.0/151.5 [C(8), C(8′)].

3,4,5-Tris((2-furylmethyl)sulfanyl)-6-methylcatechol, ***2e*** *(0.04 mmol, 2% yield) (**Figure 2**):* LC/TOF-MS, $C_{22}H_{20}O_5S_3$; MS-ESI⁻, *m/z* 459 (100, [M − H]⁻); MS/MS (−30 V), *m/z* 378 (*89*), 347 (*3*), 297 (*100*), 266 (*4*), 185 (*5*); ^{1}H NMR (400 MHz, MeOD), δ 2.15 [s, 3H, H−C(12)], 3.87/3.98/4.06 [s, 3 × 2H, H−C(7, 7′, 7″)], 5.73/5.86/5.88 [d, 3 × 1H, *J* = 3.1 Hz, H−C(9, 9′, 9″)], 6.23 [m, 3 × 1H, H−C(10, 10′, 10″)], 7.32/7.34 [dd, 3 × 1H, *J* = 0.7, 1.6 Hz, H−C(11, 11′, 11″)].

3-((2-Furylmethyl)sulfanyl)-5-methylcatechol, ***3a*** *(0.38 mmol, 21% yield) (**Figure 2**):* LC/TOF-MS, $C_{12}H_{12}O_3S$; MS-ESI⁻, *m/z* 235 (100, [M − H]⁻); MS/MS (−30 V), *m/z* 154 (*100*), 126 (*4*), 121 (*10*); ^{1}H NMR (400 MHz, MeOD), δ 2.12 [s, 3H, H−C(12)], 4.00 [s, 2H, H−C(7)], 6.00 [d, 1H, *J* = 3.2 Hz, H−C(9)], 6.23 [dd, 1H, *J* = 1.9, 3.0 Hz, H−C(10)], 6.54 [d, 1H, *J* = 2.1 Hz, H−C(3)], 6.57 [d, 1H, *J* = 2.1 Hz, H−C(5)], 7.35 [dd, 1H, *J* = 0.8, 1.9 Hz, H−C(11)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 19.4 [C(12)], 30.5 [C(7)], 107.3 [C(9)], 109.9 [C(10)], 116.1 [C(3)], 119.2 [C(6)], 124.6 [C(5)], 128.7 [C(4)], 141.6 [C(11)], 142.8 [C(1), C(2)], 151.3 [C(8)].

3,4-Bis((2-furylmethyl)sulfanyl)-5-methylcatechol, ***3b*** *(0.04 mmol, 2% yield) (**Figure 2**):* LC/TOF-MS, $C_{17}H_{16}O_4S_2$; MS-ESI⁻, *m/z* 347 (100, [M − H]⁻); MS/MS (−30 V), *m/z* 266 (*15*), 185 (*100*), 157 (*4*); ^{1}H NMR (400 MHz, MeOD), δ 2.04 [s, 3H, H−C(12)], 3.78/4.14 [s, 2 × 2H, H−C(7, 7′)], 5.68/5.93 [d, 2 × 1H, *J* = 3.0 Hz, H−C(9, 9′)], 6.19 [m, 2 × 1H, H−C(10, 10′)], 6.61 [s, 1H, H−C(3)], 7.35 [m, 2 × 1H, H−C(11,11′)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 20.2 [C(12)], 31.0/32.5 [C(7), C(7′)], 106.9/107.5 [C(9), C(9′)], 109.9 [C(10), C(10′)], 116.6 [C(3)], 124.5/126.1 [C(5), C(6)], 135.8 [C(4)], 141.6 [C(11), C(11′)], 144.7/145.6 [C(1), C(2)], 151.2/151.4 [C(8), C(8′)].

3,6-Bis((2-furylmethyl)sulfanyl)-4-methylcatechol, ***3c*** *(0.42 mmol, 21% yield) (**Figure 2**):* LC/TOF-MS, $C_{17}H_{16}O_4S_2$; MS-ESI⁻, *m/z* 347 (100, [M − H]⁻); MS/MS (−30 V), *m/z* 266 (*16*), 185 (*100*); ^{1}H NMR (400 MHz, MeOD), δ 2.05 [s, 3H, H−C(12)], 3.87/4.04 [s, 2 × 2H, H−C(7, 7′)], 5.79/6.01 [d, 2 × 1H, *J* = 2.8 Hz, H−C(9, 9′)], 6.20/6.24 [dd, 2 × 1H, *J* = 1.9, 2.8 Hz, H−C(10, 10′)], 6.60 [s, 1H, H−C(5)], 7.35 [m, 2 × 1H, H−C(11,11′)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 19.1 [C(12)], 29.7/30.5 [C(7), C(7′)], 107.5 [C(9), C(9′)], 110.0 [C(10), C(10′)], 118.0/121.3 [C(3), C(6)], 123.8 [C(5)], 133.8 [C(4)], 141.8 [C(11), C(11′)], 142.3/146.2 [C(1), C(2)], 150.9 [C(8), C(8′)].

3-((2-Furylmethyl)sulfanyl)-5-ethylcatechol, ***4a*** *(0.36 mmol, 20% yield) (**Figure 2**):* LC/TOF-MS, $C_{13}H_{14}O_3S$; MS-ESI⁻, *m/z* 249 (100, [M − H]⁻); MS/MS (−30 V), *m/z* 168 (*100*), 153 (*11*), 140 (*3*), 134 (*3*); ^{1}H NMR (400 MHz, MeOD), δ 1.10 [t, 3H, *J* = 7.6 Hz, H−C(13)], 2.40 [q, 2H, *J* = 7.6 Hz, H−C(12)], 3.98 [s, 2H, H−C(7)], 5.96 [d, 1H, *J* = 3.2 Hz, H−C(9)], 6.22 [dd, 1H, *J* = 1.8, 3.2 Hz, H−C(10)], 6.51 [d, 1H, *J* = 1.8 Hz, H−C(5)], 6.57 [d, 1H, *J* = 1.8 Hz, H−C(3)], 7.34 [dd, 1H, *J* = 0.8, 1.8 Hz, H−C(11)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 14.7 [C(13)], 27.6 [C(12)], 30.4 [C(7)], 107.2 [C(9)], 109.9 [C(10)], 114.8 [C(3)], 119.3 [C(6)], 123.7 [C(5)], 135.5 [C(4)], 141.8 [C(11)], 143.1 [C(1), C(2)], 151.3 [C(8)].

3,6-Bis((2-furylmethyl)sulfanyl)-4-ethylcatechol, ***4b*** *(0.44 mmol, 24% yield) (**Figure 2**):* LC/TOF-MS, $C_{18}H_{18}O_4S_2$; MS-ESI⁻, *m/z* 361 (100, [M − H]⁻); MS/MS (−30 V), *m/z* 280 (*40*), 199 (*100*), 165 (*18*); ^{1}H NMR (400 MHz, MeOD), δ 0.95 [t, 3H, *J* = 7.5 Hz, H−C(13)], 2.43 [q, 2H, *J* = 7.5 Hz, H−C(12)], 3.89/4.00 [s, 2 × 2H, H−C(7, 7′)], 5.78/5.96 [d, 2 × 1H, *J* = 3.0 Hz, H−C(9, 9′)], 6.17/6.22 [dd, 2 × 1H, *J* = 1.8, 3.0 Hz, H−C(10, 10′)], 6.54 [s, 1H, H−C(5)], 7.32/7.34 [dd, 2 × 1H, *J* = 0.7, 1.7 Hz, H−C(11, 11′)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 14.9 [C(13)], 26.3 [C(12)], 29.9/30.5 [C(7), C(7′)], 107.5 [C(9), C(9′)], 110.1 [C(10), C(10′)], 118.1 [C(3)], 121.4 [C(6)], 123.0 [C(5)], 139.6 [C(4)], 141.6 [C(11), C(11′)], 142.6/146.3 [C(1), C(2)], 151.1/151.5 [C(8), C(8′)].

4-((2-Furylmethyl)sulfanyl)pyrogallol, ***5a*** *(0.10 mmol, 6% yield) (**Figure 4**):* LC/TOF-MS, $C_{11}H_{10}O_4S$; MS-ESI⁻, *m/z* 237 (100, [M − H]⁻); MS/MS (−30 V), *m/z* 156 (*100*), 123 (*12*); ^{1}H NMR (400 MHz, MeOD), δ 3.83 [s, 2H, H−C(7)], 5.89 [d, 1H, *J* = 3.1 Hz, H−C(9)], 6.20 [dd, 1H, *J* = 1.9, 3.1 Hz, H−C(10)], 6.22 [d, 1H, *J* = 8.3 Hz, H−C(6)], 6.54 [d, 1H, *J* = 8.3 Hz, H−C(5)], 7.33 [dd, 1H, *J* = 0.7, 1.9 Hz, H−C(11)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 31.8 [C(7)], 106.9 [C(5), C(6)], 107.3 [C(9)], 109.2 [C(4)], 109.9 [C(10)], 126.3 [C(5), C(6)], 132.8 [C(2)], 141.6 [C(11)], 146.9 [C(1), C(3)], 151.5 [C(8)].

4,5-Bis((2-furylmethyl)sulfanyl)pyrogallol, ***5b*** *(0.06 mmol, 3% yield) (**Figure 4**):* LC/TOF-MS, $C_{16}H_{14}O_5S_2$; MS-ESI⁻, *m/z* 349 (100, [M − H]⁻); MS/MS (−30 V), *m/z* 268 (*22*), 236 (*100*), 203 (*59*), 187 (*12*); ^{1}H NMR (400 MHz, MeOD), δ 3.60/3.85 [s, 2 × 2H, H−C(7, 7′)], 5.79/5.87 [d, 2 × 1H, *J* = 3.2 Hz, H−C(9, 9′)], 6.15/6.20 [dd, 2 × 1H, *J* = 1.9, 3.1 Hz, H−C(10, 10′)], 7.24/7.33 [dd, 2 × 1H, *J* = 0.8, 1.8 Hz, H−C(11, 11′)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 31.8 [C(7), C(7′)], 106.9/107.5 [C(9), C(9′)], 109.9 [C(6), C(10), C(10′)], 114.5 [C(4)], 128.4 [C(5)], 132.3 [C(2)], 141.3/141.7 [C(11), C(11′)], 145.0 [C(1), C(3)], 150.9/151.5 [C(8), C(8′)].

3-((2-Furylmethyl)sulfanyl)hydroxyhydroquinone, ***6a*** *(0.56 mmol, 31% yield) (**Figure 4**):* LC/TOF-MS, $C_{11}H_{10}O_4S$; MS-ESI⁻, *m/z* 237 (100, [M − H]⁻); MS/MS (−30 V), *m/z* 156 (*100*), 128 (*9*), 123 (*45*); ^{1}H NMR (400 MHz, MeOD), δ 3.98 [s, 2H, H−C(7)], 6.01 [d, 1H, *J* = 3.2 Hz, H−C(9)], 6.21 [m, 2H, H−C(6, 10)], 6.25 [d, 1H, *J* = 2.8 Hz, H−C(5)], 7.32 [dd, 1H, *J* = 0.7, 1.8 Hz, H−C(11)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 30.1 [C(7)], 103.1 [C(3) or C(5)], 107.2 [C(9)], 109.4 [C(10)], 111.2 [C(3) or C(5)], 120.9 [C(6)], 138.0 [C(1)], 141.8 [C(11)], 145.6 [C(2)], 149.9 [C(4)], 151.4 [C(8)].

4-((2-Furylmethyl)sulfanyl)hydroxyhydroquinone, ***6b*** *(0.10 mmol, 6% yield) (**Figure 4**):* LC/TOF-MS, $C_{11}H_{10}O_4S$; MS-ESI⁻, *m/z* 237 (100, [M − H]⁻); MS/MS (−30 V), *m/z* 156 (*100*), 128 (*6*), 123 (*45*); ^{1}H NMR (400 MHz, MeOD), δ 3.82 [s, 2H, H−C(7)], 5.91 [d, 1H, *J* = 3.2 Hz, H−C(9)], 6.21 [dd, 1H, *J* = 2.0, 3.2 Hz, H−C(10), 6.33 [s, 1H, H−C(3)], 6.62 [s, 1H, H−C(6)], 7.33 [dd, 1H, *J* = 0.7, 2.0 Hz, H−C(11)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 31.8 [C(7)], 102.3 [C(3)], 107.2 [C(9)], 109.6 [C(10)], 121.7 [C(6)], 122.1 [C(5)], 138.0 [C(1)], 141.8 [C(11)], 147.5 [C(2)], 151.3 [C(4)], 151.4 [C(8)].

3,4-Bis((2-furylmethyl)sulfanyl)hydroxyhydroquinone, ***6c*** *(0.14 mmol, 8% yield) (**Figure 4**):* LC/TOF-MS, $C_{16}H_{14}O_5S_2$; MS-ESI⁻, *m/z* 349

(100, [M − H]$^-$); MS/MS (−30 V), *m/z* 268 (*6*), 187 (*100*), 159 (*3*); ^{1}H NMR (400 MHz, MeOD), δ 3.81/4.06 [s, 2 × 2H, H−C(7, 7′)], 5.83/5.93 [d, 2 × 1H, J = 3.1 Hz, H−C(9, 9′)], 6.19/6.20 [dd, 2 × 1H, J = 1.8, 3.1 Hz, H−C(10, 10′)], 6.36 [s, 1H, H−C(3)], 7.32 [m, 2 × 1H, H−C(11,11′)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 30.9/31.8 [C(7), C(7′)], 102.7 [C(3)], 107.3/107.5 [C(9), C(9′)], 110.1 [C(10), C(10′)], 111.1 [C(5)], 123.7 [C(6)], 140.8 [C(1)], 141.8 [C(11), C(11′)], 147.5 [C(2)], 151.2 [C(8), C(8′)], 152.3 [C(4)].

2-((2-Furylmethyl)sulfanyl)caffeic acid, ***7a*** *(0.42 mmol, 23% yield) (**Figure 6**):* LC/TOF-MS, $C_{14}H_{12}O_5S$; MS-ESI$^-$, *m/z* 291 (100, [M − H]$^-$); MS/MS (−30 V), *m/z* 247 (*3*), 210 (*11*), 181 (*8*), 165 (*100*); ^{1}H NMR (400 MHz, MeOD), δ 3.91 [s, 2H, H−C(7)], 5.80 [d, 1H, J = 3.1 Hz, H−C(9)], 6.12 [d, 1H, J = 15.9 Hz, H−C(13)], 6.13 [dd, 1H, J = 1.8, 3.1 Hz, H−C(10)], 6.81 [d, 1H, J = 8.4 Hz, H−C(6)], 7.12 [d, 1H, J = 8.5 Hz, H−C(5)], 7.27 [dd, 1H, J = 0.7, 1.8 Hz, H−C(11)], 8.11 [d, 1H, J = 15.9 Hz, H−C(12)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 32.4 [C(7)], 109.0 [C(9)], 111.1 [C(10)], 117.4 [C(6), C(13)], 119.7 [C(5)], 121.1 [C(3)], 131.5 [C(4)], 143.4 [C(11)], 145.1 [C(12)], 148.2 [C(1), C(2)], 151.8 [C(8)], 170.9 [C(14)].

2,5-Bis((2-furylmethyl)sulfanyl)caffeic acid, ***7b*** *(0.22 mmol, 12% yield) (**Figure 6**):* LC/TOF-MS, $C_{19}H_{16}O_6S_2$; MS-ESI$^-$, *m/z* 403 (100, [M − H]$^-$); MS/MS (−30 V), *m/z* 277 (*100*), 197 (*82*); ^{1}H NMR (400 MHz, MeOD), δ 3.88/4.09 [s, 2 × 2H, H−C(7, 7′)], 5.77/6.01 [d, 2 × 1H, J = 3.2 Hz, H−C(9, 9′)], 6.02 [d, 1H, J = 15.9 Hz, H−C(13)], 6.14/6.25 [dd, 2 × 1H, J = 1.8, 3.2 Hz, H−C(10, 10′)], 7.04 [s, 1H, H−C(6)], 7.27/7.38 [dd, 2 × 1H, J = 0.7, 1.8 Hz, H−C(11, 11′)], 7.99 [d, 1H, J = 15.9 Hz, H−C(12)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 29.4/31.2 [C(7), C(7′)], 107.5/108.0 [C(9), C(9′)], 109.9 [C(10), C(10′)], 116.9 [C(13)], 119.3/122.5 [C(3), C(6)], 121.7 [C(5)], 130.1 [C(4)], 142.1 [C(11), C(11′)], 142.6/146.1 [C(1), C(2)], 143.1 [C(12)], 150.2/151.1 [C(8), C(8′)], 169.3 [C(14)].

5-O-[2-((2-Furylmethyl)sulfanyl)caffeoyl]quinic acid, ***8a*** *(0.42 mmol, 23% yield) (**Figure 6**):* LC/TOF-MS, $C_{21}H_{22}O_{10}S$; MS-ESI$^-$, *m/z* 465 (100, [M − H]$^-$); MS/MS (−30 V), *m/z* 291 (*1*), 273 (*10*), 191 (*100*); ^{1}H NMR (400 MHz, MeOD), δ 1.96−2.18 [m, 4H, $H_{a/b}$−C(16,18)], 3.64 [dd, 1H, J = 3.2, 8.8 Hz, H−C(20)], 3.82 [s, 2H, H−C(7)], 4.09 [dd, 1H, J = 3.2, 7.7 Hz, H−C(19)], 5.26 [dt, 1H, J = 4.5, 9.5 Hz, H−C(15)], 5.72 [d, 1H, J = 3.2 Hz, H−C(9)], 6.05 [dd, 1H, J = 1.9, 3.2 Hz, H−C(10)], 6.10 [d, 1H, J = 15.9 Hz, H−C(13)], 6.73 [d, 1H, J = 8.4 Hz, H−C(6)], 7.04 [d, 1H, J = 8.4 Hz, H−C(5)], 7.20 [dd, 1H, J = 0.7, 1.8 Hz, H−C(11)], 8.04 [d, 1H, J = 15.9, H−C(12)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 31.0 [C(7)], 36.8/37.7 [C(16), C(18)], 70.2 [C(15)], 70.6 [C(19)], 72.3 [C(20)], 75.0 [C(17)], 107.8 [C(9)], 109.9 [C(10)], 115.7 [C(13)], 115.9 [C(6)], 118.2 [C(5)], 120.0 [C(3)], 130.0 [C(4)], 142.1 [C(11)], 143.6/146.9 [C(1), C(2)], 143.9 [C(12)], 150.3 [C(8)], 167.2 [C(14)], 175.7 [C(21)].

5-O-[2,5-Bis((2-furylmethyl)sulfanyl)caffeoyl]quinic acid, ***8b*** *(0.26 mmol, 14% yield) (**Figure 6**):* LC/TOF-MS, $C_{26}H_{26}O_{11}S_2$; MS-ESI$^-$, *m/z* 577 (100, [M − H]$^-$); MS/MS (−30 V), *m/z* 385 (*55*), 304 (*28*), 276 (*4*), 191 (*100*); ^{1}H NMR (400 MHz, MeOD), δ 2.05 [m, 2H, $H_{a/b}$−C(18)], 2.19 [m, 2H, $H_{a/b}$−C(16)], 3.72 [dd, 1H, J = 3.1, 8.7, H−C(20)], 3.87/4.09 [s, 2 × 2H, H−C(7, 7′)], 4.15 [m, 1H, H−C(19)], 5.33 [dt, 1H, J = 4.5, 9.4 Hz, H−C(15)], 5.76/6.02 [d, 2 × 1H, J = 3.1, H−C(9, 9′)], 6.09 [d, 1H, J = 15.9 Hz, H−C(13)], 6.13/6.24 [dd, 2 × 1H, J = 1.9, 3.1 Hz, H−C(10, 10′)], 7.07 [s, 1H, H−C(5)], 7.28/7.37 [dd, 2 × 1H, J = 0.7, 1.9 Hz, H−C(11, 11′)], 8.00 [d, 1H, J = 15.9 Hz, H−C(12)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 29.4/31.2 [C(7), C(7′)], 37.0 [C(16), C(18)], 70.0 [C(19)], 70.6 [C(15)], 72.3 [C(20)], 75.0 [C(17)], 107.7/108.0 [C(9), C(9′)], 109.9 [C(10), C(10′)], 116.4 [C(13)], 119.2 [C(3), C(6)], 130.0 [C(4)], 142.1 [C(11), C(11′)], 143.0/146.2 [C(1), C(2)], 143.1 [C(12)], 150.1/151.0 [C(8), C(8′)], 166.8 [C(14)], 175.7 [C(21)].

5-O-[2-((2-Furylmethyl)sulfanyl)-5-((2-furylmethyl)disulfanyl))caffeoyl]quinic acid, ***8c*** *(0.02 mmol, 1% yield) (**Figure 6**):* LC/TOF-MS, $C_{26}H_{26}O_{11}S_3$; MS-ESI$^-$, *m/z* 609 (100, [M − H]$^-$); MS/MS (−30 V), *m/z* 528 (*28*), 497 (*27*), 416 (*77*), 335 (*26*), 305 (*22*), 255 (*7*), 224 (*16*), 191 (*100*); ^{1}H NMR (400 MHz, MeOD), δ 2.09 [m, 2H, $H_{a/b}$−C(18)], 2.22 [m, 2H, $H_{a/b}$−C(16)], 3.75 [dd, 1H, J = 3.1, 9.0 Hz, H−C(20)], 3.84/4.00 [s, 2 × 2H, H−C(7, 7′)], 4.18 [d, 1H, J = 3.4 Hz, H−C(19)], 5.38 [dt, 1H, J = 4.6, 9.9 Hz, H−C(15)], 5.82/6.23 [d, 2 × 1H, J = 3.2 Hz, H−C(9, 9′)], 6.17/6.20 [dd, 2 × 1H, J = 1.9, 3.2 Hz, H−C(10, 10′)], 6.23 [d, 1H, J = 15.9 Hz, H−C(13)], 7.31/7.24 [dd, 2 × 1H, J = 0.7, 1.8 Hz, H−C(11, 11′)], 7.34 [s, 1H, H−C(5)], 8.00 [d, 1H, J = 15.9 Hz, H−C(12)]; ^{13}C NMR (100 MHz, MeOD, HMQC, HMBC), δ 31.7/35.2 [C(7), C(7′)], 37.0/37.8 [C(16), C(18)], 70.3 [C(19)], 70.4 [C(15)], 72.5 [C(20)], 75.0 [C(17)], 108.0/108.7 [C(9), C(9′)], 110.0 [C(10), C(10′)], 116.1 [C(5)], 116.6 [C(13)], 118.2/125.5 [C(3), C(6)], 130.2 [C(4)], 142.4 [C(11), C(11′)], 143.1 [C(12)], 143.9/145.3 [C(1), C(2)], 149.9/150.1 [C(8), C(8′)], 167.1 [C(14)], 175.5 [C(21)].

Reduction of Disulfide 8c Using 1,4-Dithioerythritol. 1,4-Dithioerythritol (1 mg) was added to a solution of **8c** (100 μg) in methanol/water (1 mL; 1:1, v/v). After the solution had been stirred overnight in a septum-sealed vessel, the mixture was analyzed by means of HPLC-UV−vis at 324 nm. Isocratic chromatography was performed with a mixture (60:40, v/v) of aqueous formic acid (1% in water) and acetonitrile on a 250 × 4.0 mm i.d. Microsorb C18 column (Varian, Darmstadt, Germany). A solution of **8c** (100 μg) in methanol/water (1 mL; 1:1, v/v) without 1,4-dithioerythritol was analyzed as the control.

Identification of Phenol/2-Furfurylthiol Conjugates in Roasted Coffee Brew. An aliquot (100 mL) of a freshly prepared coffee brew (Arabica, Colombia) was spiked with an aqueous solution of the odorant 2-furfurylthiol (0.2 mL; 500 μg/mL in 0.1 mol/L phosphate buffer, pH 5.7) and then maintained for 20 min at 30 °C in a septum-sealed vessel (180 mL). Thereafter, the solution was diluted with 5 times the amount of water, and sodium chloride (20 g) was added and then extracted with ethyl acetate (3 × 200 mL). The combined organic layers were freed from solvent in vacuum, and the residue was taken up in a mixture (4 mL; 1:1, v/v) of acetonitrile and aqueous formic acid (1% in water), membrane filtered (0.45 μm), and then analyzed by means of HPLC-MS/MS operating in the multiple reaction monitoring (MRM).

LC/Time-of-Flight Mass Spectrometry (LC/TOF-MS). High-resolution mass spectra of the compounds were measured on a Bruker Micro-TOF (Bruker Daltronics, Bremen, Germany) mass spectrometer and referenced on sodium formate and polyethylene glycol (PEG) 600, respectively.

High-Performance Liquid Chromatography−Tandem Mass Spectrometry (HPLC-MS/MS). The Agilent 1100 series HPLC system consisted of a pump, a degasser, and an autosampler (Agilent, Waldbronn, Germany) and was connected to a 4000 Q Trap triple-quadrupole/linear ion trap mass spectrometer (Applied Biosystems/MDS Sciex, Darmstadt, Germany) with an electrospray ionization (ESI) device running in negative ionization mode. The quadrupoles operated at unit mass resolution. For instrumentation control and data aquisition, the Sciex Analyst software (v1.4) was used.

For the structure determination of phenol/2-furfurylthiol conjugates, samples were injected by means of a Rheodyne manual injector (5 μL) into an isocratic flow (200 μL/min) of a mixture (1:1, v/v) of acetonitrile and aqueous formic acid (1% in water) without any further separation. Detection was performed in the scan mode, recording the mass-to-charge ratios (*m/z*) from 200 to 700, or in product ion mode, recording the fragments of the main signal of the corresponding scan. The declustering potential was set to −30 V, the cell exit potential was set to −15 V, and the collision energy was set to −30 V.

For identification of phenol/2-furfurylthiol conjugates in coffee, the multiple-reaction monitoring mode (MRM) was used recording the following mass transitions from the negative pseudo-molecular ion [M − H]$^-$ to the fragment after collision-induced dissociation: **1a**, 221→140; **1b**, 333→252, 333→171; **1c**, 333→252, 333→171; **1d**, 445→364, 445→283; **1e**, 445→364, 445→283; **2a**, 235→154; **2b**, 235→154; **2c**, 347→266, 347→185; **2d**, 347→266, 347→185; **2e**, 459→378, 459→297; **3a**, 235→154; **3b**, 347→266, 347→185; **3c**, 347→266, 347→185; **4a**, 249→168, 249→153; **4b**, 361→280, 361→199; **5a**, 237→156, 237→123; **5b**, 349→236, 349→203; **6a**, 237→156, 237→123; **6b**, 237→156, 237→123; **6c**, 349→268, 349→187; **7a**, 291→210/, 291→165; **7b**, 403→277, 403→197; **8a**, 465→273, 465→191; **8b**, 577→385, 577→191; **8c**, 609→416, 609→191. After sample injection (5 μL), chromatographic separation was carried out on a 150 × 2.5 mm i.d. Luna Phenyl-Hexyl column (Phenomenex, Aschaffenburg, Germany) with gradient elution at a flow rate of 0.25 mL/min. Eluent A was acetonitrile, and eluent B was 1% formic acid in water. For chromatography, eluent A was held at 15% for 5 min, then increased

linearly to 80% within 30 min, then to 100% within 1 min, and, finally, maintained at 100% for 9 min.

Nuclear Magnetic Resonance (NMR) Spectroscopy. ^{1}H, COSY, HMQC, and HMBC spectroscopic experiments were performed on a Bruker DMX-400 spectrometer (Bruker, Rheinstetten, Germany). Using methanol-d_4 or $CDCl_3$ as solvent, chemical shifts were measured from using tetramethylsilane (TMS) as the internal standard.

RESULTS AND DISCUSSION

Because transition metal ions were recently identified as important catalysts accelerating thiol binding in coffee (*11*), single solutions of catechol, 3-methylcatechol, 4-methylcatechol, 4-ethylcatechol, pyrogallol, hydroxyhydroquinone, 5-*O*-caffeoylquinic acid, and caffeic acid, respectively, were reacted with 2-furfurylthiol in the presence of iron(III) chloride and air oxygen to study whether phenol/thiol conjugates are formed via iron(III)-mediated oxidative coupling of thiols to dihydroxybenzenes. To gain more detailed insight into the exact molecular structure of phenol/thiol conjugates, the reaction mixtures were extracted with ethyl acetate, and the reaction products formed were isolated and purified by means of semipreparative RP-HPLC to give a total of 25 phenol/2-furfurylthiol conjugates. The structures of these conjugates have been determined by means of LC-MS/MS as well as one- and two-dimensional NMR experiments.

Thiol Conjugates of Dihydroxybenzenes. Reaction of catechol with 2-furfurylthiol in the presence of iron(III) ions and air oxygen led to the formation of a series of reaction products as given in **Figure 1**. Among the products formed, compounds **1a**−**1e** were identified as catechol/2-furfurylthiol conjugates by means of LC/TOF-MS, LC-MS/MS and NMR experiments. Compound **1a** showed a molecular mass of 222 Da and an elementary composition of $C_{11}H_{10}O_3S$, thus indicating that one molecule of the thiol reacted with the 1,2-dihydroxybenzene. The ^{1}H NMR spectrum obtained was typical for catechol but lacked the H−C(3) signal and exhibited additional signals for the (2-furylmethyl)sulfanyl moiety resonating at 4.01 (H−C(7)), 5.99 (H−C(9)), 6.23 (H−C(10)), and 7.35 ppm (H−C(11)). Two-dimensional NMR experiments as well as the coupling constants of the aromatic protons H−C(4)−H−C(6) confirmed C(3) as the carbon atom linking the (2-furylmethyl)-sulfanyl moiety to the catechol. By taking all of the spectroscopic data into consideration, compound **1a** was identified as the previously unreported 3-((2-furylmethyl)sulfanyl)catechol (**Figure 2**).

Compound **1b** exhibited a molecular mass of 334 Da, thus indicating that the catechol reacted with two molecules of the thiol. This was further confirmed by demonstrating that compound **1b** is formed as a reaction product when compound **1a** reacts with additional 2-furfurylthiol in the presence of air and iron(III) ions (data not shown). The ^{1}H NMR spectrum of **1b** was similar to that obtained for **1a**, but another aromatic proton of the catechol unit was lacking and the FFT proton signals were observed in duplicate. The small homonuclear coupling ($J = 2.1$ Hz) of the arene protons H−C(4) and H−C(6) indicated that the (2-furylmethyl)sulfanyl moieties are linked to C(3) and C(5) of the catechol. Therefore, the structure of compound **1b** was determined as the previously unreported 3,5-bis((2-furylmethyl)sulfanyl)catechol (**Figure 2**).

Also, compound **1c** showed a molecular mass of 334 Da, and the main fragments in the MS/MS spectrum indicated the loss of one (252 amu) or two (2-furyl)methyl moieties (171 amu), respectively. The ^{1}H NMR spectrum, showing resonance signals representing only one (2-furylmethyl)sulfanyl moiety and one arene proton singlet, indicated the presence of a symmetric molecule with (2-furylmethyl)sulfanyl moieties linked to positions C(4) and C(5) of the catechol. In consequence, the chemical structure of compound **1c** was identified as the previously unreported 4,5-bis((2-furylmethyl)sulfanyl)catechol (**Figure 2**).

Compound **1d**, exhibiting a molecular mass of 446 Da, was expected to be the reaction product involving one catechol and three 2-furfurylthiol molecules. This collaborated well with the main fragments in the MS/MS spectrum showing the loss of one (364 amu), two (283 amu), and three (2-furyl)methyl moieties (202 amu). This was further confirmed by demonstrating that compound **1b** is formed as a reaction product when compound **1a** reacts with additional 2-furfurylthiol in the presence of air and iron(III) ions (data not shown). The ^{1}H NMR spectrum of **1d** was very close to that obtained for **1b**, but only one arene proton signal was detectable and the (2-furyl)methyl protons were present in triplicate. On the basis of the calculation of increments, the remaining arene proton of the catechol resonating at 6.87 ppm was determined as H−C(5), and the structure of compound **1d** was proposed as the previously unreported 3,4,6-tris((2-furylmethyl)sulfanyl)catechol (**Figure 2**). Finally, the structure of **1d** was confirmed by demonstrating that compound **1d** is generated as the main reaction product when compound **1b** reacts with additional 2-furfurylthiol in the presence of air and iron(III) ions (data not shown).

Compound **1e** also showed a molecular mass of 446 Da, again indicating a tris[(2-furylmethyl)sulfanyl] conjugate, but the ^{1}H NMR spectrum, which was similar to that of **1d**, showed two coupling adjacent arene protons ($J = 8.4$ Hz) and one H−C(9) proton of the three (2-furyl)methyl moieties was lacking. All three homonuclear couplings in the proton spin system of two 2-furylmethyl moieties were detectable in the COSY spectrum, but only two coupling protons H−C(10′) and H−C(11′) were detectable for the third (2-furylmethyl)sulfanyl moiety, thus indicating that one molecule of 2-furfurylthiol was bound to position 3 of another (2-furylmethyl)sulfanyl group. By taking all of the 1D and 2D NMR data into consideration, the chemical structure of conjugate **1e** was proposed as the previously unreported 3-[(2-furylmethyl)sulfanyl]-4-[(2-(3-(2-furylmethyl)-sulfanyl)furylmethyl) sulfanyl]catechol given in **Figure 2**.

The iron(III)-mediated oxidative coupling of 3-methylcatechol and 2-furfurylthiol led to the formation of five phenol/2-furfurylthiol conjugates, **2a**−**2e** (**Figure 1**). Both compounds **2a** and **2b** showed a molecular mass of 236 Da, thus indicating these as 1:1 reaction products of 3-methylcatechol and 2-furfurylthiol. The ^{1}H NMR spectra obtained for both compounds were similar to the spectrum of 3-methylcatechol with one arene proton lacking and with additional signals expected for the (2-furylmethyl)sulfanyl moiety. Both compounds **2a** and **2b** showed a coupling of two adjacent arene protons, thus demonstrating that the (2-furylmethyl)sulfanyl moiety in these compounds is linked to positions C(4) and C(6) of the 3-methylcatechol, respectively. The (2-furylmethyl)sulfanyl-linked arene carbon C(4) in structure **2a** was observed to be more strongly high-field shifted when compared to the carbon C(6) in structure **2b**. On the basis of the interpretation of all spectroscopic data, the chemical structures of the thiol conjugates **2a** and **2b** were identified as the previously unknown 4-((2-furylmethyl)sulfanyl)-3-methylcatechol and 3-((2-furylmethyl)sulfanyl)-6-methylcatechol (**Figure 2**).

LC-MS analysis of compounds **2c** and **2d** revealed a molecular mass of 348 Da, thus indicating the existence of a 3-methylcatechol/(2-furfurylthiol)$_2$ conjugate. The ^{1}H NMR spectra of these compounds were similar to those of **2a** and

2b, but another arene proton signal was missing and the (2-furylmethyl)sulfanyl proton signals showed up in duplicate. In both cases, one of the (2-furylmethyl)sulfanyl-linking arene carbons was assigned to C(6) as a result of its chemical shifts of 116.7 ppm in **2c** and 116.2 ppm in **2d** as (2-furylmethyl)-sulfanyl-linking to carbons C(4) and C(5) induces a stronger high-field shift in the same range as that found for the second (2-furylmethyl)sulfanyl-linking arene carbon in both structures. The second (2-furylmethyl)sulfanyl-linked arene carbon in **2d** was assigned as C(4), as a (2-furylmethyl)sulfanyl moiety in this position leads to a downfield shift of C(12), which was observed for compound **2a** and confirmed by increment calculations. The carbon atom C(12) was observed to resonate at 14.5 ppm for **2c** and at 12.4 ppm for **2d**, thus indicating that the second (2-furylmethyl)sulfanyl moiety in **2c** was bound to the arene carbon C(5). On the basis of these considerations, the structures of compound **2c** and **2d** were identified as the previously unknown 3,4-bis((2-furylmethyl)sulfanyl)-6-methylcatechol and 3,5-bis((2-furylmethyl)sulfanyl)-6-methylcatechol, respectively (**Figure 2**).

Compound **2e** exhibited a molecular mass of 460 Da, thus indicating a 3-methylcatechol/(2-furfurylthiol)$_3$ conjugate. The ^{1}H NMR spectrum was similar to that of **2c** and **2d**, but no arene proton signal was detectable and the (2-furylmethyl)-sulfanyl proton signals were present in triplicate. In consequence, the structure of **2e** could be determined as the previously unreported 3,4,5-tris((2-furylmethyl)sulfanyl)-6-methylcatechol (**Figure 2**).

Reaction of 4-methylcatechol with 2-furfurylthiol led to three thiol conjugates, **3a**−**3c** (**Figure 1**). Compound **3a**, exhibiting a molecular mass of 236 Da, was suggested to be the expected 1:1 reaction product. The ^{1}H NMR spectrum was typical for 4-methylcatechol with the additional signals expected for the (2-furylmethyl)sulfanyl moiety and one lacking arene proton signal. As the two arene protons H−C(3) and H−C(5) showed homonuclear coupling with a small coupling constant of 2.1 Hz, compound **3a** was identified as the previously unreported 3-((2-furylmethyl)sulfanyl)-5-methylcatechol (**Figure 2**). LC-MS analysis of compounds **3b** and **3c** showed a molecular mass of 348 amu and indicated the presence of two 4-methylcatechol/(2-furfurylthiol)$_2$ conjugates. Both of the ^{1}H NMR spectra were similar to that of **3a**, but another arene proton signal was missing and the (2-furylmethyl)sulfanyl proton signals were present in duplicate. On the basis of the careful interpretation of the NMR data and increment calculations, the structures of **3b** and **3c** were identified as the previously unreported 3,4-bis((2-furylmethyl)sulfanyl)-5-methylcatechol and 3,6-bis((2-furylmethyl)-sulfanyl)-4-methylcatechol (**Figure 2**).

Iron(III)-mediated oxidative coupling of 4-ethylcatechol with 2-furfurylthiol induced the formation of two main reaction products, **4a**/**4b** (**Figure 1**). Compound **4a** showed a molecular mass of 250 Da and was identified as a 1:1 reaction product of 4-ethylcatechol and the thiol. The ^{1}H NMR spectrum was similar to that of 4-ethylcatechol with additional signals of the (2-furylmethyl)sulfanyl moiety and one lacking arene proton of the phenol moiety. As the two arene protons showed homonuclear coupling with a small coupling constant of 1.8 Hz, the (2-furylmethyl)sulfanyl moiety was assigned to be bound to carbon C(6) of the arene system. In consequence, the structure of thiol conjugate **4a** was identified as the previously unreported 3-((2-furylmethyl)sulfanyl)-5-ethylcatechol (**Figure 2**). LC-MS analysis of compound **4b** revealed a molecular mass of 362 Da and indicated the existence of a 4-ethylcatechol/(2-furfurylthiol)$_2$ conjugate. Due to the chemical shift of the nonsubstituted arene proton H−C(5) at 123.0 ppm, the (2-furylmethyl)sulfanyl-bearing arene carbon atoms were assigned to C(3) and C(6). The structure of compound **4b** was identified as the previously unreported 3,6-bis((2-furylmethyl)sulfanyl)-4-ethylcatechol (**Figure 2**).

Thiol Conjugates of Trihydroxybenzenes. The iron(III)-mediated oxidative coupling of pyrogallol and 2-furfurylthiol led to the formation of two conjugates, **5a** and **5b** (**Figure 3**). Compound **5a** was found to have a molecular mass of 238 Da, thus indicating the presence of a 1:1 reaction product. The ^{1}H NMR spectrum showed the proton signals expected for the (2-furylmethyl)sulfanyl moiety and two coupling adjacent arene protons ($J = 8.3$ Hz) of the pyrogallol system, thus demonstrating that the structure of **5a** was the previously unreported 4-((2-furylmethyl)sulfanyl)pyrogallol (**Figure 4**). LC-MS of compound **5b** revealed a molecular mass of 350 Da and indicated the presence of a pyrogallol/(2-furfurylthiol)$_2$ conjugate. The ^{1}H NMR spectrum was similar to **5a**, but another arene proton signal was missing and the (2-furylmethyl)sulfanyl proton signals were present in duplicate. As the reaction of 2-furfurylthiol at carbons C(4) and C(6) would result in a symmetric molecule with only one signal set for both the (2-furylmethyl)-sulfanyl moieties, the positions of the substituted arene carbons were determined as C(4) and C(5), and the structure of **5b** could be elucidated as the previously unknown 4,5-bis((2-furylmethyl)-sulfanyl) pyrogallol (**Figure 4**).

The use of hydroxyhydroquinone instead of pyrogallol led to the formation of three reaction products, **6a**−**6c** (**Figure 3**). LC-MS analysis of compounds **6a** and **6b** revealed a molecular mass of 238 Da, matching with that of a mono((2-furylmethyl)-sulfanyl) conjugate. The ^{1}H NMR spectrum showed the signals expected for the (2-furylmethyl)sulfanyl moiety as well as those of the hydroxyhydroquinone structure lacking one arene proton. The (2-furylmethyl)sulfanyl-bearing arene carbon of **6a** was identified as C(6) as the two remaining arene protons showed homonuclear coupling with a small coupling constant of 2.8 Hz. The (2-furylmethyl)sulfanyl-substituted arene carbon of **6b** could be assigned as C(5) as the two remaining arene protons did not show any homonuclear coupling. Therefore, the chemical structures of **6a** and **6b** were determined as 3-((2-furylmethyl)-sulfanyl) hydroxyhydroquinone and 4-((2-furylmethyl)sulfanyl)-hydroxyhydroquinone (**Figure 4**), respectively. Compound **6c**, exhibiting a molecular mass of 350 Da, was identified as a hydroxyhydroquinone/(2-furfurylthiol)$_2$ conjugate. The ^{1}H NMR spectrum was similar to that of **6a**, but another arene proton signal was missing and the proton signals of the (2-furylmethyl)-sulfanyl moiety showed up in duplicate. Considering the chemical shift of 102.7 ppm found for the arene carbon C(3), the second (2-furylmethyl)sulfanyl moiety was assigned to position C(5), and the structure of compound **6c** was identified as the previously unreported 3,4-bis((2-furylmethyl)sulfanyl)-hydroxyhydroquinone (**Figure 4**).

Thiol Conjugates of Caffeic Acid and 5-*O*-Caffeoylquinic Acid. To study whether thiols attack the aromatic ring or the double bond of caffeoyl systems, caffeic acid and 2-furfurylthiol were reacted in the presence of iron(III) ions and air oxygen. HPLC-DAD analysis demonstrated the formation of two (2-furylmethyl)sulfanyl-containing reaction products, **7a** and **7b** (**Figure 5**). LC-MS analysis of compound **7a** showed a molecular mass of 292 Da and indicated the presence of a mono-((2-furylmethyl)sulfanyl) conjugate. The ^{1}H NMR spectrum was close to that expected for caffeic acid (*19*) with the arene proton H−C(3) lacking and with the additional signals of the (2-furylmethyl)sulfanyl moiety. Strengthened by the coupling of

the proton signals H−C(5) and H−C(6) with 8.4 Hz, carbon atom C(3) was identified as the (2-furylmethyl)sulfanyl-substituted arene carbon and, in consequence, compound **7a** was determined as the previously unreported 2-((2-furylmethyl)-sulfanyl)caffeic acid (**Figure 6**). Compound **7b**, exhibiting a molecular mass of 404 Da, was identified as a caffeic acid/(2-furfurylthiol)$_2$ conjugate. The ^{1}H NMR spectrum of **7b** was similar to that of **7a**, but another arene proton signal was missing and the proton signals of the (2-furylmethyl)sulfanyl moiety were present in duplicate. On the basis of assignment of the all of the protons as well as increment calculations, the position of the second (2-furylmethyl)sulfanyl moiety was identified as carbon atom C(6) and the structure of the conjugate **7b** was determined as the previously unreported 2,5-bis((2-furylmethyl)-sulfanyl)caffeic acid (**Figure 6**).

Substitution of caffeic acid by 5-*O*-caffeoylquinic acid revealed three reaction products, **8a**−**8c** (**Figure 5**), which have been identified as thiol conjugates. LC-MS/MS analysis of compound **8a**, exhibiting a molecular mass of 466 Da and indicating a mono((2-furylmethyl)sulfanyl) conjugate, showed the quinic acid moiety as the main fragment ion with *m*/*z* 191, thus indicating the cleavage of a (2-furylmethyl)sulfanyl-substituted caffeoyl residue. The ^{1}H NMR spectrum of compound **8a** was very close to that of the 5-*O*-caffeoylquinic acid (*20*) but lacking an H−C(3) signal and an additional signal set as expected for the (2-furylmethyl)sulfanyl moiety. The arene carbon C(3) was confirmed as the (2-furylmethyl)sulfanyl-bearing carbon atom by considering the coupling constant of 8.4 Hz observed for the arene protons H−C(5) and H−C(6). Therefore, compound **8a** was identified as the previously unreported 5-*O*-[2-((2-furylmethyl)sulfanyl)caffeoyl]quinic acid (**Figure 6**). LC-MS analysis of compound **8b** revealed a molecular mass of 578 Da, indicating the presence of a 1+2 conjugate. The ^{1}H NMR spectrum of **8b** was similar to that observed for **8a**, but another arene proton signal was missing and the (2-furylmethyl)sulfanyl proton signals were present in duplicate. On the basis of increment calculation, the position of the second (2-furylmethyl)sulfanyl moiety was identified as carbon atom C(6) instead of C(5) due to the upfield shift of H−C(5) (7.07 ppm), which would be more downfield shifted for a corresponding proton in position C(6). On the basis of these considerations, the structure of thiol conjugate **8b** was proposed as the previously unreported 5-*O*-[2,5-bis((2-furylmethyl)sulfanyl)caffeoyl]quinic acid (**Figure 6**).

LC-MS analysis of compound **8c** showed a molecular mass of 610 Da, that is, 32 Da more than the conjugate **8b**, but showed a very similar ^{1}H NMR spectrum. This similarity and the mass difference of 32 indicated a third sulfur atom in this bis((2-furylmethyl)sulfanyl) conjugate, most likely via a disulfide-bridged molecule of 2-furfurylthiol. To confirm this hypothesis, a solution of compound **8c** was incubated in the presence of the reducing agent 1,4-dithiothreitol. As a control, **8c** was incubated without the reducing agent. When both vessels were opened, the control sample was still odorless, whereas the mixture including the reducing agent exhibited the intensely sulfury−roasty smell of 2-furfurylthiol. Also, HPLC-DAD analysis showed that compound **8c** remained unchanged in the control, whereas the mixture containing the reducing agent lacked any detectable amount of **8c** but showed 2-furfurylthiol besides various minor reaction products. These data clearly confirmed the disulfide moiety in the molecule and led to the identification of the thiol conjugate **8c** as the previously unreported 5-*O*-[2-((2-furylmethyl)sulfanyl)-5-((2-furylmethyl)-disulfanyl)caffeoyl]quinic acid (**Figure 6**).

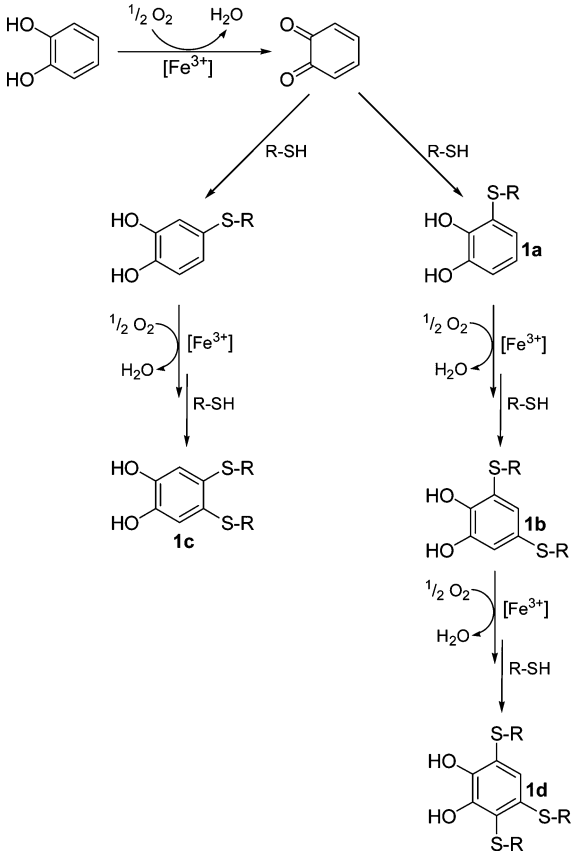

Figure 7. Reaction sequence explaining the formation of the thiol conjugates **1a**–**1d** from catechol.

On the basis of the yield of each conjugate formed in the model experiments, the favored position for the covalent attachment of the first thiol molecule to di- and trihydroxybenzene derivatives was found to be the carbon atom adjacent to the *o*-dihydroxy function, the other arene positions reacting thereafter. Whereas methyl and ethyl groups direct the thiol to position C(6) of the catechol derivative, the thiol was found to attack caffeic acid and 5-*O*-caffeoylquinic acid primarily at position C(3). As ferric ions have been reported as important chelating agents in quinone formation from dopamine (*17*, *18*), the formation of the phenol/thiol conjugates can be easily explained via a transition metal mediated oxidation of the *o*-dihydroxybenzene moiety to give the corresponding *o*-quinone which, upon nucleophilic attack of the sulfur atom of the thiol, is instantaneously converted into the corresponding phenol/thiol conjugates. Using catechol as an example, the reaction pathways leading to the formation of the conjugates **1a**−**1d** are outlined in **Figure 7**. These findings confirm earlier reports on the formation of conjugates from *o*-quinones and thiol components (*14*−*16*, *21*−*25*). For example, a caftaric acid/glutathione conjugate has been identified in grapes and wines (*14*−*16*), or various conjugates of methanethiol were identified in studies on deodorization using phenol-rich fruit and vegetable extracts (*21*−*24*). However, this was the first systematic study on the influence of the phenol structure on the formation of conjugates with the coffee odorant 2-furfurylthiol delivering the reference compounds required to understand the molecular basis for the recently observed depletion of odor-active thiols in coffee beverages (*2*, *3*).

Verification of the Formation of Phenol/2-Furfurylthiol Conjugates in Coffee Brew. To investigate whether thiols can generate such phenol/2-furfurylthiol conjugates under storage

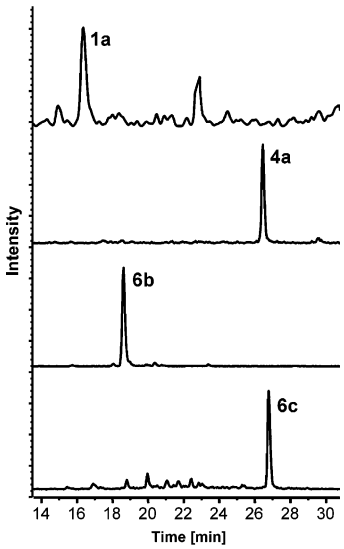

Figure 8. HPLC-MS/MS chromatogram (MRM mode) of phenol/2-furfurylthiol conjugates identified in a coffee brew (54 g/L) spiked with 2-furfurylthiol (1.0 μg/mL) and incubated for 20 min at 30 °C.

conditions, a freshly prepared standard coffee brew was incubated for 20 min at 30 °C (experiment A). In addition, another aliquot of the coffee beverage was spiked with 1.0 μg of synthetic 2-furfurylthiol per milliliter prior to storage (experiment B). Using the thiol conjugates prepared above as reference materials, both coffee samples were analyzed by means of HPLC-MS/MS using the selective and sensitive MRM method. Using this technology, conjugates **1a**, **4a**, **6b**, and **6c** could be unequivocally identified in both coffee samples on the basis of their retention times and the mass transitions (**Figure 8**). These findings clearly demonstrate that, in particular, thiol conjugates of catechol, 4-ethylcatechol, and hydroxyhydroquinone are primarily formed during coffee storage. Therefore, these phenols might play a key role in thiol degradation, inducing the decrease of the aroma quality of coffee beverages upon storage or further processing.

It is interesting to note that conjugates of 5-*O*-caffeoylquinic acid seem not to be formed upon coffee storage, although this compound is the quantitatively predominating phenol in coffee. To investigate the role of phenol/thiol conjugates in aroma staling more precisely on a quantitative basis, isotopologues of selected conjugates are currently being synthesized, and the exact concentrations of 2-furfurylthiol and the thiol-receptive phenols as well as the phenol/thiol conjugates will be determined to get a more comprehensive understanding on the molecular mechanisms underlying the storage-induced aroma staling of coffee beverages.

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