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-buten-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 melanoids 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 2H2-labeled 2-furfurylthiol, pyrazinium dications, which have been identified as key intermediates in roasting-induced melanoidin genesis, were shown to covalently bind 2-furfurylthiol (5–10). 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,
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-methyl catechol 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-methyl catechol, 3-methyl catechol, 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-ethyl catechol (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-methyl catechol, 4-methyl catechol, 4-ethyl catechol, 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 Na2SO4, filtered, and, finally, freed from solvent in vacuum.

**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-methyl catechol; (C) 4-methyl catechol; (D) 4-ethyl catechol.

**Figure 2.** Structures of 2-furfurylthiol conjugates formed from catechol (1a–1e), 3-methyl catechol (2a–2e), 4-methyl catechol (3a–3c), and 4-ethyl catechol (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 phenols (Figures 2, 4, and 6).
2-Furfurylthiol in the presence of iron(III) ions and air oxygen, respectively, with 2-furfurylthiol in the presence of iron(III) ions and air oxygen.

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, CDCl₃, HMBC, HMQC): δ 3.36 [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)].

4,5-Bis(2-furylmethyl)sulfanyl)catechol, 1e (0.62 mmol, 34% yield) (Figure 2): LC/TOF-MS, C₁₂H₁₂O₃S; MS/MS, m/z 333 (100, [M – H]⁻); MS/MS (–30 V), m/z 252 (42), 219 (81), 170 (101), 143 (15); 1H NMR (400 MHz, CDC₁₃), 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, 3)), 7.35 (dd, 2H, J = 0.9, 1.8 Hz, H–C(11, 11)); 13C NMR (100 MHz, CDC₁₃, HMBC, HMQC), δ 32.2 [C(2)], C(7, 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)].

4,6,6-Tris(2-furylmethyl)sulfanyl)catechol, 1d (0.04 mmol, 2% yield) (Figure 3): LC/TOF-MS, C₁₂H₁₀O₃S; MS/MS, m/z 445 (100, [M – H]⁻); MS/MS (–30 V), m/z 364 (10), 283 (100), 249 (5), 202 (7); 1H NMR (400 MHz, CDC₁₃), 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)); 13C NMR (100 MHz, CDC₁₃, HMBC, HMQC), δ 31.7 [C(7), C(7, 7), C(7)], 108.1/108.5 [C(9), C(9, 9), C(9)], 110.7 [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, 11)], 143.0/146.1 [C(1), C(2)], 149.8/150.1/150.4 [C(8), C(8), C(8)].

3-((2-Furfurylmethyl)sulfanyl)-4-((2-(2-furylmethyl)sulfanyl)catechol, 1e (0.06 mmol, 3% yield) (Figure 2): LC/TOF-MS, C₁₂H₁₀O₃S; MS/MS, m/z 445 (100, [M – H]⁻); MS/MS (–30 V), m/z 364 (40), 333(2), 283 (100), 252 (24), 202 (7), 171 (4); 1H NMR (400 MHz, CDCl₃), δ 3.90 [s, 2H, H–C(7, 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)); 13C NMR (100 MHz, CDCl₃, HMBC, HMQC), 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-Furfurylmethyl)sulfanyl)-3-methylcatechol, 2a (0.04 mmol, 3% yield) (Figure 2): LC/TOF-MS, C₁₂H₁₄O₃S; MS/MS, m/z 235 (100, [M – H]⁻); MS/MS (–30 V), m/z 214 (82), 192 (73), 170 (100), 143 (15); 1H NMR (400 MHz, CDCl₃, HMBC, HMQC), δ 3.96/3.98/4.04 [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)); 13C NMR (100 MHz, CDCl₃, HMBC, HMQC), δ 31.7 [C(7), C(7, 7), C(7)], 108.1/108.5 [C(9), C(9, 9), C(9)], 110.7 [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, 11)], 143.0/146.1 [C(1), C(2)], 149.8/150.1/150.4 [C(8), C(8), C(8)].
(3-((2-Furylmethyl)sulfonyl)-6-methylcatechol, 2b (0.48 mmol, 27% yield) (Figure 2): LC/TOF-MS, C_{16}H_{14}O_{5}S_{2}; MS-ESI, m/z 347 (100, [M – H]); MS/MS: (30 V), m/z 266 (65), 185 (100); 7H NMR (400 MHz, MeOD), δ 1.21 [s, 3H, H–C(12)], 3.15 [3H, H–C(10)], 6.46 [s, 1H, H–C(5)], 7.36 [dd, 1H, J = 1.7, 1.9 Hz, H–C(11)]; 13C NMR (100 MHz, MeOD), δC(12) 131.0, δC(10) 111.6, δC(11) 141.6, δC(11') 145.8, δC(1) 150.1/151.5, δC(8), δC(8').

3-(3-(2-Furylmethyl)sulfonyl)-4-methylcatechol, 3a (0.38 mmol, 21% yield) (Figure 2): LC/TOF-MS, C_{16}H_{14}O_{5}S; MS-ESI, m/z 459 (100, [M – H]); MS/MS: (30 V), m/z 378 (89), 347 (3), 297 (100), 266 (4), 185 (3); 7H NMR (400 MHz, MeOD), δ 2.15 [s, 3H, H–C(12)], 3.87/3.94/4.06 [s, 3×H, H–C(10), H–C(7), 7'] 5.73/5.65/6.88 [d, 3×H, J = 1.3, H–C(9, 9'), 9'']. 7.32/7.37 [dd, 3×H, J = 0.7, 1.6 Hz, H–C(11, 11', 11'')].

3-(3-(2-Furylmethyl)sulfonyl)-5-methylcatechol, 3b (0.04 mmol, 2% yield) (Figure 2): LC/TOF-MS, C_{16}H_{14}O_{5}S; MS-ESI, m/z 459 (100, [M – H]); MS/MS: (30 V), m/z 378 (89), 347 (3), 297 (100), 266 (4), 185 (3); 7H NMR (400 MHz, MeOD), δ 2.15 [s, 3H, H–C(12)], 3.87/3.94/4.06 [s, 3×H, H–C(10), H–C(7), 7'] 5.73/5.65/6.88 [d, 3×H, J = 1.3, H–C(9, 9'), 9'']. 7.32/7.37 [dd, 3×H, J = 0.7, 1.6 Hz, H–C(11, 11', 11'')].

4-(3-(2-Furylmethyl)sulfonyl)-5-ethylcatechol, 4a (0.06 mmol, 3% yield) (Figure 2): LC/TOF-MS, C_{16}H_{18}O_{5}S; MS-ESI, m/z 399 (100, [M – H]); 7H NMR (400 MHz, MeOD), δ 3.83 [s, 2H, H–C(7)], 5.89 [d, 1H, J = 1.3, H–C(9)], 6.20 [dd, 1H, J = 1.9, 3.1 Hz, H–C(10)], 6.22 [dd, 1H, J = 1.8, 3.2 Hz, H–C(10)], 6.57 [dd, 1H, J = 1.8, 3.2 Hz, H–C(10)], 7.34 [dd, 1H, J = 0.8, 1.8 Hz, H–C(11)]; 13C NMR (100 MHz, MeOD, HMOC, HMBC), δ 14.9 [C(13)], 26.1 [C(12)], 29.2/30.5 [C(7), C(7')], 107.2 [C(9)], 109.9 [C(10)], 114.8 [C(9)], 119.3 [C(6)], 127.3 [C(5)], 135.5 [C(4)], 141.8 [C(11), C(11')], 143.1 [C(1)], 151.3 [C(8)].

4-(3-(2-Furylmethyl)sulfonyl)-4-ethylcatechol, 4b (0.14 mmol, 8% yield) (Figure 4): LC/TOF-MS, C_{16}H_{14}O_{5}S; MS-ESI, m/z 419 (100, [M – H]); 7H NMR (400 MHz, MeOD), δ 3.83 [s, 2H, H–C(7)], 5.89 [d, 1H, J = 1.3, H–C(9)], 6.20 [dd, 1H, J = 1.9, 3.1 Hz, H–C(10)], 6.22 [dd, 1H, J = 1.8, 3.2 Hz, H–C(10)], 6.57 [dd, 1H, J = 1.8, 3.2 Hz, H–C(10)], 7.34 [dd, 1H, J = 0.8, 1.8 Hz, H–C(11)]; 13C NMR (100 MHz, MeOD, HMOC, HMBC), δ 14.9 [C(13)], 26.1 [C(12)], 29.2/30.5 [C(7), C(7')], 107.2 [C(9)], 109.9 [C(10)], 114.8 [C(9)], 119.3 [C(6)], 127.3 [C(5)], 135.5 [C(4)], 141.8 [C(11), C(11')], 143.1 [C(1)], 151.3 [C(8)].
1H NMR (400 MHz, MeOD), δ 3.83/4.06 [2 × 2H, H-C(7)], 3.73 [2m, 2 × H, H-C(11,11)]; 13C NMR (100 MHz, MeOD, MeOCD, HMBC, HMQC), δ 30/39.38 [C(7), C(7)], 102.7 [C(7)], 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(7)], 141.8 [C(11), C(11)], 147.5 [C(2)], 151.2 [C(8), C(8)], 153.2 [C(4)].

2-(2-Furylmethyl)sulfanyl)caffeic acid, 7a (0.42 mmol, 23% yield) (Figure 6): LC/TOF-MS, C17H16O7S; MS-ESI, m/z 291 (M+H)+, 277 (M+K+), 210 (11), 181 (8), 165 (100); 1H NMR (400 MHz, MeOD), δ 4.06/4.12 [2 × 2H, H-C(7)], 3.86 [2H, H-C(10)], 6.36 [3H, H-C(3)], 7.32 [2m, 2 × H, H-C(11,11)]; 13C NMR (100 MHz, MeOD, MeOCD, HMBC, HMQC), δ 30.9/31.8 [C(7), C(7)], 102.7 [C(7)], 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(7)], 141.8 [C(11), C(11)], 147.5 [C(2)], 151.2 [C(8), C(8)], 153.2 [C(4)].

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-MS/MS operating in the multiple reaction monitoring (MRM) mode.

LC/Time-of-Fly Mass Spectrometry (LC/TOF-MS). High-resolution mass spectra of the compounds were measured on a Bruker Micro-TOF (Bruker Daltonics, Bremen, Germany) mass spectrometer and referenced on sodium formate and polyethylene glycol (PEG) 6000, 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 acquisition, 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 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; 2a, 445→364, 445→283; 2a, 235→154; 2b, 235→154; 2c, 237→266, 237→185; 2d, 247→266, 247→185; 2e, 247→266, 247→185; 2c, 247→366, 247→185; 2e, 247→366, 247→185; 2c, 247→366, 247→185; 2d, 247→366, 247→185; 2e, 247→366, 247→185; 2f, 249→168, 249→153, 2f, 261→280, 261→199; 2a, 237→156, 237→123; 2b, 349→236, 349→203; 2d, 237→156, 237→123; 6b, 237→156, 237→123; 6c, 439→268, 439→187; 7a, 291→210, 291→165; 2b, 403→277, 403→197; 2a, 465→273, 465→191; 2b, 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.
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_{3}S, thus indicating that one molecule of the thiol reacted with the 1,2-dihydroxybenzene. The 1H 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 1H NMR spectrum of 1b was very close to that obtained for 1a, 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 4,5-bis((2-furylmethyl)sulfanyl)catechol (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 1H 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 1H NMR spectra of these compounds were similar to those of 2a and
Compound 2e exhibited a molecular mass of 460 Da, thus indicating a 3-methylcatechol/(2-furfurylthiol)$_2$ 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)-3-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-(1-(2-furylmethyl)sulfanyl) hydroxyhydroquinone and 4-(1-(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
Substitution of caffeic acid by 5-O-cafeoylquinic 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 1H NMR spectrum of compound 8a was very close to that of 5-O-cafeoylquinic acid (20) but lacking an H–C(3) signal and an additional signal set as expected for the (2-furylmethyl)sulfanyl moiety. The aromatic 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]caffeoylquinic 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 1H 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) 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-cafeoylquinic 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 1H NMR spectrum of compound 8a was very close to that of 5-O-cafeoylquinic acid (20) but lacking an H–C(3) signal and an additional signal set as expected for the (2-furylmethyl)sulfanyl moiety. The aromatic 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]caffeoylquinic 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 1H 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) and the structure of the conjugate 7b was determined as the previously unreported 2,5-bis((2-furylmethyl)sulfanyl)caffeic acid (Figure 6).

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 conditions, 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-cafeoylquinic 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).

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-cafeoylquinic 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), and 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).
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 MR 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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