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# Identification of (furan-2-yl)methylated benzene diols and triols as a novel class of bitter compounds in roasted coffee

Stefanie Kreppenhofer, Oliver Frank, Thomas Hofmann *

*Chair of Food Chemistry and Molecular Sensory Science, Technische Universität München, Liese–Meitner-Strasse 34, 85354 Freising, Germany*

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ABSTRACT

Preliminary studies demonstrated that the identification of unknown bitter taste compounds in roasted coffee, by means of an analytical fractionation approach, is hampered by their limited oxidative, as well as chemical stability. A synthetic-constructive strategy was followed in the present investigation by performing targeted reactions of putative coffee-related precursors to give candidate bitter taste molecules. Binary mixtures of a di and trihydroxybenzene, namely pyrogallol, hydroxyhydroquinone, catechol, or 3- and 4-methylcatechole, and a furan derivative, namely furfuryl alcohol, furan-2-aldehyde, or 5-(hydroxymethyl)furan-2-aldehyde, all of which are known to be present in roasted coffee, were thermally treated. The reaction products were identified as (furan-2-yl)methylated benzene diols and triols, by means of LC–MS and NMR experiments, and their bitter taste thresholds determined by means of sensory analysis. Finally, LC–MS/MS studies verified the natural occurence of 4-(furan-2-ylmethyl)benzene-1,2-diol, 4-(furan-2-ylmethyl)benzene-1,2,3-triol, 4-(furan-2-ylmethyl)-5-methylbenzene-1,2-diol, and 3-(furan-2-ylmethyl)-6-methylbenzene-1,2-diol as a novel class of bitter taste compounds in roasted coffee. Depending on their chemical structure, the bitter taste recognition threshold of these compounds ranged between 100 and 537 μmol/l.



## 1. Introduction

Throughout history, from 14th century Arabian merchants, to the famous coffee houses of Vienna, to the comfort of your own home, a freshly brewed coffee has been associated with pleasure, reward, and enjoyment by stimulating our senses from the alluring smell of the composition of aroma-active volatiles to the first sip inducing a well-balanced taste impression centering around bitterness and sourness.

Although it is the roasting procedure which is reported to generate an orchestra of volatile key odourants (Mayer, Czerny, & Grosch, 2000; Semmelroch & Grosch, 1995, 1996) and non-volatile bitter taste compounds (Blumberg, Frank, & Hofmann, 2010; Frank, Blumberg, Krümpel, & Hofmann, 2008; Frank, Blumberg, Kunert, Zehentbauer, & Hofmann, 2007; Frank, Zehentbauer, & Hofmann, 2006), the knowledge available on the later group of molecules is far from comprehensive. By application of a sensomics approach to coffee beverages and coffee-related model systems, followed by LC–MS/MS and 1D/2D NMR studies, coffee roasting was found to induce the transesterification, epimerization, and lactonisation of non-bitter 3-*O*-, 4-*O*-, and 5-*O*-caffeoylquinic acids, as well as dicaffeoylquinic acids giving rise to a series of intensely bitter tasting caffeoyl quinides (Blumberg et al., 2010; Clifford, 1979; Frank et al., 2006, 2008). Moreover, the caffeoylquinic acids and, once formed, their corresponding lactones, have been recently reported to be degraded to 4-vinylcatechol, which oligomerises to produce a family of polyhydroxylated phenylindans exhibiting a harsh and lingering bitter taste profile (Blumberg et al., 2010; Frank et al., 2007).

Although *O*-caffeoylquinic acids are long-known to be decomposed into a series of di and trihydroxybenzenes such as pyrogallol, hydroxyhydroquinone, catechol, 3- and 4-methylcatechol, and 4-ethylcatechol (Clifford, 1979; Haffenden & Yaylayan, 2005; Lang, Müller, & Hofmann, 2006; Tressl, Bahri, Koeppler, & Jensen, 1978), their role as transient intermediates in the generation of candidate bitter tastants upon coffee roasting is unknown. Similarly, furan derivatives such as furfuryl alcohol (Shibamoto et al., 1981) and 5-(hydroxymethyl)furan-2-aldehyde (Belitz, 1977; Moon & Shibamoto, 2009), the later of which originates from Maillard-type and caramelisation reactions of carbohydrates (Antal, Mok, & Richards, 1990; Lewkowski, 2001; Richards, 1956), respectively, have been suggested to contribute to the development of the coffee's bitterness. Any information, however, on the mechanisms involved in transforming such reactive furans into candidate bitter taste molecules upon coffee roasting is lacking.

* Corresponding author. Tel.: +49 8161/71 2902; fax: +49 8161/71 2949.
*E-mail address:* thomas.hofmann@wzw.tum.de (T. Hofmann).

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As the high reactivity and chemical instability of many bitter compounds have limited their unequivocal identification in roasted coffee, by means of iterative fractionation experiments in previous studies, the objective of the present investigation was to thermally treat binary mixtures of di/trihydroxybenzenes and reactive furan derivatives identified in coffee, namely furfuryl alcohol, furan-2-aldehyde, and 5-(hydroxymethyl)furan-2-aldehyde, respectively. The reaction products formed should be identified in their chemical structure by means of LC–MS/MS and 1D/2D NMR experiments, their bitter taste threshold concentrations determined by means of human sensory studies, and finally, their natural occurrence in freshly prepared coffee brew verified by means of HPLC–MS/MS.

## 2. Materials and methods

### 2.1. Chemicals and materials

The following compounds were obtained commercially: pyrogallol, hydroxyhydroquinone, catechol, 4-methylcatechol, 3-methylcatechol, resorcinol, caffeic acid, chlorogenic acid, furfuryl alcohol, furan-2-aldehyde, 5-(hydroxymethyl)furan-2-aldehyde (Sigma–Aldrich, Steinheim, Germany), ethanol, methanol, formic acid, acetic acid (Merck KGA, Darmstadt, Germany). Solvents were of HPLC grade (J.T. Barker, Deventer, Netherlands). Deionized water used for the chromatography was purified by means of Milli-Q-Gradient A10 system (Millipore, Billerica, USA). Deuterated solvents were supplied by Euriso-Top (Gif-Sur-Yvette, France). For gradient flash chromatography RP-18-Bulk material (LiChroprep® RP-18, 25–40 μm, Merck KGaA, Darmstadt, Germany) was used. Sensory analyses were performed with bottled water (Evian, Danone, Wiesbaden, Germany). Coffee beans (Arabica Brazil, Santos), roasted for 240 s at 230 °C, were obtained from the food industry. Reference compounds of 3-((2-furylmethyl)sulfanyl)benzene-1,2-diol and 3-((2-furylmethyl)sufanyl)benzene-1,2,4-triol were synthesised and purified as reported previously (Müller, Hemmersbach, Van't Slot, & Hofmann, 2006).

### 2.2. Sensory analyses

#### 2.2.1. Panel training

Twelve subjects (seven women and five men, 25–38 years of age), who gave informed consent to participate in the sensory tests of the present investigation with no history of known taste disorders, participated for at least two years in sensory training sessions with purified reference compounds by using the sip-and-spit method as earlier reported in detail (Brock and Hofmann, 2008; Frank et al., 2006; Frank et al., 2007; Frank et al., 2008; Haseleu, Intelmann, & Hofmann, 2009; Scharbert, Holzmann, & Hofmann, 2004; Stark, Bareuther, & Hofmann, 2005).

#### 2.2.2. Bitter taste recognition threshold concentrations

To overcome carry-over effects of astringent and bitter compounds, threshold concentrations of test compounds were determined in bottled water by means of the recently developed half-tongue test (Brock and Hofmann, 2008; Frank et al., 2007; Frank et al., 2008; Hufnagel and Hofmann, 2008; Scharbert et al., 2004; Stark et al., 2005, 2010). Serial 1:1 dilutions of the samples (1 ml) were presented in order of increasing concentrations to a trained panel of twelve persons in three different sessions, using the sip-and-spit method. The geometric mean of the last and second to last concentrations was calculated and taken as the individual recognition threshold. At the start of the session and before each trial, the subject rinsed with water and expectorated. Values between individuals and three separate sessions differed by not more than plus or minus one dilution step; that is, a threshold value of 134 μmol/l for 4-(furan-2-ylmethyl)benzene-1,2-diol represents a range from 67 to 268 μmol/l for the astringent impression.

### 2.3. Roasting experiments with a binary mixture of catechol and furfuryl alcohol

A binary mixture of catechol (0.04 mmol) and furfuryl alcohol (0.2 mmol), was intimately mixed with silica gel (900 mg) and acetic acid (500 μl; 1% in water). After drying this mixture for 120 min at 50 °C in a lab oven, the temperature was raised to 180 °C and kept for 10 min. After cooling, the reaction mixture was extracted twice with acetone/water (7/3, v/v; 5 ml), the organic solvent was separated in vacuum and then analysed by means of RP-HPLC/DAD.

### 2.4. Model reactions with mixtures of di/trihydroxybenzenes and furan derivatives in solution

#### 2.4.1. Analytical scale

A binary solution of 5-*O*-caffeoylquinic acid, caffeic acid, catechol, 3-methylcatechol, 4-methylcatechol, resorcinol, pyrogallol, or hydroxyhydroquinone (0.04 mmol each), respectively, with furfuryl alcohol, furan-2-aldehyde, or 5-(hydroxymethyl)furan-2-aldehyde (0.04–0.4 mmol each), respectively, in aqueous acetic acid (1 ml, 1% in water) was thermally treated in a closed Pyrex glass vial (10 ml) at 100 °C using a heated alumina block (Table 1). After various time intervals (10 min, and 1, 2, 4, and 8 h), the reaction mixture was cooled to room temperature, membrane filtered, and aliquots (30 μl) were directly monitored for reaction products by means of RP-HPLC/DAD.

**Table 1**
Reaction conditions used and reaction products formed in binary model systems of di/trihydroxybenzenes and furfuryl alcohol, furan-2-aldehyde, and 5-(hydroxymethyl)furan-2-aldehyde, respectively.

| Di/trihydroxybenzene[a] | Time [min][b] at 100 °C | Phenol/furan ratio[c] | Reaction product[d] |
|---|---|---|---|
| *Furfuryl alcohol* | | | |
| Catechol | 120 | 1/5 | **1** |
| Resorcinol | 120 | 1/5 | **2** |
| Pyrogallol | 60 | 1/5 | **3** |
| Hydroxyhydroquinone | 10 | 1/5 | **4** |
| 4-Methylcatechol | 120 | 1/1 | **5** |
| 3-Methylcatechol | 120 | 1/1 | **6** |
| Caffeic acid | n.r. | 1/1–1/10 | |
| Chlorogenic acid | n.r. | 1/1–1/10 | |
| *Furan-2-aldehyde* | | | |
| Catechol | n.r. | 1/1–1/10 | |
| Resorcinol | 120 | 1/5 | **7** |
| Pyrogallol | 60 | 1/10 | **8** |
| Hydroxyhydroquinone | n.r. | 1/1–1/10 | |
| 4-Methylcatechol | n.r. | 1/1–1/10 | |
| 3-Methylcatechol | n.r. | 1/1–1/10 | |
| Caffeic acid | n.r. | 1/1–1/10 | |
| Chlorogenic acid | n.r. | 1/1–1/10 | |
| *5-(Hydroxymethyl)furan-2-aldehyde* | | | |
| Catechol | n.r. | 1/1–1/10 | |
| Resorcinol | 120 | 1/5 | **9** |
| Pyrogallol | 120 | 1/5 | **10** |
| Hydroxyhydroquinone | n.r. | 1/1–1/10 | |
| 4-Methylcatechol | n.r. | 1/1–1/10 | |
| 3-Methylcatechol | n.r. | 1/1–1/10 | |
| Caffeic acid | n.r. | 1/1–1/10 | |
| Chlorogenic acid | n.r. | 1/1–1/10 | |

[a] Binary mixtures of di/trihydroxybenzenes (0.04 mmol) and furan derivatives (0.04–0.4 mmol) in aqueous acetic acid (1 ml, 1% in water) were thermally treated at 100 °C for different time intervals (10–480 min).
[b] Optimum heating time was determined in analytical scale model systems.
[c] Optimum ratio of reactants was determined in analytical scale model systems.
[d] Structures of reaction products are given in Figs. 1–3. n.r.: no reaction product detected.

*2.4.2. Preparative scale*

A binary solution of catechol, 3-methylcatechol, 4-methylcatechol, resorcinol, pyrogallol, or hydroxyhydroquinone (2.0 mmol each), respectively, and furfuryl alcohol (2–10 mmol), furan-2-aldehyde (10–20 mmol), or 5-(hydroxymethyl)furan-2-aldehyde (10 mmol), respectively, in aqueous acetic acid (15 ml, 1% in water) was thermally treated in a Pyrex glass vial (60 ml) at 100 °C for the times given in Table 1. After cooling, the reaction mixtures were diluted with methanol (10 ml) and separated by means of gradient flash chromatography using a Buechi sepacore system (Flawil, Switzerland) equipped with a 150 × 40 mm i.d. column filled a slurry of RP-18 material (LiChroprep, 25–40 μm, Merck KGaA Darmstadt, Germany). Monitoring the effluent at 220 nm, chromatography was performed at a flow rate of 40 ml/min, starting with a mixture (100/0, v/v) of aqueous formic acid (0.1% in water; solvent A) and methanol (solvent B) for 8 min, then increasing B to 80% within 34 min, followed by an increase of B to 100% within 2 min, and finally, maintaining B at 100% for 10 min. Using an automated fraction collector, crude fractions showing UV absorbance were collected individually, freed from organic solvents in vacuum and then further purified by means of preparative RP-HPLC using a 250 × 21.2 mm i.d., 5 μm, Microsorb 100–5 C18 column (Varian, Darmstadt, Germany). Monitoring the effluent at 220 and 280 nm, chromatography was performed at a flow rate of 18 ml/min with a mixture (25/75, v/v) of aqueous formic acid (0.1% in water; solvent A) and methanol (solvent B) for 5 min, increasing the methanol content to 60% within 20 min, then to 100% within 5 min, and finally, maintaining solvent B at 100% for 5 min. After separation of the solvent in vacuum, the residue was suspended in water (10 ml), freeze-dried twice, then analysed by means of LC–MS/MS and 1D/2D NMR experiments.

*2.4.3. 4-(Furan-2-ylmethyl)benzene-1,2-diol, **1** (Fig. 1)*

UV/Vis (MeOH): $\lambda_{max}$ = 220 nm, 284 nm; LC–TOF–MS: *m/z* 189.05457 (measured for $[C_{11}H_9O_3]^-$), 189.055718 (calculated for $[C_{11}H_9O_3]^-$); MS–ESI$^-$: *m/z* 189 (100, $[M-H]^-$); MS/MS (−54 V): *m/z* 161 (100), 67 (65), 49 (35), 41 (20); $^1$H NMR (400 MHz; $d_3$-MeOD, COSY): $\delta$ [ppm] 3.77 [s, 2H, H−C(7)], 5.96 [d, 1H, *J* = 3.1 Hz, H−C(9)], 6.27 [pt, 1H, *J* = 1.8 Hz, H−C(10)], 6.52 [dd, 1H, *J* = 1.9, 8.0 Hz, H−C(5)], 6.64 [d, 1H, *J* = 1.9 Hz, H−C(1)], 6.68 [d, 1H, *J* = 8.0 Hz, H−C(4)], 7.33 [d, 1H, *J* = 1.8 Hz H−C(11)]; $^{13}$C NMR (100 MHz, $d_3$-MeOD, 135-DEPT, HMQC, HMBC): $\delta$ [ppm] 34.6 [$CH_2$, C(7)], 106.8 [CH, C(9)], 111.3 [CH, C(10)], 116.4 [CH, C(4)], 117.0 [CH, C(1)], 121.1 [CH, C(5)], 131.4 (C, C(6)], 142.5 [CH, C(11)], 144.9 [C, C(2)], 146.3 [C, (C3)], 156.8 [C, C(8)].

*2.4.4. 4-(Furan-2-ylmethyl)benzene-1,3-diol, **2** (Fig. 1)*

UV/Vis (MeOH): $\lambda_{max}$ = 212 nm, 272 nm; LC–TOF–MS: *m/z* 189.055545 (measured for $[C_{11}H_9O_3]^-$), 189.055718 (calculated for $[C_{11}H_9O_3]^-$); MS–ESI$^-$: *m/z* 189 (100, $[M-H]^-$); MS/MS (−54 V): *m/z* 161 (80), 67 (100), 49 (55), 41 (40); $^1$H NMR (400 MHz; $d_3$-MeOD, COSY): $\delta$ [ppm] 3.80 [s, 2H, H−C(7)], 5.80 [dd, 1H, *J* = 0.9, 3.0 Hz, H−C(9)], 6.22 [dd, 1H, *J* = 2.4, 8.0 Hz, H−C(4)], 6.25 [dd, 1H, *J* = 1.8, 3.0 Hz, H−C(10)], 6.30 [d, 1H, *J* = 2.4 Hz, H−C(2)], 6.80 [d, 1H, *J* = 8.0 Hz, H−C(5)], 7.32 [s, 1H, *J* = 1.8 Hz H−C(11)]; $^{13}$C NMR (100 MHz, $d_3$-MeOD, 135-DEPT, HMQC, HMBC): $\delta$ [ppm] 28.6 [$CH_2$, C(7)], 103.5 [CH, C(2)], 106.7 [CH, C(9)], 107.6 [CH, C(4)], 111.3 [CH, C(10)], 117.4 [C, C(6)], 131.8 [CH, C(5)], 142.1 [CH, C(11)], 156.9 [C, C(8)], 157.1 [C, C(1)], 158.1 [C, C(3)].

*2.4.5. 4-(Furan-2-ylmethyl)benzene-1,2,3-triol, **3** (Fig. 1)*

UV/Vis (MeOH): $\lambda_{max}$ = 220 nm, 280 nm; LC–TOF–MS: *m/z* 205.051234 (measured for $[C_{11}H_9O_4]^-$), 205.050632 (calculated for $[C_{11}H_9O_4]^-$); MS–ESI$^-$: *m/z* 205 (100, $[M-H]^-$); MS/MS (−54 V): *m/z* 161 (100), 67 (65), 49 (35), 41 (20); $^1$H NMR (400 MHz; $d_3$-MeOD, COSY): $\delta$ [ppm] 3.83 [s, 2H, H−C(7)], 5.90 [dd, 1H, *J* = 0.9, 3.2 Hz, H−C(9)], 6.24 [dd, 1H, *J* = 1.8, 3.1 Hz, H−C(10)], 6.27 [d, 1H, *J* = 8.3 Hz, H−C(4)], 6.37 [d, 1H, *J* = 8.3 Hz, H−C(5)], 7.31 [dd, 1H, *J* = 0.9, 1.8 Hz H−C(11)]; $^{13}$C NMR (100 MHz, $d_3$-MeOD, 135-DEPT, HMQC, HMBC): $\delta$ [ppm] 28.8 [$CH_2$, C(7)], 106.6 [CH, C(9)], 107.7 [CH, C(4)], 111.1 [CH, C(10)], 118.2 [C, C(6)], 121.1 [CH, C(5)], 134.2 [C, C(2)], 142.0 [CH, C(11)], 145.3 [C, C(1)], 145.7 [C, (C3)], 156.7 [C, C(8)].

*2.4.6. 5-(Furan-2-ylmethyl)benzene-1,2,4-triol, **4** (Fig. 1)*

UV/Vis (MeOH): $\lambda_{max}$ = 204 nm, 296 nm; LC–TOF–MS: *m/z* 205.051234 (measured for $[C_{11}H_9O_4]^-$), 205.050632 (calculated for $[C_{11}H_9O_4]^-$); MS–ESI$^-$: *m/z* 205 (100, $[M-H]^-$); MS/MS (−54 V): *m/z* 137 (100), 124 (50), 67 (15), 41 (20); $^1$H NMR (400 MHz; $d_3$-MeOD, COSY): $\delta$ [ppm] 3.77 [s, 2H, H−C(7)], 5.93 [d, 1H, *J* = 3.1 Hz, H−C(9)], 6.26 [dd, 1H, *J* = 1.8, 3.1 Hz, H−C(10)], 6.34 [s, 1H, H−C(2)], 6.47 [s, 1H, H−C(5)], 7.32 [d, 1H, *J* = 1.8 Hz, H−C(11)]; $^{13}$C NMR (100 MHz, $d_3$-MeOD, 135-DEPT, HMQC, HMBC): $\delta$ [ppm] 27.1 [$CH_2$, C(7)], 103.1 [CH, C(2)], 105.3 [CH, C(9)], 109.8 [CH, C(10)], 115.3 [C, C(6)], 116.6 [CH, C(5)], 137.5 [C, C(1)], 140.7 [CH, C(11)], 143.9 [C, C(3)], 147.5 [C, (C4)], 155.2 [C, C(8)].

*2.4.7. 4-(Furan-2-ylmethyl)-5-methylbenzene-1,2-diol, **5** (Fig. 1)*

UV/Vis (MeOH): $\lambda_{max}$ = 220 nm, 282 nm; LC–TOF–MS: *m/z* 203.07151 (measured for $[C_{12}H_{11}O_3]^-$), 203.071368 (calculated for $[C_{12}H_{11}O_3]^-$); MS–ESI$^-$: *m/z* 203 (100, $[M-H]^-$); MS/MS

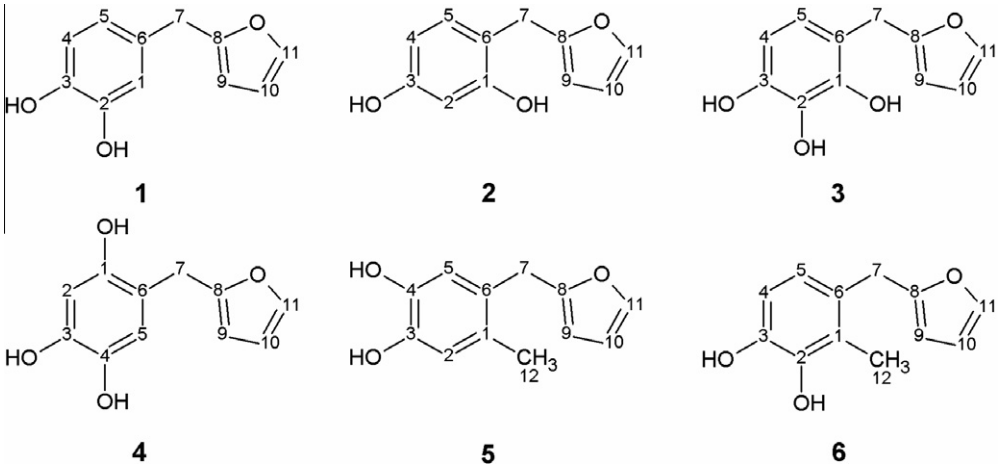



**Fig. 1.** Chemical structures of reaction products **1**–**6** formed upon reaction of di/trihydroxybenzenes with furfuryl alcohol.

(−64 V): *m/z* 159 (70), 67 (100), 49 (50), 41 (40); $^1$H NMR (400 MHz; $d_3$-MeOD, COSY): $\delta$ [ppm] 2.11 [s, 3H, H−C(12)], 3.76 [s, 2H, C−H(7)], 5.85 [d, 1H, *J* = 3.0 Hz, H−C(9)], 6.26 [t, 1H, *J* = 2.0, 3.0 Hz, H−C(10)], 6.55 [s, 1H, H−C(5)], 6.57 [s, 1H, H−C(2)], 7,33 [s, 1H, *J* = 2.0 Hz, H−C(11)]]; $^{13}$C NMR (100 MHz, $d_3$-MeOD, 135-DEPT, HMQC, HMBC): $\delta$ [ppm] 18.7 [$CH_3$, C(12)], 32.3 [$CH_2$, C(7)], 106.7 [CH, C(9)], 111.2 [CH, C(10)], 118.0 [CH, C(5)], 118.3 [CH, C(2)], 128.5 [C, C(6)], 128.8 [C, C(1)], 142.3 [CH, C(11)], 144.1 [C, C(3)], 144.7 [C, (C4)], 156.4 [C, C(8)].

*2.4.8. 3-(Furan-2-ylmethyl)-6-methylbenzene-1,2-diol,* ***6*** *(Fig. 1)*

UV/Vis (MeOH): $\lambda_{max}$ = 220 nm, 280 nm; LC–TOF–MS: *m/z* 203.070836 (measured for $[C_{12}H_{11}O_3]^-$), 203.071368 (calculated for $[C_{12}H_{11}O_3]^-$); MS–ESI$^-$: *m/z* 203 (100, $[M-H]^-$); MS/MS (−56 V): *m/z* 175 (90), 67 (100), 49 (45), 41 (25); $^1$H NMR (400 MHz; $d_3$-MeOD, COSY): $\delta$ [ppm] 2.11 [s, 3H, H−C(12)], 3.82 [s, 2H, H−C(7)], 5.80 [dd, 1H, *J* = 0.8, 3.1 Hz, H−C(9)], 6.25 [dd, 1H, *J* = 1.8, 3.1 Hz, H−C(10)], 6.48 [d, 1H, *J* = 8.0 Hz, H−C(5)], 6.57 [d, 1H, *J* = 8.0 Hz, H−C(4)], 7.32 [d, 1H, *J* = 1.8 Hz, H−C(11)]; $^{13}$C NMR (100 MHz, $d_3$-MeOD, 135-DEPT, HMQC, HMBC): $\delta$ [ppm] 12.0 [$CH_3$, C(12)], 33.0 [$CH_2$, C(7)], 106.7 [CH, C(9)], 111.3 [CH, C(10)], 113.1 [CH, C(4)], 121.8 [CH, C(5)], 124.7 [C, C(6)], 129.7 [C, C(3)], 142.2 [CH, C(11)], 144.7 [C, C(1)], 144.8 [C, (C2)], 156.8 [C, C(8)].

*2.4.9. 4,4′-(Furan-2-ylmethanediyl)dibenzene-1,3-diol,* ***7*** *(Fig. 2)*

UV/Vis (MeOH): $\lambda_{max}$ = 224 nm, 280 nm; LC–TOF–MS: *m/z* 297.077153 (measured for $[C_{17}H_{13}O_5]^-$), 297.076847 (calculated for $[C_{17}H_{13}O_5]^-$); MS−ESI$^-$: *m/z* 297 (100, $[M-H]^-$); MS/MS (−56 V): *m/z* 253 (55), 187 (60), 109 (45), 41 (20); $^1$H NMR (400 MHz; $d_3$-MeOD, COSY): $\delta$ [ppm] 5.70 [d, 1H, *J* = 3.1 Hz, H−C(9)], 5.84 [s, 1H, H−C(7)], 6.18 [dd, 2H, *J* = 2.4, 8.0 Hz, H−C(4, 4′)], 6.25 [dd, 1H, *J* = 1.8, 3.1 Hz, H−C(10)], 6.28 [d, 2H, *J* = 2.4 Hz, H−C(2, 2′)], 6.60 [d, 2H, *J* = 8.0 Hz, H−C(5, 5′)], 7.34 [d, 1H, *J* = 1.8 Hz, H−C(11)]; $^{13}$C NMR (100 MHz, $d_3$-MeOD, 135-DEPT, HMQC, HMBC): $\delta$ [ppm] 36.1 [CH, C(7)], 102.1 [CH, C(4, 4′)], 105.6 [CH, C(5, 5′)], 106.8 [CH, C(9)], 109.4 [CH, C(10)], 120.1 [C, C(6, 6′)], 129.1 [CH, C(2, 2′)], 140.7 [CH, C(11)], 155.3 [C, C(1, 1′)], 156.3 [C, C(3, 3′)], 158.2 [C, C(8)].

*2.4.10. 4,4′-(Furan-2-ylmethanediyl)dibenzene-1,2,3-triol,* ***8*** *(Fig. 2)*

UV/Vis (MeOH): $\lambda_{max}$ = 220 nm, 272 nm; LC–TOF–MS: *m/z* 329.067259 (measured for $[C_{17}H_{13}O_7]^-$), 329.066676 (calculated for $[C_{17}H_{13}O_7]^-$); MS–ESI$^-$: *m/z* 329 (100, $[M-H]^-$); MS/MS (−64 V): *m/z* 283 (25), 203 (35), 125 (100), 41 (10); $^1$H NMR (400 MHz; $d_3$-MeOD, COSY): $\delta$ [ppm] 5.72 [d, 1H, *J* = 3.5 Hz, H−C(9)], 5.88 [s, 1H, H−C(7)], 6.17 [d, 2H, *J* = 8.5 Hz, H−C(5, 5′)], 6.21−6.26 [m, 3H, H−C(6, 6′, 10)], 7.33 [d, 1H, *J* = 1.8 Hz, H−C(11)]; $^{13}$C NMR (100 MHz, $d_3$-MeOD, 135-DEPT, HMQC, HMBC): $\delta$ [ppm] 38.2 [CH, C(7)], 107.4 [CH, C(4, 4′)], 108.4 [CH, C(9)], 110.8 [CH, C(10)], 120.6 [CH, C(5,5′)], 122.3 [C, C(6, 6′)], 134.2 [C, C(2, 2′)], 142.1 [CH, C(11)], 144.9 [C, C(1, 1′)], 145.6 [C, C(3, 3′)], 159.3 [C, C(8)].

*2.4.11. 2-(Bis-(2,4-dihydroxyphenyl)methyl)-5-(2,4-dihydroxybenzyl))furan* ***9*** *(Fig. 3)*

UV/Vis (MeOH): $\lambda_{max}$ = 216 nm, 280 nm; LC–TOF–MS: *m/z* 419.116258 (measured for $[C_{24}H_{23}O_7]^-$), 419.113627 (calculated for $[C_{24}H_{23}O_7]^-$); MS–ESI$^-$: *m/z* 419 (100, $[M-H]^-$); MS/MS (−54 V): *m/z* 309 (100), 253 (25), 159 (75), 149 (40), 109 (50), 41 (20); $^1$H NMR (400 MHz; $d_3$-MeOD, COSY): $\delta$ [ppm] 3.75 [s, 2H, H−C(7)], 5.57 [d, 1H, *J* = 3.1 Hz, H−C(10)], 5.74 [d, 1H, *J* = 3.1 Hz, H−C(9)], 5.80 [s, 1H, H−C(12)], 6.19 [dd, 2H, *J* = 2.3, 8.2 Hz, H−C(17, 17′)], 6.20 [dd, 1H, *J* = 2.3, 8.0 Hz, H−C(4)], 6.28 [m, 3H, H−C(2, 15, 15′)], 6.63 [d, 2H, *J* = 8.2 Hz, H−C(18, 18′)], 6.81 [d, 1H; *J* = 8.2 Hz, H−C(5)]; $^{13}$C NMR (100 MHz, $d_3$-MeOD, 135-DEPT, HMQC, HMBC): $\delta$ [ppm] 28.6 [$CH_2$, C(7)], 37.6 [CH, C(12)], 103.4 [CH, C(2)], 103.5 [CH, C(15, 15′)], 107.0 [CH, C(9)], 107.1 [CH, C(17, 17′)], 107.5 [CH, C(4)], 109.0 [CH, C(10)], 117.7 [C, C(6)], 121.9 [C, C(13, 13′)], 131.1 [CH, C(18, 18′)], 131.7 [CH, C(5)], 155.1 [C, C(1)], 156.6 [C, C(14, 14′)], 156.9 [C, C(16, 16′)], 157.6 [C, C(8)], 157.6 [C, C(11)], 157.8 [C, C(3)].

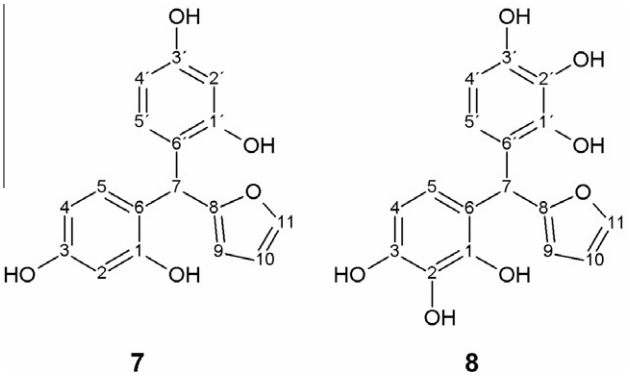



**Fig. 2.** Chemical structures of reaction products **7** and **8** formed upon reaction of resorcinol and pyrogallol, respectively, with furan-2-aldehyde.

*2.4.12. 2-(Bis-(2,3,4-trihydroxyphenyl)methyl)-5-(2,3,4-trihydroxybenzyl))furan* ***10*** *(Fig. 3)*

UV/Vis (MeOH): $\lambda_{max}$ = 216 nm, 272 nm; LC–TOF–MS: *m/z* 467.0997 (measured for $[C_{24}H_{19}O_{10}]^-$), 467.098360 (calculated for $[C_{24}H_{19}O_{10}]^-$); MS–ESI$^-$: *m/z* 419 (100, $[M-H]^-$); MS/MS (−66 V): *m/z* 341 (100), 175 (65), 125 (40), 109 (15), 41 (5); $^1$H NMR (400 MHz; $d_3$-MeOD, COSY): $\delta$ [ppm] 3.77 [s, 2H, H−C(7)], 5.59 [dd, 1H, *J* = 0.9, 3.1 Hz, H−C(9)], 5.73 [d, 1H, *J* = 3.1 Hz, H−C(10)], 5.82 [s, 1H, H−C(12)], 6.21 [d, 2H, *J* = 8.5 Hz, H−C(17, 17′)], 6.24 [d, 2H, *J* = 8.0 Hz, H−C(18, 18′)], 6.36 [d, 1H, *J* = 8.3 Hz, H−C(4)], 6.39 [d, 1H, *J* = 8.3 Hz; H−C(5)]; $^{13}$C NMR (100 MHz, $d_3$-MeOD, 135-DEPT, HMQC, HMBC): $\delta$ [ppm] 28.9 [CH2, C(7)], 38.9 [CH, C(12)], 107.0 [CH, C(17, 17′)], 107.4 [CH, C(9)], 107.8 [CH, C(4)], 109.2 [CH, C(10)], 118.5 [C, C(6)], 120.7 [CH, C(18, 18′)], 121.2 [CH, C(5)], 122.6 [C, C(13, 13′)], 134.2 [C, C(15, 15′)], 134.3 [C, C(2)], 144.8 [CH, C(14, 14′)], 145.1 [C, C(1)], 145.5 [C, C(16, 16′)], 145.6 [C, C(3)], 155.1 [C, C(8)], 157.3 [C, (C(11)].

*2.5. Preparation of the coffee beverage*

After grinding the coffee beans by means of a batch mill (IKA, Staufen, Germany), a portion (54 g) of coffee powder, placed in a coffee filter (No. 4, Melitta, Germany), was percolated with boiling water until the filtrate reached a volume of 1.0 l. The coffee beverage obtained was immediately cooled to room temperature in an ice-bath prior to HPLC–MS/MS analysis.

*2.6. High-performance liquid chromatography (HPLC)*

The analytical HPLC apparatus (Kontron, Eching, Germany) consisted of a low-pressure gradient system 525 HPLC pump, an M800 gradient mixer, a type 560 autosampler, and a DAD type 540 + diode array detector. Chromatography was performed on 250 × 4.6 mm i.d., 5 μm, Luna Phenyl-Hexyl column (Phenomenex, Aschaffenburg, Germany), operated with a flow rate of 0.8 ml/min. Semi-preparative chromatography was done using a HPLC system consisting of two Sykam S1122 high pressure pumps (Eresing, Germany), an Sunchrom Spectraflow 600 DAD detector (Friedrichsdorf, Germany), a Rheodyne injector with a 2 ml loop

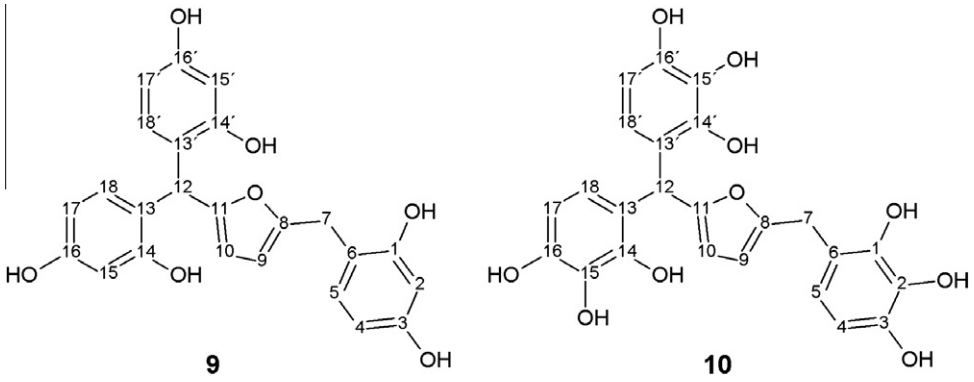



**Fig. 3.** Chemical structures of reaction products **9** and **10** formed upon reaction of resorcinol and pyrogallol, respectively, with 5-(hydroxymethyl)furan-2-aldehyde.

(Alsbach a.d. Bergstrasse, Germany), and a 250 × 21.2 mm i.d., 5 μm, Microsorb 100–5 C18 column (Varian, Darmstadt, Germany) operated with a flow rate of 18 ml/min.

### 2.7. Gradient flash chromatography

The gradient flash chromatography apparatus (Buechi, Flawil, Switzerland) consisted of two C-605 pumps with a C-615 pump manager, a C-635 UV/Vis detector, and a C-660 fraction collector. Chromatography was performed with a flow rate of 40 ml/min on a self-packed 150 × 40 mm i.d. polypropylene cartridge filled with LiChroprep, 25–40 μm, RP-18 Material (Merck KGaA, Darmstadt, Germany).

### 2.8. LC/time-of-flight mass spectrometry (LC/TOF–MS)

High resolution mass spectra were measured on a Bruker Micro–TOF mass spectrometer (Bruker Daltronics, Bremen, Germany) and referenced with sodium formate.

### 2.9. High-performance liquid chromatography / tandem mass spectrometry (HPLC–MS/MS)

The Agilent 1200 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 ionisation (ESI) device running in negative ionisation mode, with a spray voltage of −4500 V. The quadruples operated at unit mass resolution. Nitrogen served as a curtain gas (20 psi) with the declustering potential was set at −10 to −80 V in the $ESI^-$ mode. The mass spectrometer operated in the full scan mode monitoring negative ions. Fragmentation of $[M-H]^-$ molecular ions into specific product ions was induced by collision with nitrogen ($5 \times 10^{-5}$ Torr) and a collision energy of −10 to −70 V. For instrumentation control and data acquisition, the Sciex Analyst software (v1.5) was used.

For HPLC–MS/MS analysis of the furan-2-ylmethylated benzene diols and triols in a coffee beverage, an analytical 150 × 2.1 mm i.d., 5 μm, Luna Phenyl–Hexyl column (Phenomenex, Aschaffenburg, Germany) was connected to the mass spectrometer operating in the multiple reaction monitoring (MRM), detecting negative ions. For a duration of 50 ms, the mass transitions *m/z* 189 → 161 and 189 → 67 were used for the analysis of **1** and **2**, *m/z* 205 → 67 and 205 → 41 for compound **3**, *m/z* 205 → 67 and 205 → 43 for compound **4**, *m/z* 203 → 67 and 203 → 159 for compound **5**, *m/z* 203 → 67 and 203 → 175 for compound **6**, *m/z* 297 → 187 and 297 → 253 for compound **7**, *m/z* 329 → 125 and 329 → 203 for compound **8**, *m/z* 419 → 309 and 419 → 159 for compound **9**, and *m/z* 467 → 341 and 467 → 174 for compound **10**. Nitrogen served as nebulizer gas (65 psi) and as turbo gas (350 °C) for solvent drying (55 psi). After injection of the sample (5 μl), chromatography was performed with a flow rate of 250 μl using a solvent gradient starting with aqueous formic acid (0.1% in water) for 3 min, then increasing the methanol content to 40% within 10 min, then to 60% within 5 min, to 70% within 12 min, and finally, to 100% within 2 min, thereafter maintaining the methanol content for an additional 3 min.

### 2.10. Nuclear magnetic resonance (NMR) spectroscopy

The $^1H$ and $^{13}C$ NMR experiments were conducted on a DRX 400 spectrometer (Bruker, Rheinstetten, Germany). The samples were dissolved in $d_3$-MeOD (Euriso-Top, Giv-Sur-Yvette, France) and then analysed in 178 × 5 mm NMR tubes (Norell, Sp5000 Landisville, USA) at 298 K. Chemical shifts were determined using tetramethylsilane (TMS) as the internal standard in the proton dimension and from the carbon signal of $d_3$-MeOD (49.3 ppm) in the carbon dimension. While data processing was performed using Topspin Version 1.3 (Bruker, Rheinstetten), the individual data interpretation was done by means of MestReNova® 5.1.0–2940 (Mestrelab Research S.L., Santiago de Compostela, Spain).

## 3. Results and discussion

### 3.1. Preliminary studies

Preliminary studies demonstrated that the identification of previously unknown bitter taste compounds in roasted coffee, by means of an analytical fractionation approach is hampered by their limited oxidative, as well as chemical stability. In consequence, a synthetic-constructive strategy based on the targeted reaction of putative taste precursors to give candidate bitter taste molecules, followed by the verification of their natural occurrence in roasted coffee, is believed to be a more promising approach. As di and trihydroxybenzenes such as pyrogallol, hydroxyhydroquinone, catechol, 3- and 4-methylcatechole, and 4-ethylcatechol (Clifford, 1979; Haffenden & Yaylayan, 2005; Lang et al., 2006; Tressl et al., 1978) as well as furan derivatives such as furfuryl alcohol, furan-2-aldehyde, 5-(hydroxymethyl)-furan-2-aldehyde (Belitz, 1977; Moon & Shibamoto, 2009; Shibamoto et al., 1981) have been identified as rather reactive molecules in roasted coffee samples, the screening of thermally treated binary mixtures of di/trihydroxybenzenes and furan derivatives was supposed to be a fruitful strategy for the discovery of previously undetermined, candidate bitter tasting reaction products in roasted coffee.

### 3.2. Reaction of catechol and furfuryl alcohol under low-moisture and aqueous conditions, respectively

As preliminary quantitative studies on roasted coffee revealed catechol and furfuryl alcohol as the quantitatively predominating dihydroxybenzene and furan derivative, respectively, a binary mixture of these compounds was thermally treated at 180 °C for 10 min on silica gel, before the presence of catalytic amounts of acetic acid. After cooling, the reaction mixture was extracted with acetone/water to separate the reaction products from the silica gel and the extract obtained was analysed by means of RP-HPLC connected to a diode array detector or a mass spectrometer, respectively. HPLC analysis revealed one main reaction product (**1**), eluting after 21 min and exhibiting UV absorption maxima at 220 and 284 nm, besides the educts furfuryl alcohol and catechol. LC–MS analysis (ESI$^-$) revealed *m/z* 189 as the pseudomolecular ion ([M−H]$^-$) and high resolution mass spectrometry indicated an elemental composition of $C_{11}H_{10}O_3$ for the target molecule.

As the amounts of **1** were too low for an unequivocal structure elucidation by means of NMR spectroscopy, a binary mixture of catechol and furfuryl alcohol was heated at 100 °C for 120 min in aqueous solution containing small amounts of acetic acid. Comparison of the HPLC chromatograms recorded for the dry-heated and the aqueous model system revealed the same peak pattern for both reaction systems. As the up-scaling of the model reaction was rather simple for the aqueous system, this later reaction was performed in preparative scale. After cooling, the reaction mixture was separated by means of gradient flash chromatography on RP-18 material, followed by final purification of reaction product **1** using preparative RP-18 HPLC, and subsequent lyophilisation to give the target compound, showing the pseudomolecular ion *m/z* 189 ([M−H]$^-$) as a pale, amorphous powder in a purity of more than 98% (HPLC, NMR).

The $^1$H NMR spectrum recorded for **1** showed seven resonance signals integrating for eight protons. The resonance signals observed at 3.77, 5.96, 6.26, and 7.31 ppm were assigned as the protons of a (furan-2-yl)methyl moiety, whereas the three signals detected at 6.68, 6.52, and 6.64 ppm were proposed to belong to the aromatic protons H−C(1), H−C(4), and H−C(5) of catechol. The large coupling constant of 8 Hz found for the protons H−C(4) and H−C(5) demonstrated the vicinal position of these two protons in the catechol moiety and indicated the linkage of the (furan-2-yl)methyl moiety to C(6) of the catechol ring. This was further strengthened by the heteronuclear connectivity found in the HMBC spectrum between the carbon atom C(7) of the (furan-2-yl)methyl moiety and the protons H−C(1) and H−C(5) of the catechol moiety. Taking all the spectroscopic data into consideration, compound **1** was unequivocally identified as 4-(furan-2-ylmethyl)benzene-1,2-diol (Fig. 1). Although the structure of compound **1** was proposed already in 1960 on the basis of IR experiments (Kenzo, 1960; Kenzo, 1961), a report on its final confirmation by means of LC–MS and NMR spectroscopy is still lacking to the best of our knowledge.

### 3.3. Reaction of di/trihydroxybenzenes and furfuryl alcohol

In order to investigate the reaction products formed from other coffee-related di and trihydroxybenzenes and furfuryl alcohol, first comparative analytical scale experiments were performed with binary mixtures of catechol, resorcin, pyrogallol, hydroxyhydroquinone, 3-methylcatechol, 4-methylcatechol, caffeic acid, or 5-*O*-caffeoyl quinic acid, respectively, combined with furfuryl alcohol in 1% aqueous acetic acid heated at 100 °C, varying in the phenol/furan ratio (1:1, 1:5, 1:10) and the heating time (10, 60, 120, 240, 480 min). Each reaction mixture was analysed by means of RP-HPLC coupled to a diode array detector and a mass spectrometer, respectively. Interestingly, reaction products were generated exclusively in the model systems containing the di and trihydroxybenzenes, whereas caffeic acid and 5-*O*-caffeoyl quinic acid did not show any reactivity under the conditions applied in the model experiments. The optimised reaction conditions, which afforded the reaction products from di/trihydroxybenzenes and furfuryl alcohol in the highest yields, are summarised in Table 1.

For structure determination of the respective target compounds, the individual model reactions were carried out in a preparative scale, the crude reaction mixtures were separated by means of gradient flash chromatography, followed by semi-preparative RP-HPLC to give the target molecules **2**–**6** (Fig. 1). The structure of this reaction product was unequivocally determined by means of LC–MS/MS, LC–TOF–MS, and 1D/2D NMR spectroscopic experiments.

Reaction of resorcinol and furfuryl alcohol revealed compound **2** (Fig. 1) exhibited a pseudmolecular ion of *m/z* 189 ([M−H]$^-$), fitting well with that of compound **1**. Differing from the $^1$H NMR spectrum of **1**, the signal of the proton H−C(4) was high-field shifted to 6.22 ppm in compound **2** and also the homonuclear couplings $^3J_{4,5}$ (8.0 Hz) and $^4J_{2,4}$ (2.4 Hz) confirmed the linkage of the (furan-2-yl)methyl moiety to C(6) of the resorcinol moiety. Strengthened by the heteronuclear connectivities (HMBC) observed between C(7), H−C(5), as well as H−C(9), compound **2** was identified as 4-(furan-2-ylmethyl)benzene-1,3-diol (Fig. 1), thus confirming data reported earlier (Kenzo, 1961).

LC–MS and LC–TOF–MS analysis of compounds **3** and **4** (Fig. 1), isolated from the reaction mixture of furfuryl alcohol with pyrogallol and hydroxyhydroquinone, respectively, showed a pseudomolecular ion of *m/z* 205 ([M−H]$^-$) and an elemental composition of $C_{11}H_{10}O_4$ for both isomers. The $^1$H NMR spectrum of **3** and **4**, respectively, showed the proton signals expected for the (furan-2-yl)methyl moiety, two coupling adjacent arene protons ($^3J$ = 8.3 Hz) of the pyrogallol moiety of compound **3,** as well as two non-coupling, isolated aromatic protons in the hydroxyhydroquinone moiety of compound **4**. Taking all spectroscopic data into account, these compounds were identified as the previously not reported 4-(furan-2-ylmethyl)benzene-1,2,3-triol (**3**) and 5-(furan-2-ylmethyl)benzene-1,2,4-triol (**4**), respectively (Fig. 1).

Compounds **5** and **6** (Fig. 1), isolated from the reaction mixture of furfuryl alcohol with 4-methylcatechol and 3-methylcatechol, respectively, both showed a molecular weight of 204 Da. The $^1$H NMR spectra of **5** and **6** exhibited the proton signals assigned for the (furan-2-yl)methyl moiety and the proton resonances expected for 4-methylcatechol and 3-methylcatechol, respectively, lacking one arene proton each. Signal assignment by means of an HMBC experiment led to the unequivocal identification of the previously not reported 4-(furan-2-ylmethyl)-5-methylbenzene-1,2-diol (**5**) and 3-(furan-2-ylmethyl)-6-methylbenzene-1,2-diol (**6**), respectively (Fig. 1).

### 3.4. Reaction of di/trihydroxybenzenes and furan-2-aldehyde

To compare the compounds formed from coffee-related di and trihydroxybenzenes in the reaction with furfuryl alcohol, with those formed in the presence of furan-2-aldehyde, analytical scale reactions were performed with binary mixtures of catechol, resorcinol, pyrogallol, hydroxyhydroquinone, 3-methylcatechol, 4-methylcatechol, caffeic acid, or 5-*O*-caffeoyl quinic acid, respectively, and furan-2-aldehyde in 1% aqueous acetic acid heated at 100 °C varying in the phenol/furan ratio (1:1, 1:5, 1:10) and the heating time (10, 60, 120, 240, 480 min). HPLC analysis revealed that reaction products were generated exclusively in the model systems containing resorcin and pyrogallol, respectively, the aromatic ring of both molecules is substituted with hydroxyl groups in the *meta*-position (Table 1).

For structure determination, the individual model reactions were carried out in a preparative scale, the crude reaction mixtures were separated by means of gradient flash chromatography, followed by semi-preparative RP-HPLC to afford the purified target molecules **7** and **8** (Fig. 2), which were identified by means of LC–MS/MS, LC–TOF–MS, and 1D/2D NMR spectroscopy.

LC–MS and LC–TOF–MS analysis of compound **7**, isolated from the model reaction of resorcinol and furan-2-aldehyde, revealed a pseudomolecular ion of *m/z* 297 ($[M-H]^-$) and an elemental composition of $C_{17}H_{14}O_5$, thus indicating a reaction product formed from one molecule of furan-2-aldehyde and two molecules of resorcin. The $^1$H NMR spectrum of **7** exhibited seven resonance signals integrating for nine protons. The proton signals of the (furan-2-yl)-methyl moiety showed integrals of one, whereas the resonance signals observed at 6.18, 6.28, and 6.58 ppm integrated for two protons each, thus confirming one (furan-2-yl)methyl moiety and two resorcinol moieties in the target molecule. In addition, the homonuclear coupling constants $^3J_{4,5}$ = 8.0 Hz and $^3J_{2,4}$ = 1.5 Hz, as well as the heteronuclear couplings observed between C(7) and H–C(5)/H–C(5′), in the HMBC spectrum indicated that the (furan-2-yl)methyl moiety is bridging two resorcin molecules at position C(6) and led to the unequivocal identification of molecule **7** as 4,4′-(furan-2-ylmethanediyl) dibenzene-1,3-diol (Fig. 2).

LC–MS analyses of compound **8**, generated from pyrogallol and furan-2-aldehyde, revealed a molecular weight of 330 Da and indicated the presence of two pyrogallol moieties and one furan-2-aldehyde moiety in the target molecule. Differing from the $^1$H NMR spectrum of compound **7**, the spectrum of **8** was lacking the arene protons H–C(2)/H–C(2′), thus leading to the identification of the target compound as the previously unknown 4,4′-(furan-2-ylmethanediyl)dibenzene-1,2,3-triol (**8**, Fig. 2).

### 3.5. Reaction of di/trihydroxybenzenes and 5-(hydroxymethyl)furan-2-aldehyde

As 5-(hydroxymethyl)furan-2-aldehyde is combining the reactivity of furfuryl alcohol and furan-2-aldehyde in the same molecule, the question arose as to which reaction products were formed in the reaction with the coffee-related di and trihydroxybenzenes. Among all the analytical model reactions performed (Table 1), resorcinol and pyrogallol each exclusively gave a specific reaction product when reacted with 5-(hydroxymethyl)furan-2-aldehyde. After semi-preparative up-scaling of these reaction systems, the reaction products **9** and **10** were isolated by means of gradient flash chromatography, followed by RP-HPLC, before their chemical structures were identified by means of LC–MS/MS, LC–TOF–MS and 1D/2D NMR spectroscopic experiments.

LC–MS and LC–TOF–MS analysis of compound **9**, generated from resorcin and 5-(hydroxymethyl)furan-2-aldehyde, revealed the pseudomolecular ion *m/z* 419 ($[M-H]^-$) and the elemental composition $C_{24}H_{24}O_7$, thus indicating that three molecules of resorcin reacted with one molecule 5-(hydroxymethyl)furan-2-aldehyde. The $^1$H NMR spectrum exhibited 10 resonance signals integrating for a total of 14 carbon-bound protons. The resonance signal observed at 3.74 ppm integrated for two protons and was assigned as the methylene group H–C(7), whereas the three protons resonating at 5.57, 5.74, and 5.80 ppm were assigned as the protons H–C(9), H–C(10), and H–C(12) of the former 5-(hydroxymethyl)furan-2-aldehyde moiety. The remaining proton signals observed in the $^1$H NMR spectrum corresponded to the arene protons of three resorcin moieties. The signals detected at 6.19, 6.28, and 6.63 ppm integrated for two protons each, showed the coupling constants $^3J_{17/18}$ = 8.2, $^3J_{17'/18'}$ = 8.2, $^3J_{17/15}$ = 2.3, $^3J_{17'/15'}$ = 2.3, and were assigned as H–C(17)/H–C(17′), H–C(15)/H–C(15′), and H–C(18)/H–C(18′). The third resorcin moiety showed three resonances at 6.20, 6.28, and 6.81 ppm corresponding to the protons H–C(4), H–C(2) and H–C(5) of compound **9**. By means of a heteronuclear multiple-bond coherence experiment (HMBC), heteronuclear correlations were observed between carbon C(7) and proton H–C(5), as well as between the methin carbon atom C(12) and the arene protons H–C(18) and H–C(18́), respectively, thus demonstrating the structure of compound **9** as the previously not reported 2-(bis-(2,4-dihydroxyphenyl)methyl)-5-(2,4-dihydroxybenzyl) furan (Fig. 3).

Compound **10**, isolated from the reaction mixture of pyrogallol and 5-(hydroxymethyl)furan-2-aldehyde, showed a pseudomolecular ion of *m/z* 419 ($[M-H]^-$) in the LC–MS spectrum and an elemental composition of $C_{24}H_{20}O_{10}$ by means of LC–TOF–MS analysis. The $^1$H NMR data of compound **10** were rather similar to those found for compound **9,** with the exception of the lack of one arene proton for each benzene moiety. Taking all the spectroscopic data into account, the structure of compound **10** was clearly determined as the previously unknown 2-(bis-(2,3,4-trihydroxyphenyl)methyl)-5-(2,3,4-trihydroxybenzyl)furan (Fig. 3).

### 3.6. Taste recognition threshold concentrations

Prior to sensory analysis, the purity of the reaction products **1**–**10** as well as their parent di/trihydroxybenzenes, were checked by $^1$H NMR spectroscopy as well as HPLC–MS. To overcome carry-over effects of astringent and bitter compounds, taste threshold concentrations of the individual compounds were determined in bottled water (pH 6.0) by means of the half-tongue test using the sip-and-spit method (Brock and Hofmann, 2008; Frank et al., 2007; Frank et al., 2008; Scharbert et al., 2004; Stark et al., 2005). The trained panel of twelve sensory subjects recognised an astringent mouthfeel as well as a clear bitter taste for each of the compounds evaluated. Depending on their chemical structure, the recognition thresholds ranged between 16 and 900 μmol/L for astringency and between 100 and 1800 μmol/l for bitter taste (Table 2). For each class of compounds, the modification of the di and trihydroxybenzenes with the different furan moieties induced a significant change in their taste threshold concentrations. With the

**Table 2**
Human taste threshold concentrations of di/trihydroxybenzenes and selected reaction products.

| Compound[a] | Threshold concentration [μmol/l][b] | |
|---|---|---|
| | Bitter | Astringent |
| Benzene-1,2-diol (catechol) | 1313 | 671 |
| 4-(Furan-2-ylmethyl)benzene-1,2-diol (**1**) | 537 | 134 |
| 3-((2-Furylmethyl)sulfanyl)benzene-1,2-diol (**11**) | 350 | n.d. |
| Benzene-1,3-diol (resorcinol) | 1800 | 900 |
| 4-(Furan-2-ylmethyl)benzene-1,3-diol (**2**) | 812 | 333 |
| 4,4′-(Furan-2-ylmethanediyl)dibenzene-1,3-diol (**7**) | 177 | 66 |
| 2-(Bis-(2,4-dihydroxyphenyl)methyl)-5-(2,4-dihydroxybenzyl) furan (**9**) | 340 | 110 |
| Benzene-1,2,3-triol (pyrogallol) | 297 | 78 |
| 4-(Furan-2-ylmethyl)benzene-1,2,3-triol (**3**) | 328 | 62 |
| 4,4′-(Furan-2-ylmethanediyl)dibenzene-1,2,3-triol (**8**) | 457 | 42 |
| 2-(Bis-(2,3,4-trihydroxyphenyl)methyl)-5-(2,3,4-trihydroxybenzyl) furan (**10**) | 455 | 16 |
| Benzene-1,2,4-triol (hydroxyhydroquinone) | 1336 | 74 |
| 5-(Furan-2-ylmethyl)benzene-1,2,4-triol (**4**) | 317 | 20 |
| 3-((2-Furylmethyl)sufanyl)benzene-1,2,4-triol (**12**) | 135 | n.d. |
| 4-Methylbenzene-1,2-diol (4-methylcatechol) | 540 | 363 |
| 4-(Furan-2-ylmethyl)-5-methylbenzene-1,2-diol (**5**) | 279 | 60 |
| 3-Methylbenzene-1,2-diol (3-methylcatechol) | 371 | 131 |
| 3-(Furan-2-ylmethyl)-6-methylbenzene-1,2-diol (**6**) | 100 | 79 |

[a] Compound numbering refers to the chemical structures given in Figs. 1–4.
[b] Taste threshold concentrations were determined by means of half-tongue test in bottled water (pH 6.0), n.d.: not determined.

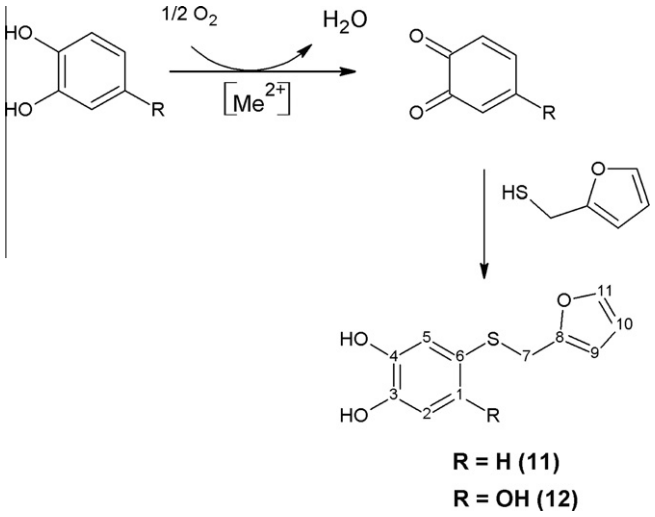



**Fig. 4.** Chemical structures and formation of the thio ethers **11** and **12**, previously identified in roasted coffee (17, 18).

exception of the pyrogallol derivatives (**3**, **8**, **10**), the various reaction products exhibited lower bitter taste threshold concentrations than the parent di and trihydroxybenzenes, e.g. the bitter taste threshold of resorcin decreased by a factor of 2.2 (**2**), 10.2 (**7**), and 5.3 (**9**) after reaction with furfuryl alcohol, furan-2-aldehyde, and 5-(hydroxymethyl)furan-2-aldehyde, respectively (Table 2).

Interestingly, the chemical structures of compound **1** and **4** shared the identical carbo skeleton with that of 3-((2-furylmethyl)sulfanyl)benzene-1,2-diol (**11**) and 3-((2-furylmethyl)sufanyl)benzene-1,2,4-triol (**12**), which were recently identified in coffee beverages and found to be formed upon oxidative coupling of catechol and hydroxyhydroquinone, respectively, with the odour-active 2-furfurylthiol as shown in Fig. 4 (Müller, Hemmersbach, Van't Slot, & Hofmann, 2006; Müller and Hofmann, 2007). This structural similarity prompted us to investigate the taste activity of the thio ethers **11** and **12** (Table 2). Compounds **11** and **12** showed bitter taste recognition thresholds of 350 and 135 μmol/l being rather close to the data found for **1** (537 μmol/l) and **4** (317 μmol/l), thus demonstrating that the additional sulphur atom in **11** and **12** does not significantly influence the bitter taste activity of that class of compounds.

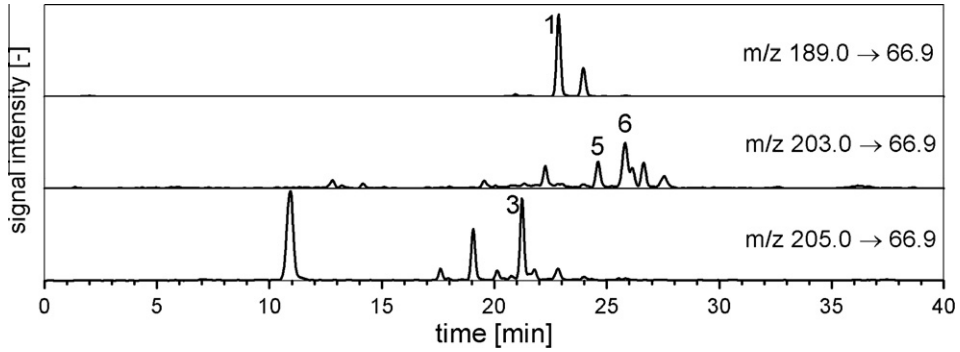
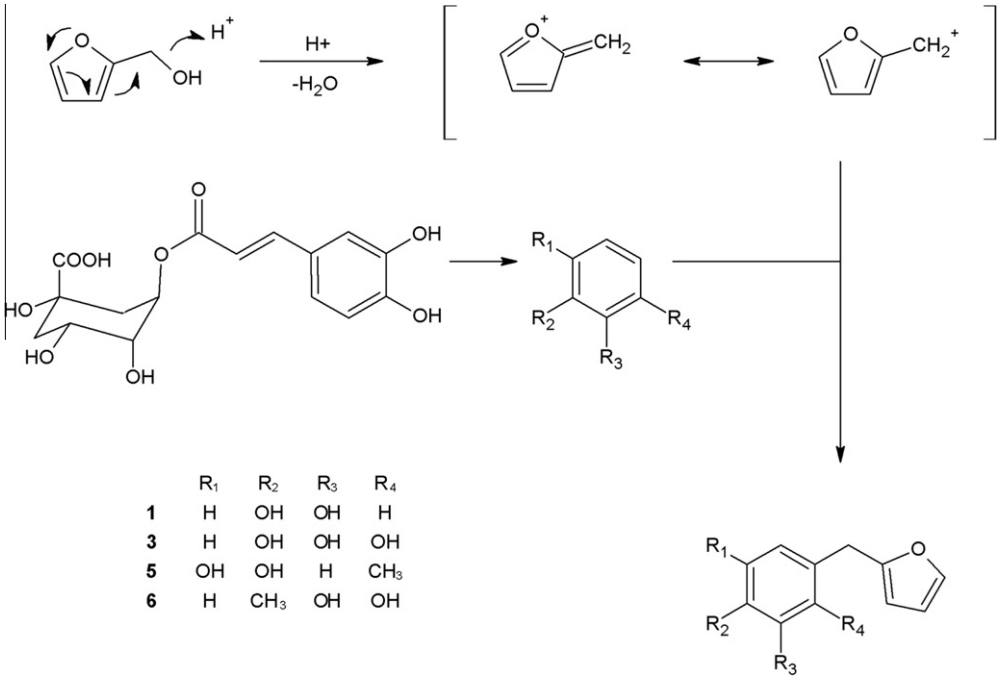



**Fig. 5.** HPLC–MS/MS chromatogram (MRM mode) of the analysis of (furan-2-yl)methylated benzene diols and triols **1**, **3**, **5**, and **6** in coffee brew.




| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| **1** | H | OH | OH | H |
| **3** | H | OH | OH | OH |
| **5** | OH | OH | H | $CH_3$ |
| **6** | H | $CH_3$ | OH | OH |

**Fig. 6.** Reaction mechanism leading to the formation of (furan-2-yl)methylated benzene diols and triols **1**, **3**, **5**, **6** from furfuryl alcohol and 5-*O*-chlorogenic acid upon coffee roasting.

### 3.7. Identification of bitter compounds in coffee brew

In order to verify the occurrence of the bitter tasting reaction products formed from di/trihydroxybenzenes and furan derivatives in coffee beverages, a beverage freshly prepared from medium roasted coffee beans was analysed for compounds **1**–**10** by means of HPLC–MS/MS ($ESI^-$) operating in the multiple reaction monitoring (MRM) mode. Prior to analysis, characteristic mass transitions and optimised instrument settings were selected for each individual compound in tuning runs.

Comparison of the retention time as well as the characteristic mass transitions of each target compound detected in coffee with those of the corresponding reference compound led to the unequivocal identification of the bitter tastants **1**, **3**, **5**, and **6** in the coffee sample (Fig. 5). In addition, the identity of these compounds in coffee was confirmed by means of co-chromatography with the corresponding reference compound isolated from the model reaction systems. Interestingly, all the compounds (**1**, **3**, **5**, **6**) detected in roasted coffee are generated upon reaction of di and trihydroxybenzenes with furfuryl alcohol, whereas the compounds **2**, **4**, and **7**–**10**, which are generated from furan-2-aldehyde and 5-(hydroxymethyl)furan-2-aldehyde, respectively, could not be detected in the coffee brew.

## 4. Conclusions

The data obtained in this investigation clearly demonstrate that, once formed upon coffee roasting by degradation of *O*-caffeoylquinic acids, the di and trihydroxybenzenes catechol, pyrogallol, 3-methylcatechol, and 4-methylcatechol do react with furfuryl alcohol to give (furan-2-yl)methylated benzene diols and triols as a novel class of bitter taste compounds in roasted coffee. On the basis of the model reactions performed, it can be concluded that acid-catalysed dehydration of furfuryl alcohol gives the furfuryl cation as a transient intermediate, which reacts via an electrophilic substitution with di and trihydroxybenzenes to yield the bitter tasting (furan-2-yl)methylated benzenes **1**, **3**, **5**, and **6** (Fig. 6). In contrast, the sulphur-containing bitter compounds **11** and **12**, earlier identified in roasted coffee (Müller, Hemmersbach, Van't Slot, & Hofmann, 2006; Müller and Hofmann, 2007) are generated upon oxidative coupling of the odour-active 2-furfurylthiol to the di and trihydroxybenzene catechol and hydrohydroquinone, respectively (Fig. 4).

To demonstrate the percent abundance of these four novel bitter taste compounds to the bitterness of coffee beverages, the development of a stable isotope dilution analysis for their accurate quantification is currently in progress.

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