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Structure Determination of 3-*O*-Caffeoyl-*epi*-γ-quinide, an Orphan Bitter Lactone in Roasted Coffee

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Recent investigations on the bitterness of coffee as well as 5-*O*-caffeoyl quinic acid roasting mixtures indicated the existence of another, yet unknown, bitter lactone besides the previously identified bitter compounds 5-*O*-caffeoyl-*muco*-γ-quinide, 3-*O*-caffeoyl-γ-quinide, 4-*O*-caffeoyl-*muco*-γ-quinide, 5-*O*-caffeoyl-*epi*-δ-quinide, and 4-*O*-caffeoyl-γ-quinide. In the present study, this orphan bitter lactone was isolated from the reaction products generated by dry heating of 5-*O*-caffeoylquinic acid model, and its structure was determined as the previously unreported 3-*O*-caffeoyl-*epi*-γ-quinide by means of liquid chromatography−mass spectrometry (LC-MS) and one-/two-dimensional NMR experiments. The occurrence of this bitter lactone, exhibiting a low bitter recognition threshold of 58 μmol/L, in coffee beverages could be confirmed by LC-MS/MS (negative electrospray ionization) operating in the multiple reaction monitoring mode.

KEYWORDS: Coffee; bitter taste; chlorogenic acid; 5-*O*-caffeoylquinic acid; lactones; caffeoyl quinides

INTRODUCTION

The secret to developing the attractive flavor of coffee is found in the roasting of the coffee beans, and the duration as well as the temperature of the roast are critical to develop a premium quality cup of coffee attracting the consumer by its desirable aroma and its well-balanced taste profile. Whereas sensory-based screening of odorous volatiles by means of gas chromatography/olfactometry followed by their quantitative analysis and aroma reconstitution/omission experiments have impressively demonstrated that not much more than 30 odorants are required to evoke the typical odor profile of a coffee beverage (*1*−*3*), the knowledge on the molecules imparting the typical bitter taste developed upon roasting of coffee beans is far from comprehensive.

First, systematic analytical and sensory studies revealed that not the alkaloids caffeine and trigonelline (*4*) but thermally generated compounds such as furfurylalcohol (*5*), 5-hydroxymethyl-2-furancarboxaldehyde (*6*), various pyrazines (*6*), and 2,5-diketopiperazines (*7*) are the key elicitors of coffee bitter taste. Very recently, sensory-guided deconstruction of a beverage prepared from medium roasted coffee, followed by liquid chromatography−tandem mass spectrometry (LC-MS/MS) and one- (1D)/two-dimensional (2D) nuclear magnetic resonance (NMR) spectroscopy, as well as synthesis revealed that the nonbitter 5-*O*-caffeoylquinic acid (**1**, chlorogenic acid) in the raw coffee bean is converted upon bean roasting into the five bitter-tasting chlorogenic acid lactones 5-*O*-caffeoyl-*muco*-γ-quinide (**2**), 3-*O*-caffeoyl-γ-quinide (**3**), 4-*O*-caffeoyl-*muco*-γ-quinide (**4**), 5-*O*-caffeoyl-*epi*-δ-quinide (**5**), and 4-*O*-caffeoyl-γ-quinide (**6**) involving transesterification, epimerization, and lactonization reactions (*8*) (**Figure 1**). Similarily, the bitter-tasting 3-*O*-feruloyl-γ-quinide and 4-*O*-feruloyl-γ-quinide were found to be generated from *O*-feruloylquinic acids, and the bitter-tasting 3,4-*O*-dicaffeoyl-γ-quinide, 3,5-*O*-dicaffeoyl-*epi*-δ-quinide, and 4,5-*O*-dicaffeoyl-*muco*-γ-quinide were found to be generated from the corresponding *O*-dicaffeoylquinic acids (*8*). Human sensory studies revealed that depending on their chemical structure, the bitter threshold levels of these lactones ranged between 9.8 and 180 μmol/L (*8*). In addition to these lactones, a group of lingering, harsh, bitter-tasting 4-vinylcatechol oligomers derived from the thermal breakdown of the caffeoyl moiety of *O*-caffeoylquinic acids and/or the corresponding quinides have been recently identified in coffee beverages (*9*).

As preliminary studies on coffee as well as 5-*O*-caffeoyl quinic acid roasting mixtures suggested the existence of another, yet unknown bitter *O*-caffeoylquinide, the objectives of the present study were to isolate and determine the chemical structure of this orphan lactone, to determine the bitter recognition threshold of that lactone, and to confirm the occurrence of

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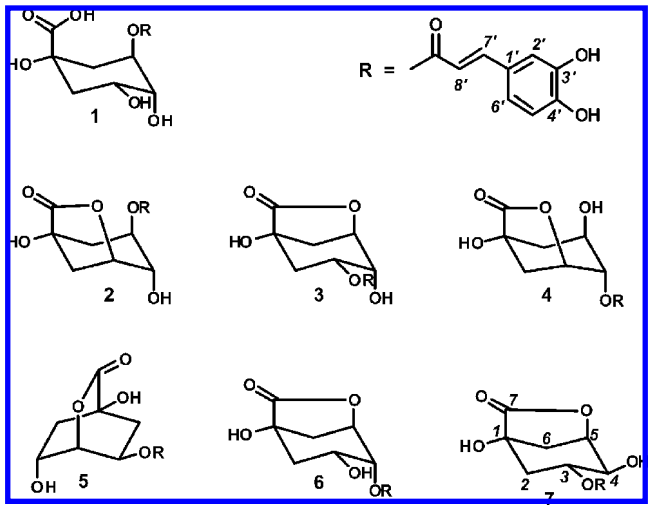

Figure 1. Chemical structures of the previously identified bitter lactones 5-*O*-caffeoyl-*muco*-γ-quinide (**2**), 3-*O*-caffeoyl-γ-quinide (**3**), 4-*O*-caffeoyl-*muco*-γ-quinide (**4**), 5-*O*-caffeoyl-*epi*-δ-quinide (**5**), and 4-*O*-caffeoyl-γ-quinide (**6**), as well as the novel 3-*O*-caffeoyl-*epi*-γ-quinide (**7**) formed upon thermal treatment (30 min, 230 °C) of 5-*O*-caffeoylquinic acid (**1**).

this compound in a coffee beverage by means of LC-MS/MS experiments.

MATERIALS AND METHODS

Chemicals. All of the chemicals were purchased from Sigma-Aldrich (Steinheim, Germany), solvents were obtained in high-performance liquid chromatography (HPLC)-grade from Merck (Darmstadt, Germany), and deuterated solvents were supplied by Euriso-top (Saarbrücken, Germany). Roasted coffee beans were obtained from the coffee industry. Reference compounds of 5-*O*-caffeoyl-*muco*-γ-quinide (**2**), 3-*O*-caffeoyl-γ-quinide (**3**), 4-*O*-caffeoyl-*muco*-γ-quinide (**4**), 5-*O*-caffeoyl-*epi*-δ-quinide (**5**), and 4-*O*-caffeoyl-γ-quinide (**6**) were prepared and purified following the procedure reported earlier (*8*).

Analytical Sensory Experiments. Twelve assessors (five male and seven female; ages 23−40 years), who had given their informed consent to participate in the sensory tests of the present investigation and had no history of known taste disorders, were trained in sensory experiments at regular intervals for at least 2 years as described earlier (*10*−*14*) and were, therefore, familiar with the techniques applied. Sensory analyses were performed in a sensory panel room at 22−25 °C in three different sessions. To prevent cross-modal interactions with odorants, the panelists used nose clips.

Precautions Taken for Sensory Analysis of Food Fractions and Taste Compounds. Prior to sensory analysis, solvent traces were removed from the freeze-dried fractions. To achieve this, the individual fractions were dissolved in water, and the remaining volatiles and solvent traces were removed under high vacuum (<5 mPa, 35 °C). The individual residue was then again taken up in water and freeze-dried twice. High-resolution gas chromatography-MS analysis and ion chromatographic analysis revealed that food fractions treated with that procedure were essentially free of the solvents and buffer compounds used.

Taste Recognition Threshold Concentrations. Threshold concentrations of purified bitter compounds were determined in bottled water adjusted to pH 5.2 with trace amounts of formic acid (1% in water) using triangle tests with ascending concentrations of the stimulus following the procedure reported previously (*10, 12*). The threshold values of the sensory group were approximated by averaging the threshold value of the individuals in three independent sessions. Values between individuals and separate sessions differed by not more than plus or minus one dilution step; that is, a threshold value of 0.5 mmol/L for caffeine represented a range from 0.25 to 1.0 mmol/L.

Model Roasting Experiments. 5-*O*-Caffeoylquinic acid (3.0 mmol) was suspended in water (2 mL), dried at 80 °C for 30 min, and was then dry-heated in a laboratory oven at 230 °C for 18, 24, 30, and 36 min, respectively. The reaction products were dissolved in water (40 mL; pH 5.2) at 80 °C, cooled to room temperature, and then split into two aliquots. One aliquot (20 mL) was used for sensory analysis, and the other aliquot (20 mL) was extracted five times with ethyl acetate (50 mL each). The combined organic layers were freed from solvent in high vacuum (<5 mPa), and the residue was taken up in a mixture of methanol/water (1/1, v/v; 6 mL) and separated by means of HPLC/diode array detection (DAD).

Isolation of a Novel Bitter Lactone 7. The solvent extractables, obtained from the roasting experiment detailed above, were separated by means of preparative HPLC using a 250 mm × 21.2 mm i.d., 5 μm, phenyl−hexyl column (Phenomenex, Aschaffenburg, Germany). Monitoring the effluent at 324 nm, chromatography was performed starting with a mixture (75/25, v/v) of aqueous formic acid (0.25 mol/L, pH 3.5) and methanol, thereafter increasing the methanol content to 28% within 38 min, then to 100% within 7 min, and finally maintaining the methanol content for additional 5 min. The effluents of the HPLC peaks 1−7 (**Figure 2**) were collected individually in several runs, and the isolates of the corresponding fractions were combined. After the solvent was removed in vacuum, each fraction was finally purified by means of solid phase extraction using C-18 E cartridges (10 g) (Phenomenex), which were preconditioned with acetonitrile (3 × 50 mL), followed by water (3 × 50 mL). After sample application, the cartridge was rinsed with water (50 mL), nitrogen was sucked through the cartridge for 15 min by means of a vacuum pump, and finally, the target compounds were eluted with acetonitrile (100 mL). After the solvent was removed in high vacuum (<5 mPa), the isolates of peaks 2−7 were studied by means of UV/vis, LC-MS/MS, and 1D/2D NMR experiments. The spectroscopic data of the compounds **2**−**6** were well in agreement with the data published previously for 5-*O*-caffeoyl-*muco*-γ-quinide (**2**), 3-*O*-caffeoyl-γ-quinide (**3**), 4-*O*-caffeoyl-*muco*-γ-quinide (**4**), 5-*O*-caffeoyl-*epi*-δ-quinide (**5**), and 4-*O*-caffeoyl-γ-quinide (**6**) (*8*), whereas compound **7** was identified as the previously unreported 3-*O*-caffeoyl-*epi*-γ-quinide.

3-*O*-Caffeoyl-*epi*-γ-quinide (the arbitrary numbering of the carbon atoms refers to structure **7** given in **Figure 1**): UV/vis (water/MeOH; 5/5, v/v): λ_{max} = 235, 300, 324 nm. LC-TOF-MS: *m/z* 335.2971 ([M − H]$^-$, measured), *m/z* 335.2976 ([M − H]$^-$, calcd for $C_{16}H_{16}O_8$). LC/MS (ESI$^-$): *m/z* 335.1 (100; [M − H]$^-$). ^{1}H NMR [400 MHz; MeOD-d_3; correlation spectroscopy (COSY)]: δ 1.87 [dd, 1H, J = 10.8, 11.8 Hz, H−C(2_{ax})], 2.22 [d, 1H, J = 11.7 Hz, H−C(6_{ax})], 2.36−2.44 [m, 1H, J = 3.4, 7.4, 11.8 Hz, H−C(2_{eq})], 2.48−2.56 [m, 1H, J = 3.4, 6.8, 11.7 Hz, H−C(6_{eq})], 3.94 [dd, 1H, J = 1.0, 8.3 Hz, H−C(4_{ax})], 4.71 [dd, 1H, J = 1.0, 6.8 Hz, H−C(5_{eq})], 4.88−4.96 [m, 1H, J = 7.4, 8.3, 10.8 Hz, H−C(3_{ax})], 6.30 [d, 1H, J = 15.9 Hz, H−C(8')], 6.80 [d, 1H, J = 8.2, H−C(5′)], 6.96 [dd, 1H, J = 1.9, 8.2 Hz, H−C(6′)], 7.06 [d, 1H, J = 1.9, H−C(2′)], 7.61 [d, 1H, J = 15.9, H−C(7′)]. ^{13}C NMR (100 MHz; MeOD-d_3; HMQC, HMBC): δ 37.1 [CH_2, C(2)], 40.1 [CH_2, C(6)], 68.3 [C, C(1)], 71.7 [CH, C(4)], 72.0 [CH, C(3)], 78.9 [CH, C(5)], 113.1 [CH, C(8′)], 113.6 [CH, C(2′)], 115.1 [CH, C(5′)], 121.6 [CH, C(6′)], 126.2 [C, C(1′)], 145.2 [C, C(3′)], 146.2 [CH, C(7′)], 148.2 [C, C(4′)], 166.9 [C, C(9′)], 177.8 [C, C(7)].

Preparation of the Coffee Beverage. After the coffee beans were ground by means of an ultracentrifuge mill (Retsch, Haan, Germany) equipped with a sieve (2 mm pore diameter), aliquots (54 g) of the coffee powder were placed in a coffee filter (Melitta, Minden, Germany) and percolated with hot water by using a "Melitta Look" coffee maker (Melitta) until the filtrate reached a volume of 1 L. The resulting beverage (54 g/L) had a temperature of 75 °C and was rapidly cooled in an ice bath prior to HPLC-MS/MS analysis.

HPLC. The HPLC apparatus (Jasco, Gross-Umstadt, Germany) consisted of two PU 2087 type pumps, a Rheodyne injector with a 100 or 1000 μL loop, and a MD 2010 plus type DAD, monitoring the effluent in a range between 220 and 500 nm. Separations were performed on a 250 mm × 4.6 mm i.d. or a 250 mm × 21.2 mm i.d., 5 μm, phenyl−hexyl column (Luna, Phenomenex) operated with a flow rate of 0.8 or 18.0 mL/min mL/min, respectively.

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

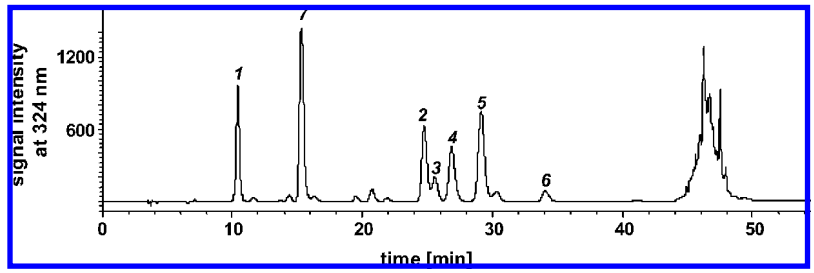

Figure 2. RP-HPLC chromatogram (λ = 324 nm) of the ethyl acetate extractables isolated from 5-*O*-caffeoylquinic acid dry-heated for 30 min at 230 °C.

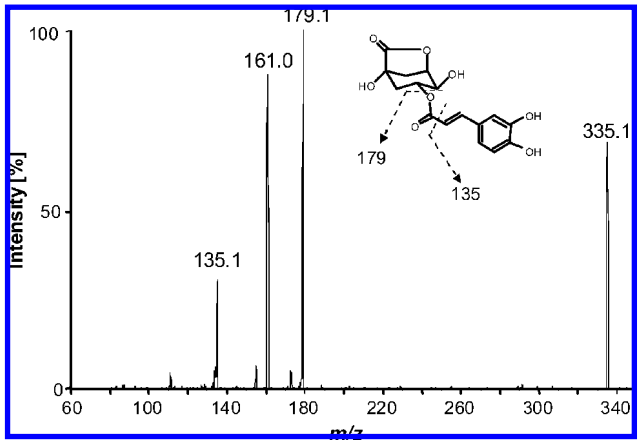

Figure 3. LC-MS/MS (ESI$^-$; collision energy, −50 V) spectrum of 3-*O*-caffeoyl-*epi*-γ-quinide (**7**).

Table 1. ^{1}H NMR Data (400 MHz) of 3-*O*-Caffeoyl-*epi*-γ-quinide (**7**) and *epi*-γ-Quinide

	3-*O*-caffeoyl-*epi*-γ-quinide (**7**)					*epi*-γ-quinide[c]	
H at relevant C atom[a]	M[c]	δ (ppm)[b]	*I*[c]	*J* (Hz)[c]		δ (ppm)[b]	*J* (Hz)[c]
H−C(2_{ax})	dd	1.87	1	10.8, 11.8		1.92	10.5, 12.0
H−C(6_{ax})	d	2.22	1	11.7		2.25	11.9
H−C(2_{eq})	m	2.40	1	3.4, 7.4, 11.8		2.27	3.5, 6.8, 12.0
H−C(6_{eq})	m	2.52	1	3.4, 6.8, 11.7		2.59	3.5, 6.8, 11.9
H−C(4_{ax})	dd	3.94	1	1.0, 8.3		3.74	1.1, 8.1
H−C(5_{eq})	dd	4.71	1	1.0, 6.8		4.81	1.1, 6.8
H−C(3_{ax})	m	4.92	1	7.4, 8.3, 10.8		3.69	6.8, 8.1, 10.5
H−C(8′)	d	6.30	1	15.9			
H−C(5′)	d	6.80	1	8.2			
H−C(6′)	dd	6.96	1	1.9, 8.2			
H−C(2′)	d	7.06	1	1.9			
H−C(7′)	d	7.61	1	15.9			

[a] IUPAC numbering of carbon atoms refers to structure **7** (**Figure 4**). [b] The ^{1}H chemical shifts are given in relation to methanol-d_4. [c] Determined from 1D spectrum. [d] NMR data for the *epi*-γ-quinide are taken from the literature (*15*).

HPLC/Tandem Mass Spectrometry (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) with an electrospray ionization (ESI) device running in negative ionization mode with a spray voltage of −4500 V. The quadrupoles operated at unit mass resolution. Nitrogen served as a curtain gas (20 psi), and the declustering potential was set at −10 to −85 V in the ESI$^-$ mode. The mass spectrometer was 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 (5×10^{-5} Torr) and a collision energy of −15 to −70 V. For instrumentation control and data aquisition, the Sciex Analyst software (v 1.4.2) was used.

For HPLC-MS/MS analysis of lactone **7** in a coffee beverage, a 150 mm × 2 mm i.d., 4 μm, Synergi Fusion column (Phenomenex) was connected to the mass spectrometer operating in the multiple reaction monitoring mode (MRM) detecting negative ions. For a duration of 150 ms, the mass transitions *m*/*z* 335→133, *m*/*z* 335→135, and 335→161 were used for the analysis of the lactone. Nitrogen served as the nebulizer gas (30 psi) and as the turbo gas (350 °C) for solvent drying (50 psi). After injection of the sample (5 μL), chromatography was performed with a flow rate of 250 μL/min using a gradient starting with a mixture (75/25, v/v) of aqueous formic acid (1% in water) and methanol and increasing the methanol content to 40% within 10 min, then to 60% within 15 min, and finally to 100% within 13 min, thereafter maintaining the methanol content for additional 7 min.

NMR Spectroscopy. 1D and 2D NMR experiments were performed on a Bruker DMX-400 (Bruker, Rheinstetten, Germany). Chemical shifts were determined using tetramethylsilane (TMS) as the internal standard in the proton dimension and from the carbon signal of MeOD-d_3 (49.3 ppm) in the carbon dimension.

RESULTS AND DISCUSSION

Recent investigations on coffee as well as 5-*O*-caffeoyl quinic acid roasting mixtures indicated the existence of another, yet unknown dehydration product of *O*-caffeoylquinic acid besides the previously reported bitter lactones **2**−**6** (*8*). To identify this orphan compound, 5-*O*-caffeoylquinic acid was dry-heated at 230 °C for 18, 24, 30, and 36 min, respectively, and the reaction products were taken up in water and then extracted the ethyl acetate. HPLC/DAD analysis of the organic extract showed a time-dependent generation of the orphan compound **7** eluting between the 5-*O*-chlorogenic acid and the peak pattern of the five previously reported lactones assigned as 5-*O*-caffeoyl-*muco*-γ-quinide (**2**), 3-*O*-caffeoyl-γ-quinide (**3**), 4-*O*-caffeoyl-*muco*-γ-quinide (**4**), 5-*O*-caffeoyl-*epi*-δ-quinide (**5**), and 4-*O*-caffeoyl-γ-quinide (**6**) upon comparison of the chromatographic (RP-HPLC) and spectroscopic data (LC-MS, UV/vis) with those of the reference compounds (*8*) (**Figure 2**). As the highest yields of compound **7** were found when 5-*O*-caffeoylquinic acid was heated for 30 min at 230 °C, this reaction mixture was used for the following structure determination experiments. Compound **7** was isolated from the ethyl acetate extract by means of preparative RP-HPLC, followed by a final purification by means of solid phase extraction on RP18 cartridges. Sensory analysis of the purified compound **7** in aqueous solution revealed an intense bitter taste, similar to that reported for the lactones **2**−**6** (*8*).

Structure Determination of Bitter Lactone 7. UV/vis spectroscopy of a solution of compound **7** showed the same absorption maxima at 235, 300, and 324 nm as found for the previously reported *O*-caffeoylquinides **2**−**6** (**Figure 1**). In addition, LC-MS analysis revealed an intense pseudo molecular ion [M − H]$^-$ with *m*/*z* 335, which is well in the line with a caffeoylquinide formed by the loss of one molecule of water from chlorogenic acid. LC-MS/MS experiments in the ESI$^-$ mode showed the daughter ions *m*/*z* 179.1, 161.0, and 135.1, being well in line with the cleavage of a molecule of caffeic

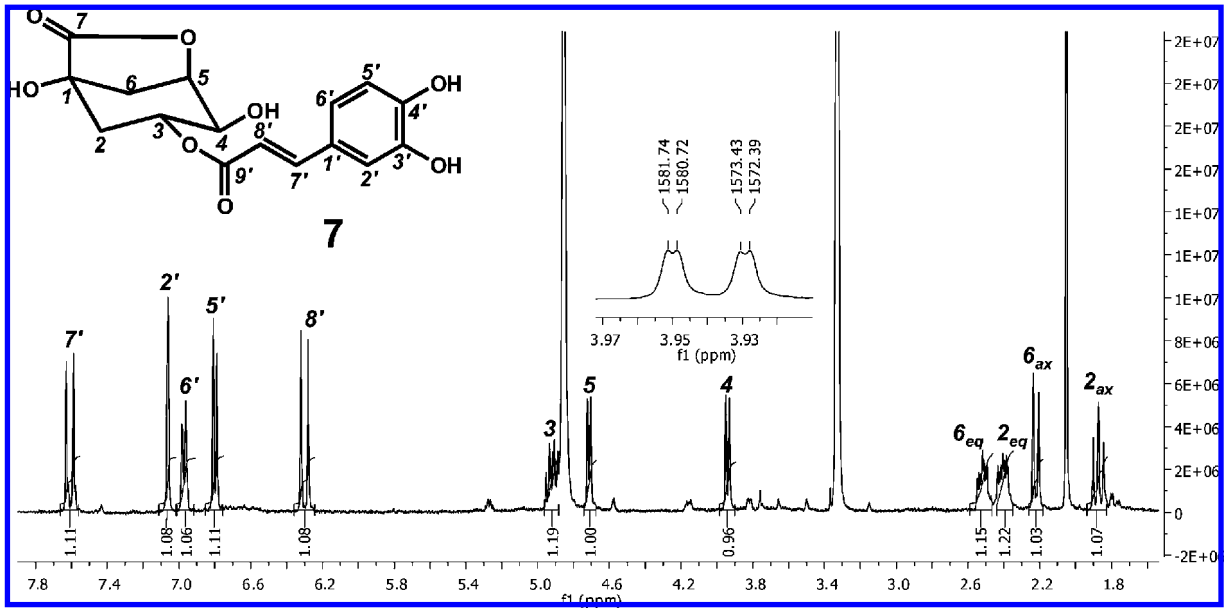

Figure 4. ^{1}H NMR spectrum (400 MHz, MeOD-d_4) of 3-*O*-caffeoyl-*epi*-γ-quinide (**7**).

Table 2. Human Bitter Recognition Thresholds of *O*-Caffeoylquinides

compound (no.)[a]	bitter threshold concentration[b] μmol/L	mg/L
5-*O*-caffeoyl-*muco*-γ-quinide (**2**)[c]	29.0	9.7
4-*O*-caffeoyl-*muco*-γ-quinide (**4**)[c]	30.0	11.2
4-*O*-caffeoyl-γ-quinide (**6**)[c]	36.0	12.1
3-*O*-caffeoyl-γ-quinide (**3**)[c]	40.0	13.4
3-*O*-caffeoyl-*epi*-γ-quinide (**7**)	58.0	19.5
5-*O*-caffeoyl-*epi*-δ-quinide (**5**)[c]	180.0	60.5

[a] The structures of the numbered compounds are given in **Figure 1**. [b] Taste threshold concentrations were determined by means of a triangle test in bottled water (pH 5.6). [c] Bitter threshold concentrations published recently (*8*).

acid and vinylcatechol, respectively (**Figure 3**). Comparison of the LC-MS/MS data of **7** with those reported recently for 3-*O*-caffeoyl-γ-quinide (*8*) revealed the same fragmentation pattern, thus giving further evidence that compound **7** is formed upon lactonization from chlorogenic acid. Analysis of the ^{1}H NMR spectroscopic data showed a total of 12 resonance signals, each integrating for one proton (**Table 1** and **Figure 4**). The signals in the aromatic/olefinic region between 6.2 and 7.7 ppm showed the typical coupling pattern of a caffeoyl acid moiety with two olefinic protons H−C(8′) and H−C(7′) at 6.30 and 7.61 ppm, respectively, exhibiting the expected coupling constant of 15.9 Hz indicating an (*E*)-configuration of the caffeic acid residue in compound **7**. In addition, the protons resonating at 6.30, 6.96, and 7.06 ppm showing coupling constants of 8.2, 8.2/1.9, and 1.9 Hz, respectively, could be assigned as H−C(5′), H−C(6′), and H−C(2′). Homonuclear (*H*,*H*) correlated *gs*-COSY spectroscopy revealed a strong coupling between the methine proton resonating at 3.94 ppm and the H−C(3) or H−C(5), respectively. This methine proton could be assigned as H−C(4) since only this proton is able to show homonuclear couplings with H−C(3) and H−C(5) but not to H−C(2) and H−C(6), respectively. This proposal was further strengthened by the observation that H−C(3) showed connectivities to H−C(2) and H−C(4), whereas proton H−C(5) is connected to the methylene group H−C(6) and the methine proton H−C(4). The ^3J coupling constant of 8.3 Hz measured between H−C(3) and H−C(4) undoubtedly identified an axial−axial conformation of the respective protons, whereas the coupling constant of 1.0 Hz observed between the protons H−C(4) and H−C(5) indicated

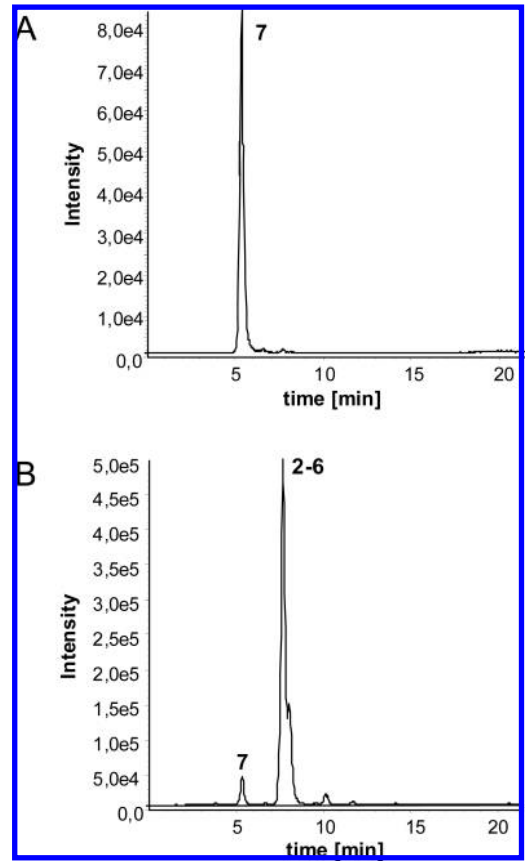

Figure 5. HPLC-MS/MS analysis (ESI$^-$; MRM, *m*/*z* 335→161) of an aqueous solution of the purified compound **7** (**A**) and of a freshly prepared coffee brew (**B**).

an axial−equatorial orientation of these protons in the proposed structure of lactone **2** (**Figure 1**). All of these findings were well in line with data reported for *epi*-γ-quinide (*15*) with the exception of the downfield shift of H−C(4), which is most likely due to the esterification with caffeic acid (**Table 1**). This assumption was further strengthened by heteronuclear multiple bond correlation spectroscopy (HMBC) optimized for $^2J_{C,H}$ and $^3J_{C,H}$ coupling constants and heteronuclear multiple quantum correlation (HMQC) optimized for $^1J_{C,H}$ coupling constants,

respectively. The HMBC experiment revealed correlations between the carbonyl group resonating at 166.9 ppm and the protons H−C(3), H−C(7′), and H−C(8′) and confirmed the connection of the caffeoyl moiety via an ester bond to carbon atom C(3) of the quinide residue. HMBC correlations of H−C(5), H−C(2), and H−C(6) with the carbon signal at 177.8 ppm were well in the line with the proposed lactone structure since a ^3J coupling between H−C(5) and C(7) is only possible after lactonization. Taking all of the spectroscopic data into consideration, the bitter compound isolated from HPLC fraction 7 (**Figure 2**) could be unequivocally identified as the previously not reported 3-caffeoyl-*epi*-γ-quinide (**7**, **Figure 1**).

Bitter Recognition Threshold Concentrations. Prior to sensory analysis, the purity of lactone **7** was checked by ^{1}H NMR spectroscopy as well as HPLC-MS. To determine the human threshold concentrations for bitter taste, aqueous solutions (pH 5.2) of lactone **2** were evaluated by means of a triangle test (**Table 2**). The bitter threshold concentration of the novel 3-caffeoyl-*epi*-γ-quinide (**2**) was determined to be 58 μmol/L, being in the same range as found for the other γ-quinides **2**, **3**, **4**, and **6**, and was three times below the threshold found for the 5-*O*-caffeoyl-*epi*-δ-quinide (**Table 2**).

Identification of the Bitter Lactone 7 in Coffee Brew. To verify the occurrence of the 3-caffeoyl-*epi*-γ-quinide in coffee beverages, a freshly prepared coffee brew was screened for compound **7** by means of LC-MS/MS (ESI$^-$) operating in the MRM mode. Prior to analysis, characteristic mass transitions were selected for the taste compound in tuning runs. Thereafter, the retention time as well as the characteristic mass transitions of compound **7** in coffee were compared to those of the reference compound. As shown in **Figure 5** for the mass transition *m*/*z* 335→161, the target compound **7** could be unequivocally detected in coffee brew by means of HPLC-MS/MS (MRM). Under the chromatographic conditions selected, the other lactones **2**−**6** were not resolved and coeluted after 8 min. In addition, the identity of the bitter tastant **7** in coffee was confirmed by means of cochromatography with the corresponding reference compound isolated from the roasted 5-*O*-caffeoylquinic acid model system. To the best of our knowledge, the bitter lactone **7**, which could be detected in various Arabica as well as in Robusta coffees (data not shown), has not been previously reported in the literature.

To demonstrate the contribution of the six monocaffeoylquinides **2**−**7** to the overall bitterness of coffee beverages, quantitative studies, followed by taste reconstruction experiments using these tastants in their "natural" concentrations, are currently in progress and will be published elsewhere.

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