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A New Method for the Preparative Isolation of Chlorogenic Acid Lactones from Coffee and Model Roasts of 5-Caffeoylquinic Acid

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Supporting Information

ABSTRACT: Chlorogenic acid lactones (CQL) are known to contribute to the bitter taste of roasted coffee. CQL might also have beneficial biological activities. Until now, there is a lack of pure standard compounds for quantification and biological testing. Using high-speed countercurrent chromatography, milligram amounts of lactones could be isolated. The structures of 3-*O*-caffeoyl-γ-quinide, 4-*O*-caffeoyl-muco-γ-quinide, and 5-*O*-caffeoyl-epi-δ-quinide were confirmed by 1D and 2D NMR spectroscopy including ^{13}C NMR data, which were previously not available from the literature. An UHPLC method was developed that enabled the separation of the lactones from roasted coffee in significantly shorter time than conventional HPLC.

KEYWORDS: *chlorogenic acid lactones, high-speed countercurrent chromatography, NMR, LC-MS, UHPLC*

INTRODUCTION

Chlorogenic acids (CGA) are among the most abundant constituents of green coffee. More than 60 chlorogenic acid-like compounds, including caffeoyl- (CQA), feruloyl- (FQA), and *p*-coumaroylquinic acids (CouQA), dicaffeoyl- and feruloylquinic acids, caffeoyl-feruloylquinic acids, and derivatives have been identified. Quantitative analysis of CGA is still particularly problematic because there is a lack of reference compounds of the isomers in suitable quality. Therefore, in most papers quantitative data are calculated as 5-CQA. For the isolation of chlorogenic acids countercurrent chromatography has already been used.[1] During the roasting process the chlorogenic acid fraction follows different reaction pathways such as epimerization, acyl migration, and dehydration.[2] Lactones are formed within the quinic acid moiety, yielding lactones, for example, caffeoyl- and feruloylquinides.

In addition to their bitter taste, chlorogenic lactones have shown a range of bioactivities in animal studies such as enhancing the insulin action in rats and interacting with the opioid receptor in mice.[3−5] Using GC-MS of the trimethylsilyl derivatives, four caffeoylquinic acid lactones and later feruloylquinides were identified.[6,7] Only mass spectroscopic data were available that time. Bennat et al.[8] employed LC-MS and identified the 3- and 4-caffeoylquinic acid lactones in coffee and published ^{1}H NMR data. In the following years other NMR data were published.[9,10] No ^{13}C NMR data for the isolated lactones were available until Frank et al.[11] gave data for 3-*O*-caffeoyl-epi-γ-lactone.

Quantitative data for 3- and 4-CQL in commercial coffee samples are still scarce. Bennat et al.[8] determined 3- and 4-CQL in a few commercial coffee samples (n = 5). The content of 3-CQL was 0.78−1.86 g/kg (average = 1.24 g/kg), whereas 4-CQL was a bit lower (0.77−1.58, average = 1.06 g/kg). In medium-roasted coffees 3-CQL was on average 2.13 g/kg (1.72−2.41, n = 12); 4-CQL, 0.96 g/kg (0.74−1.10 g/kg); 3-CQA, 5.01 g/kg (4.75−5.94 g/kg, n = 12); 4-CQA, 6.20 g/kg (5.49−7.31, n = 12); and 5-CQA, 11.40 g/kg (9.96−12.84 g/kg, n = 12).[12] These data are very similar to those of Farah et al.[13] In the latter paper also data are given for 3- and 4-FQL (amounts also roughly 2:1 and 10% of CQL content) and a 3,4-di-CQL. Surprisingly, 3,4-di-CQL was found in green coffee. Alves et al.[14] gave data (peak areas only) for lactones in espresso coffee. Chu et al.[15] determined 3-CQL (3.71 g/kg and nd), 4-CQL (nd and 11.34 g/kg), and a 5-CQL (3.71 and 10.87 g/kg, respectively) in two coffee samples.

The aim of this work was a fast isolation of milligram amounts of pure individual chlorogenic acid lactones (CQL) in high purity for use as calibration compounds and biological tests. Moreover, a full set of NMR data should be provided as currently no ^{13}C NMR data are available for most of the lactones.

MATERIALS AND METHODS

Chemicals. Chemical were obtained from the suppliers provided in parentheses: acetonitrile (ACN), HPLC gradient grade (Sigma-Aldrich, Steinheim, Germany); acetonitrile, LC-MS grade (Honeywell, Seelze, Germany); acetic acid, HPLC grade (Fisher Scientific, Loughborough, UK); acetic acid, LC-MS grade (Sigma-Aldrich); 5-caffeoylquinic acid (5-CQA), ≥95% (Sigma-Aldrich); ethyl acetate, p.a. (Carl Roth, Karlsruhe, Germany); formic acid, 99−100% p.a. (VWR, Leuven, Belgium); methanol, HPLC gradient grade (VWR); methyl *tert*-butyl ether (MTBE), for synthesis (Carl Roth); water, suitable for HPLC (Werner, Reinstwassersysteme, Leverkusen, Germany); MeOH-d_4 (Deutero, Kastellaun, Germany).

Roasting of Coffee Beans. An amount of 100 g of a green decaffeinated Vietnamese robusta coffee was roasted in a hot-air-stream roaster (model Nescafé Rohkaffeeröster, Siemens Elektrogeräte GmbH Typ TR 1000; 220 V, 1200 W) for 7 min (maximum temperature = 230 °C). Decaffeinated coffee was used because it is easier to handle in the CCC. Otherwise, there would be a high

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concentration of caffeine in some of the fractions. The dry matter of the green beans and the roasted coffee were determined, and the roasting loss and the organic roasting loss were calculated.[16] The water content of the green coffee beans was 7.04% and that of the roasted coffee, 1.55%. Total roasting loss was 11.19%, and the organic roasting loss, 4.15%.

Roasting of 5-CQA. Roasting of 5-CQA was carried out as described in a previous paper.[17] Briefly, sea sand (50 g) and 2 g of 5-CQA were mixed and roasted in a 300 mL Erlenmeyer flask at 290 °C for 7 min.

Cleanup of Roasted Coffee. *Extraction.* An amount of 125 g of the roasted and ground coffee was extracted with 1 L of boiling water and filtered. After cooling, the brew was filtered again and lyophilized.

Column Chromatography. A column (840 × 60 mm i.d.) was filled with Amberlite XAD-7 (bed height = 520 mm). After equilibration with water, 60 g of the above lyophilisate (in 500 mL water) was loaded on the column and washed with water (5 L), followed by elution with methanol (2.5 L). The methanol solution was concentrated on a rotary evaporator at 40 °C and lyophilized.

Cleanup of Model Roasts. *Extraction.* The roasted 5-CQA/sand mixture was extracted with 200 mL of boiling water. After cooling, the solution was filtered again and lyophilized.

Column Chromatography. A column (700 × 35 mm i.d.) was filled with polyamide (bed height = 600 mm, bed volume approximately 0.6 L) and equilibrated with water. The extract was loaded on the column, washed with water (5 L), and eluted with methanol (2.5 L). The methanol solution was concentrated on a rotary evaporator at 40 °C and lyophilized.

High-Speed Countercurrent Chromatography. A HSCCC model CCC-1000 (Pharmatech Research Corp., Baltimore, MD, USA) with a total coil volume of 850 mL was used (tube diameter = 2.5 mm). A system Gold Pump 116 and UV detector 166 (Beckman Instruments) were connected with the HSCCC. The separation was carried out at 900 rpm with a flow rate of 4 mL/min using a two-phase solvent system consisting of MTBE/ACN/H_2O (3:1:3, v/v/v).[17] The effluent was monitored at 324 nm. The elution mode was head-to-tail, with upper phase as stationary phase. Sample introduction was done by a manual Rheodyne valve equipped with a 30 mL loop. The sample was dissolved in a 1:1 mixture of upper and lower phases. Fractions were collected in steps of 3 min using a 2111 MULTIRAC fraction collector (LKB Bromma, Sweden).

Roasted Coffee. For HSCCC separations the lyophilized methanol eluate from XAD-7 column was taken.

Model Roasts. The lyophilized methanol eluate from polyamide column was used for HSCCC.

Analytical High-Performance Liquid Chromatography (HPLC). The apparatus used was an Agilent 1100/1200 system (Agilent, Waldbronn, Germany) consisting of a binary pump, thermostated autosampler (injection volume = 10 μL), column oven (set = 20 °C), and a DAD detector (set = 324 and 280 nm, spectra from 200 to 400 nm). The column was a Zorbax Eclipse XDB-C18, 150 mm × 2.1 mm, 3.5 μm (Agilent). Eluent A was 2% (aq) acetic acid and eluent B, acetonitrile. Flow rate was 0.25 mL/min. The gradient was as follows: 5% B for 9 min, ramped in 3 min to 12% B, ramped in 38 min to 25% B, and isocratic elution for 15 min.

Semipreparative HPLC. The apparatus consisted of a System Gold programmable solvent module 126 and a 168 DAD detector (Beckman) set at 324 nm. The injector was a Rheodyne 7125 (loop volume = 100 μL). Eluent was 0.1% (aq) formic acid/acetonitrile (8:2, v/v). The flow rate was 5 mL/min. The column was a Zorbax Eclipse XDB-C18, Custom HT, 250 mm × 21.2 mm i.d., 5 μm (Agilent).

This system was used to purify 3-γ-CQL and 5-epi-δ-CQL from HSCCC fractions.

Analytical Ultrahigh-Performance Liquid Chromatography (UHPLC). The apparatus used was an Agilent 1290 Infinity LC system equipped with an autosampler (injection volume = 2 μL), a column oven (set = 30 °C), and a DAD detector (set = 324 nm, spectra from 190 to 400 nm). The column was a Zorbax Eclipse Plus-C18, 50 mm × 2.1 mm, 1.8 μm (Agilent). The flow rate was 0.25 mL/min. Eluent A was 1% (aq) phosphoric acid and eluent B, acetonitrile. The gradient used was as follows: 5% B for 3 min, ramped in 1 min to 9% B, ramped in 12.7 min to 25% B, isocratic elution for 8.3 min.

High-Performance Liquid Chromatography−Electrospray Ionization Mass Spectrometry (HPLC-ESI-MS). An Agilent 1100/1200 system consisting of a binary pump, thermostated autosampler (injection volume = 2−5 μL), and a DAD detector (set = 324 and 280 nm, spectra from 200 to 400 nm) was connected to a ion-trap mass spectrometer via an ESI source (Bruker Daltonics HCT Ultra, Bremen, Germany), operating in scan mode from m/z 100 to 1000 and auto MS^n mode. The MS operating conditions were as follows: negative ion mode; capillary voltage, −3500 V; nebulizer, 60 psi; dry gas flow rate, 11 L/min; and dry temperature, 365 °C.

For LC conditions, see Analytical HPLC.

Nuclear Magnetic Resonance Spectrometry (NMR). 1- and 2-dimensional NMR experiments were performed on a Bruker Avance II-600 or a Bruker Avance III-400 at 25 °C in methanol-d_4. Chemical shifts are given in parts per million (δ) relative to the residual proton signals of the solvent (MeOH, δ_H 3.30 and δ_C 49.0) and coupling constants (J) are given in hertz (Hz).

3-O-Caffeoyl-γ-quinide. HPLC-ESI-MS^n, m/z **335.0** $[M - H]^-$, 160.9, 133.0; ^{1}H NMR [600 MHz, MeOH-d_4; correlation spectroscopy (HSQC, HMBC, COSY, NOESY)] δ 2.08 [t, 1H, J = 11.7, H−C(2_{ax})], δ 2.16 [ddd, 1H, J = 11.4, 6.6, 2.7, H−C(2_{eq})], δ 2.3 [ddd, 1H, J = 10.1, 6.0, 2.8, H−C(6_{eq})], δ 2.56 [d, 1H, J = 11.6, H−C(6_{ax})], δ 4.29 [t, 1H, J = 4.6, H−C(4_{eq})], δ 4.74 [t, 1H, J = 5.5, H−C(5_{eq})], δ 4.91 [ddd, 1H, J = 11.7, 6.9, 4.5, H−C(3_{ax})], δ 6.20 [d, 1H, J = 15.9, H−C(8′)], δ 6.68 [d, 1H, J = 8.2, H−C(5′)], δ 6.86 [dd, 1H, J = 2.0, 8.3, H−C(6′)], δ 6.95 [d, 1H, J = 2.0, H−C(2′)], δ 7.52 [d, 1-H, J = 15.9, H−C(7′)]; ^{13}C NMR [150 MHz, MeOH-d_4; correlation spectroscopy (HSQC, HMBC)] δ 36.9 [CH_2, C(2)], δ 37.8 [CH_2, C(6)], δ 64.9 [CH, C(4)], δ 70.2 [CH, C(3)], δ 73.1 [C, C(1)], δ 77.7 [CH, C(5)], δ 114.7 [CH, C(8′)], δ 115.2 [CH, C(2′)], δ 116.5 [CH, C(5′)], δ 123.1 [CH, C(6′)], δ 127.7 [C, C(1′)], δ 146.9 [C, C(3′)], δ 147.6 [CH, C(7′)], δ 149.8 [C, C(4′)], δ 168 [C, C(9′)], δ 178.9 [C, C(7)].

5-Caffeoyl-epi-δ-quinide. (NMR data of 5-O-caffeoyl-epi-δ-quinide and 4-O-caffeoyl-muco-γ-quinide were assigned in a mixture of both compounds.) HPLC-ESI-MS^n, m/z **335.0** $[M - H]^-$, 179.1, 134.9; ^{1}H NMR [400 MHz, MeOH-d_4; correlation spectroscopy (HSQC, HMBC, COSY, NOESY)] δ 1.78 [m, 1H, H−C(2_{eq})], δ 1.81 [m, 1H, H−C(6_{eq})], δ 2.39 [d, 1H, J = 11.9, H−C(6_{ax})], δ 2.51 [dd, 1H, J = 13.7, 9.4, H−C(2_{ax})], δ 4.48 [ddd, 1H, J = 9.4, 2.9, 1.7, H−C($_{3eq}$)], δ 4.66 [dd, 1H, J = 4.4, 1.6, H−C(4_{eq})], δ 5.20 [dt, 1H, J = 10.1, 5.7, 4.3, H−C(5_{ax})], δ 6.28 [d, 1H, J = 15.9, H−C(8′)] δ 6.78 [d, 1H, J = 8.2, H−C(5′)], δ 6.97 [dd, 1H, J = 8.2, 1.9, H−C(6′)], δ 7.06 [d, 1H, J = 1.8, H−C(2′)], δ 7.60 [d, 1H, J = 15.9, H−C(7′)]; ^{13}C NMR [100 MHz, MeOH-d_4; correlation spectroscopy (HSQC, HMBC)] δ 38.0 [CH_2, C(6)], δ 42.8 [CH_2, C(2)], δ 64.3 [CH, C(3)], δ 67.2 [CH, C(5)], δ 70.0 [C, C(1)], δ 80.0 [CH, C(4)], δ 114.4 [CH, C(8′)], δ 115.3 [CH, C(2′)], δ 116.5 [CH, C(5′)], δ 123.3 [CH, C(6′)], δ 127.5 [C, C(1′)], δ 146.9 [C, C(3′)], δ 148.2 [CH, C(7′)], δ 149.9 [C, C(4′)], δ 167.7 [C, C(9′)], δ 177.5 [C, C(7)].

4-Caffeoyl-muco-γ-quinide. (NMR data of 5-O-Caffeoyl-epi-δ-quinide and 4-O-caffeoyl-muco-γ-quinide were assigned in a mixture of both compounds.) HPLC-ESI-MS^n m/z **335.0** $[M - H]^-$; ^{1}H NMR [400 MHz, MeOH-d_4; correlation spectroscopy (HSQC, HMBC, COSY, NOESY)] δ 2.05−2.45 [m, 4H, H−C($2_{eq/ax}$), H−C($6_{eq/ax}$)], δ 4.13 [dd, 1H, J = 1.3, 5.2, H−C(5_{eq})], δ 5.08 [d, 1H, J = 3.9, H−C(4_{eq})], δ 6.30 [d, 1H, J = 15.8, H−C(8′)], δ 6.79 [d, 1H, J = 8.2, H−C(5′)], δ 7.02 [dd, 1H, H−C(6′)] (coupling constants could not be estimated because of overlapping signals from 5-epi-δ-CQL; δ 7.06 [d, 1H, H−C(2′)] (coupling constants could not be estimated because of overlapping signals from 5-epi-δ-CQL, δ 7.61 [d, 1H, J = 15.88 Hz, H−C(7′); ^{13}C NMR [100 MHz, MeOH-d_4; correlation spectroscopy (HSQC, HMBC)] δ 39.7 [CH_2, C(6)], δ 42.5 [CH_2, C(2)], δ 69.5 [CH, C(4)], δ 70.5 [CH, C(4)], δ 75.5 [CH, C(3)], δ 114.1 [CH, C(8′)], δ 115.3 [CH, C(2′)], δ 116.5 [CH, C(5′)], δ 123.1 [CH, C(6′)], δ 148 [CH, C(7′)]. ^{1}H NMR and MS^n spectra for all isolated quinides, see Figures 4−8 and Table 1 in the Supporting Information).

■ RESULTS AND DISCUSSION

Optimization of Roasting Conditions. First, the conditions for the model roast and green coffee had to be optimized by variation of roasting time and temperature to get the highest yield of CQL. Analysis was carried out by HPLC-DAD. Optimum conditions for coffee were 230 °C and 7 min. In the model roasts of 5-CQA, 290 °C and 7 min gave the best results.

HSCCC Separation. Prior to HSCCC XAD-7 column chromatography proved to be necessary for roasted coffee to purify and concentrate the lactones. For model roasts of 5-CQA polyamide column chromatography was also possible. For the HSCCC fractionation a suitable solvent system had to be developed by determination of the partition coefficients of the target compounds. According to Ito,[18] the coefficients should be between 0.5 and 1. Most of the chlorogenic acid lactones have partition coefficients between 0.5 and 0.99 for the elution mode head-to-tail in the two-phase solvent system MTBE/ACN/H_2O (3:1:3, v/v/v). This solvent system contains no acid, and hence there is no risk of acid-catalyzed hydrolysis.

It was possible to separate the chlorogenic acid lactones by HSCCC from roasted coffee after cleanup and concentration by XAD-7 into four fractions in the elution mode and two fractions in the extrusion mode (Figure 1 in the Supporting Information). The fractions were characterized by analytical HPLC and HPLC-ESI-MS.

Elution Mode. The fractions obtained from two runs (2 g of prepurified coffee in total) were pooled. All fractions contained CQL: Fraction 1 yielded 37 mg of a CQL (molecular ion $[M - H]^-$ m/z 335, purity 65%); fraction 2, 54 mg (purity 85%); fraction 3, 128 mg (purity 70%); fraction 4, 46 mg (purity 60%). Purity was determined by HPLC-DAD (λ = 324 and 280 nm).

Extrusion Mode. The pooled fractions from the extrusion mode yielded fraction 5 with 128 mg of a CQL in a purity of 80% and fraction 6 with a feruloylquinide (molecular ion $[M - H]^-$ m/z 349, purity 72%). Fraction 5 was further purified by semipreparative HPLC, and the chromatographic purity was then enhanced up to 96%.

Model Roast of 5-CQA. The HSCCC separation (Figure 2 in the Supporting Information) provided three fractions in the elution mode. Fraction 1 was unchanged 5-CQA (purity 98%), fraction 2 contained 109 mg of a CQL mixture, and fraction 3 gave 53 mg of a CQL (purity 87%). The CQL in fraction 3 was further purified by semipreparative HPLC.

In the extrusion mode another two fractions were obtained. Fraction 4 is a mixture of different CQLs (108 mg), whereas fraction 5 (62 mg) was tentatively identified as 3,5-diCQL (molecular ion $[M - H]^-$ m/z 497, purity 83%).

The fractions of the HSCCC separations from coffee extract (63 mg) and model roasts (210 mg) with nonresolved lactones were pooled. Those fractions were characterized by LC-MS/MS and UHPLC-DAD. In Figures 1 and 2 the separation of a quinide fraction by HPLC and UHPLC is shown. It was possible to separate four caffeoylquinides, two feruloylquinides, and a dicaffeoylquinide. Mass spectra (LC-MS^n) and comparison of retention times with the isolated lactones were used for identification. With the UHPLC method developed it was possible to reduce the total run time to 27 min (15 min for the separation); however, due to the presence of phosphoric acid, this method is not suitable for LC-MS. Conventional HPLC required a total run time of 75 min.

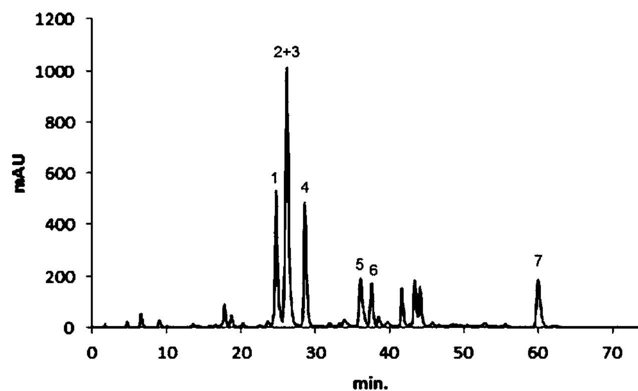

Figure 1. HPLC separation of the mixed lactone fractions after HSCCC of roasted coffee (detection: UV at 324 nm). Peaks: 1, CQL; 2/3, 3-γ-CQL and 5-epi-δ-CQL; 4, CQL; 5, FQL; 6, FQL; 7, tentatively identified as 3,5-diCQL.

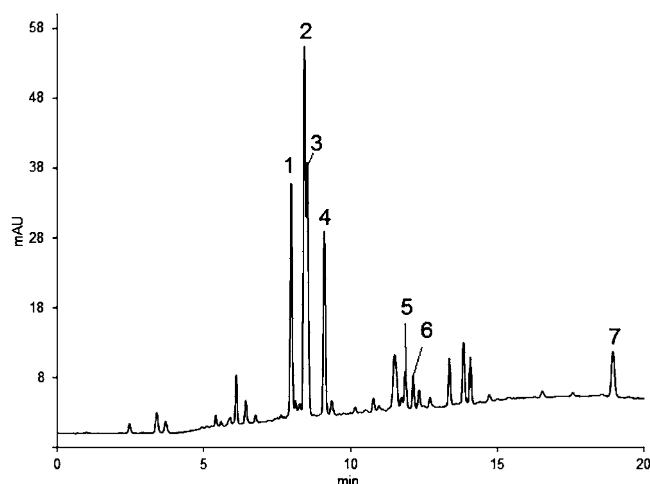

Figure 2. UHPLC separation of the mixed lactone fractions from HSCCC of roasted coffee (detection: UV at 324 nm). Peaks (cf. Figure 3): 2, 3-γ-CQL; 3, 5-epi-δ-CQL.

Structure Elucidation of 5-*O*-Caffeoyl-epi-δ-quinide and 3-*O*-Caffeoyl-γ-quinide. Prior to NMR spectroscopy fractions 3 (model roast) and 5 (coffee extract) were purified by semipreparative HPLC. 1D and 2D NMR (COSY, HSQC, HMBC, and NOESY) experiments were carried out with the purified lactones. For structures of the identified chlorogenic acid lactones, see Figure 3.

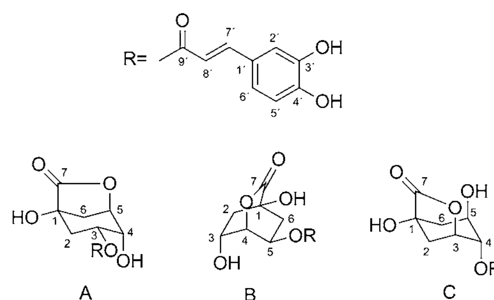

Figure 3. Structures of the identified chlorogenic acid lactones: (A) 3-*O*-caffeoyl-γ-quinide; (B) 5-*O*-caffeoyl-epi-δ-quinide; (C) 4-*O*-caffeoyl-muco-γ-quinide. R = caffeic acid.

In the case of 5-*O*-caffeoyl-epi-δ-quinide purity was 95% in HPLC and UHPLC measurements (area at λ = 324 and 280 nm). LC-MS^n gave a base peak of m/z 335 $[M - H]^-$ and fragment ions of m/z 179, 135, and 109 in MS^2. NMR experiments showed that this purified lactone fraction consisted of two different quinides in a ratio of 2:1. The more abundant quinide was identified as 5-*O*-caffeoyl-epi-δ-quinide. The NMR data obtained are in good accordance with the literature[9,19] with the exception of protons 2 and 6 of the quinide moiety. We assigned a signal pattern of overlapping signals from 2_{eq} and $6_{eq,}$ in which the signal of 2_{eq} could be assigned by HSQC in the more high-field area, followed by a doublet of 6_{ax} and a dd signal for 2_{ax}. In addition to the ^{1}H NMR data available from the literature, we were able to estimate the full ^{13}C NMR data for 5-epi-δ-CQL. The coeluting quinide was determined to be 4-*O*-caffeoyl-muco-γ-quinide by literature comparison;[9] here, the ^{13}C NMR signal assignment could be achieved in most cases by HSQC data. In the case of the quaternary carbons the signal intensities were too low to analyze. Other HPLC phase materials were tested to achieve a separation of the quinides. Finally, a separation (shoulder) on a phenyl-hexyl phase (Luna phenyl-hexyl, 150 mm × 2.00 mm i.d., particle size 3 μm, Phenomenex, Aschaffenburg, Germany) was obtained (Figure 3 in the Supporting Information).

The quinide purified from fraction 5 (coffee extract) gave a base peak in LC-MS^n of m/z 335 $[M - H]^-$ and fragment ions of 161 (100%), 135 (55%), 133 (10%), and 178 (6.6%) in MS^2. According to the obtained NMR data, we estimated the quinide as 3-*O*-caffeoyl-γ-quinide. The NMR data are well in line with the literature[9,10] except the multiplicity of proton 5_{eq}, which we found to be a triplet. In addition to the data of the literature, we could estimate the multiplicity and coupling constants of the protons in positions 2 and 6 of the quinide moiety, which appear in the order 2_{ax} as triplet, 6_{ax} doublet, 2_{eq} as ddd, and 6_{eq} as ddd. The conformation of proton 4 as equatorial could be assigned by the J^3 coupling constant of 4.6 Hz and was confirmed by NOESY because of the coupling to the proton 3_{ax}. In the case of 3-*O*-caffeoyl-γ-quinide the full ^{13}C NMR data were assigned.

To our best knowledge we report the isolation of quinides by HSCCC for the first time. Prepurification on XAD-7 adsorber resin was found to be essential for the roasted coffee extract. Using HSCCC it was possible to separate gram amounts of coffee extract and enrich several monocaffeoylquinides in the elution mode and also a dicaffeoylquinide in the extrusion mode. The high concentration of the quinides facilitates the final purification step by semipreparative HPLC. A mixture of 4-*O*-caffeoyl-muco-γ-quinide and 5-*O*-caffeoyl-epi-δ-quinide could be isolated from a model roast and characterized by NMR experiments. Another quinide was isolated from a roasted coffee extract, and the structure was determined to be 3-*O*-caffeoyl-γ-quinide by 1D and 2D NMR techniques.

This work shows that high-speed countercurrent chromatography is a powerful tool for the separation of caffeoylquinides and offers the possibility to isolate milligram amounts in a relatively short time. The isolated quinides can be used for individual biotests and serve as standard compounds for quantitative analyses. Up to now, quantitative data are available for 3- and 4-CQL,[8,9,12,20] whereas data for individual 5-CQLs in coffee brews were published only by Blumberg et al.[21] On the basis of the isolation of quinide standards by HSCCC, it will now be possible to improve these data. The availability of standard compounds also offers the possibility to develop a LC-MS^n identification scheme for the quinides in the manner of Clifford et al.[22] Furthermore, it was possible to enhance spectral data of some quinides by the until now unreported in the literature ^{13}C NMR data. It was shown that complex quinide mixtures could be separated by UHPLC in a significantly shorter time with the same or even better resolution compared to common HPLC. For the future additional applications of HSCCC and spiral coil countercurrent chromatography with coil volumes of 5.5 L and sample loads up to 40 g will be explored to enhance the separation and isolate higher amounts of quinides.

■ ASSOCIATED CONTENT

Supporting Information

Figures 1−8 and Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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