

# Formation of Ester and Amine Derivatives of 5-*O*-Caffeoylquinic Acid in the Process of Its Simulated Extraction

Andrzej L. Dawidowicz\* and Rafal Typek

Faculty of Chemistry, Maria Curie Skłodowska University, 20-031 Lublin, Pl. Maria Curie Skłodowska 3, Poland

**ABSTRACT:** Chlorogenic acid (CQA), the ester of caffeic acid with quinic acid, supplied to human organisms mainly through coffee, tea, fruits, and vegetables, is one of the most studied polyphenols. It is potentially useful in pharmaceuticals, foodstuffs, feed additives, and cosmetics due to its recently discovered biomedical activity. This finding caused new interest in its properties, its isomers, and its natural occurrence. The presented study shows that 5-*O*-caffeoylquinic acid, during its buffered water extraction, not only undergoes such transformation as isomerization, water molecule addition, and hydrolysis but also reacts with buffer components forming its derivatives. The amount of each formed component depends on the heating time, buffer pH, and buffer type. Although the concentrations of these components are low, they can be mistakenly treated as a new component not previously found in the examined plant or can be a cause of erroneous quantitative estimations of plant composition.

**KEYWORDS:** chlorogenic acid, citric 5-CQA derivatives, acetic 5-CQA derivatives, amine 5-CQA derivatives, 5-CQA buffered extraction

## ■ INTRODUCTION

Chlorogenic acid, the ester of caffeic acid with quinic acid, supplied to human organisms mainly by coffee, tea, fruits, and vegetables,<sup>1,2</sup> is one of the most studied polyphenols.<sup>3,4</sup> As shown in ref 5, the metabolic track of chlorogenic acid synthesis starts with the deaminase of phenylalanine by the specific enzyme forming *trans*-cinnamic acid, which is then transformed into the caffeoyl moiety of chlorogenic acid.<sup>6,7</sup> Antioxidant and anti-carcinogenic properties of CQA have been well established in numerous in vitro studies.<sup>8–12</sup> Chlorogenic acid in the human body is metabolized to ferulic and isoferulic acids, *m*-coumaric acid, and the derivatives of phenylpropionic, benzoic, and hippuric acids.<sup>13</sup> Coffee, a beverage rich in chlorogenic acids, modifies gastrointestinal hormone secretion and glucose tolerance in humans, although the mechanism has not been fully elucidated.<sup>14,15</sup> According to recent studies, attempts have been made to use CQA as a UVA and UVB filter.<sup>16</sup> These newly found properties of CQA caused unabated interest in 5-CQA, its isomers, and its natural occurrence.

According to previous reports,<sup>17</sup> the heating of 5-CQA water solution in the temperature range of 100–200 °C causes chlorogenic acid isomerization and transformation. It has been found that as many as 14 derivative compounds and reaction products with water can be formed from 5-*O*-caffeoylquinic acid by heating its water solution at different pH.<sup>17</sup> The cited investigations were performed using phosphate buffers of different pH. The question asked in the present study is about the influence of buffer type on 5-CQA transformation during its heating in buffered water solutions. It is a valid question as the 5-CQA molecule possesses free hydroxyl and carboxyl groups able to react with different buffer components. The presented results may be valuable for researchers examining plant materials in which chlorogenic acid derivatives formed during extraction can be mistaken for natural components of the examined plants.

## ■ MATERIALS AND METHODS

**Reagents.** Acetonitrile (HPLC), sodium phosphate, phosphoric acid, ammonium chloride, ammonium hydroxide, sodium borate (borax), boric acid, sodium citrate, citric acid, sodium acetate, and acetic acid (all of analytical grade) were purchased from the Polish Chemical Plant POCh (Gliwice, Poland); formic acid from Sigma Aldrich (Seelze, Germany); and chlorogenic acid from Loba-Chemie Australan Preparate (Austria). Water was purified on the Milli-Q system from Millipore (Millipore, Bedford, MA, USA).

**Methods.** The investigations of pH influence on the 5-*O*-caffeoylquinic acid transformation process were performed by heating 5-*O*-caffeoylquinic acid buffered water solution under reflux. Glass equipment composed of a boiling flask (100 mL) and a small condenser was used for this purpose. The following pH values were applied during the experiments: phosphoric buffers of pH 4.0; 5.0; 6.0; 7.0; 8.0; and 9.0; boric buffers of pH 6.0; 7.0; 8.0; and 9.0; citric buffers of pH 4.0; 5.0; 6.0; and 7.0; acetic buffers of pH 4.0; 5.0; 6.0; and 7.0; and ammonia buffers of pH 6.0; 7.0; 8.0; and 9.0. The heated 5-*O*-caffeoylquinic acid solutions contained 10 mg of 5-*O*-caffeoylquinic acid in 50 mL of buffer. Portions of the acid were inserted to the already boiling buffer (~100 °C). The solvents were heated for 10 min or 1, 3, or 5 h.

**HPLC Measurements.** Chromatographic measurements were performed using LC/ESI/IT/MS from Finnigan (LCQ Advantage Max) equipped with the ion-trap mass spectrometric system (Thermo-Electron Corporation, San Jose, CA, USA) and a diode array detector from Finnigan (Surveyor PDA Plus Detector). The column used was a 100 mm × 4.6 mm i.d., 3 μm, Gemini C18 (Phenomenex, Torrance, CA, USA). Chromatographic separation was performed using gradient elution in isothermic conditions (25 °C). Mobile phase A was 25 mM formic acid in water; mobile phase B was 25 mM formic acid in acetonitrile. The gradient program started at 5% B increasing to 35% for 30 min, next 35% B to 100% B for 5 min, followed by isocratic elution (100% B) for 5 min. The total run time was 40 min at the mobile phase flow rate 0.4 mL/min.

**Received:** July 10, 2012

**Revised:** November 7, 2012

**Accepted:** November 25, 2012

**Published:** November 25, 2012



**Table 1. Names, Shortcuts, and Peak Numbers of Estimated 5-O-Caffeoylquinic Transformation Products Obtained in Phosphorous Buffer**

name of compounds	shortcut	peak number <sup>a</sup>
(1S,3R,4S,5R)-1,3,4,5-tetrahydroxycyclohexanecarboxylic acid "quinic acid"	(QA)	1
(1S,3R,4R,5R)-3-[3-(3,4-dihydroxyphenyl)-3R-hydroxypropanoyl]-1,4,5-trihydroxycyclohexanecarboxylic acid	(3-CQA-3R-OH)	2 or 3
(1S,3R,4R,5R)-3-[3-(3,4-dihydroxyphenyl)-3S-hydroxypropanoyl]-1,4,5-trihydroxycyclohexanecarboxylic acid	(3-CQA-3S-OH)	2 or 3
(1S,3R,4R,5R)-5-[3-(3,4-dihydroxyphenyl)-3R-hydroxypropanoyl]-1,4,5-trihydroxycyclohexanecarboxylic acid	(5-CQA-3R-OH)	4 or 5
(1S,3R,4R,5R)-5-[3-(3,4-dihydroxyphenyl)-3S-hydroxypropanoyl]-1,4,5-trihydroxycyclohexanecarboxylic acid	(5-CQA-3S-OH)	4 or 5
(1S,3R,4R,5R)-4-[3-(3,4-dihydroxyphenyl)-3R-hydroxypropanoyl]-1,4,5-trihydroxycyclohexanecarboxylic acid	(4-CQA-3R-OH)	6 or 7
(1S,3R,4R,5R)-4-[3-(3,4-dihydroxyphenyl)-3R-hydroxypropanoyl]-1,4,5-trihydroxycyclohexanecarboxylic acid 3,4-dihydroxybenzoic acid "protocatechuic acid"	(PA)	8
(1S,3R,4R,5R)-1-[[2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-3,4,5-trihydroxycyclohexanecarboxylic acid "1-O-caffeoylquinic acid"	(1-CQA)	9
(1S,3R,4R,5R)-3-[[2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,4,5-trihydroxycyclohexanecarboxylic acid "3-O-caffeoylquinic acid"	(3-CQA)	10
(1S,3R,4R,5R)-5-[[2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,3,4-trihydroxycyclohexanecarboxylic acid "5-O-caffeoylquinic acid"	(5-CQA)	11
(1S,3R,4R,5R)-4-[[2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,3,5-trihydroxycyclohexanecarboxylic acid "4-O-caffeoylquinic acid"	(4-CQA)	12
(E)-3-(3,4-dihydroxyphenyl)-2-propenoic acid "caffeic acid"	CA	13
(Z)-3-(3,4-dihydroxyphenyl)-2-propenoic acid "cis-caffeic acid"	cis-CA	14
(1S,3R,4R,5R)-5-[[2Z)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,3,4-trihydroxycyclohexanecarboxylic acid "cis-5-O-caffeoylquinic acid"	cis-5-CQA	15

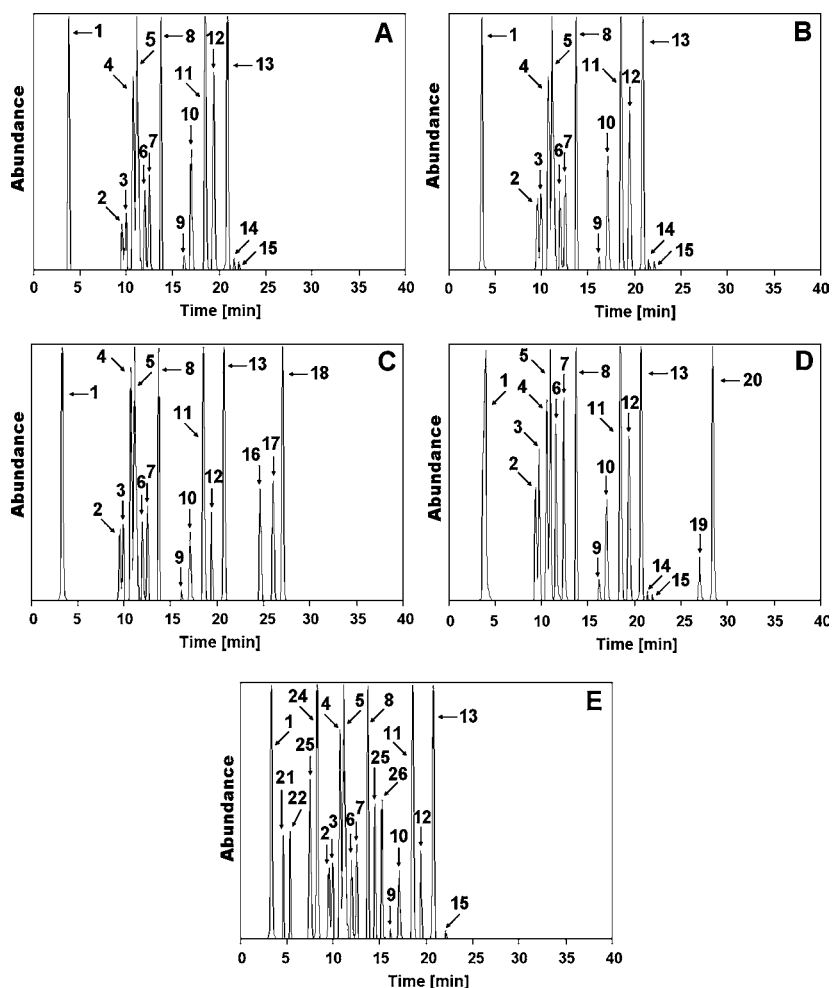
<sup>a</sup>See Figure 1.**Figure 1.** Chromatograms of 5-CQA heated under reflux for 5 h in water solutions buffered at pH 7.0 by the following buffers: phosphoric (A); boric (B); citric (C); and acetic (D), and buffered at pH 9.0 by ammonium buffer (E). Peak numbers correspond to compound numbers in Figure 2. The sequence of diastereoisomeric components in the 2/3, 4/5, 6/7, 16/17, 21/22, 23/24, or 25/26 pairs are tentative as it was impossible to differentiate the R/S components and the ortho-/para-position in the given pair.

Table 2. Time Periods and Ions Monitored in Buffered Solutions of 5-*O*-Caffeoylquinic Acid

	Phosphoric Buffer							
time range (min)	0–9	9–13.5	13.5–16	16–20	20–22	22–40		
<i>m/z</i>	191	371	153	353	179	353		
	Boric Buffer							
time range (min)	0–9	9–13.5	13.5–16	16–20	20–22	22–40		
<i>m/z</i>	191	371	153	353	179	353		
	Citric Buffer							
time range (min)	0–9	9–13.5	13.5–16	16–20	20–22	22–40		
<i>m/z</i>	191	371	153	353	179	527		
	Acetic Buffer							
time range (min)	0–9	9–13.5	13.5–16	16–20	20–22	22–23	23–40	
<i>m/z</i>	191	371	153	353	179	353	395	
	Ammonia Buffer							
time range (min)	0–4.5	4.5–9	9–13.5	13.5–14	14–16	16–20	20–22	22–40
<i>m/z</i>	191	352	371	153	353	353	179	353

Table 3. Names, Shortcuts, and Peak or Structure Numbers of Estimated 5-*O*-Caffeoylquinic Transformation Products

name of compounds	shortcut	peak number <sup>a</sup>	structure number <sup>b</sup>
(1 <i>S</i> ,3 <i>R</i> ,4 <i>R</i> ,5 <i>R</i> )-3-[(4-carboxy-3 <i>R</i> -hydroxybutanoyl)oxy]-5-[[ <i>(2E)</i> -3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,4-dihydroxycyclohexanecarboxylic acid	(5-CQA-3-1-3 <i>R</i> -Cit)	16 or 17	1
(1 <i>S</i> ,3 <i>R</i> ,4 <i>R</i> ,5 <i>R</i> )-3-[(4-carboxy-3 <i>S</i> -hydroxybutanoyl)oxy]-5-[[ <i>(2E)</i> -3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,4-dihydroxycyclohexanecarboxylic acid	(5-CQA-3-1-3 <i>S</i> -Cit)	16 or 17	2
(1 <i>S</i> ,3 <i>R</i> ,4 <i>R</i> ,5 <i>R</i> )-3-hydroxypentanedioic-5-[[ <i>(2E)</i> -3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,4-dihydroxycyclohexanecarboxylic acid	(5-CQA-3-3-Cit)	18	3
(1 <i>S</i> ,3 <i>R</i> ,4 <i>R</i> ,5 <i>R</i> )-4-acetyloxy-5-[[ <i>(2E)</i> -3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,3-dihydroxycyclohexanecarboxylic acid	(5-CQA-4-Ac)	19	4
(1 <i>S</i> ,3 <i>R</i> ,4 <i>R</i> ,5 <i>R</i> )-3-acetyloxy-5-[[ <i>(2E)</i> -3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,4-dihydroxycyclohexanecarboxylic acid	(5-CQA-3-Ac)	20	5
(1 <i>S</i> ,3 <i>R</i> ,4 <i>R</i> ,5 <i>R</i> )-3-[[ <i>(2E)</i> -3-(3-amine-4-hydroxyphenyl)prop-2-enoyl]oxy]-1,4,5-trihydroxycyclohexanecarboxylic acid	(3-CQA- <i>m</i> -NH <sub>2</sub> )	21 or 22	6
(1 <i>S</i> ,3 <i>R</i> ,4 <i>R</i> ,5 <i>R</i> )-3-[[ <i>(2E)</i> -3-(4-amine-3-hydroxyphenyl)prop-2-enoyl]oxy]-1,4,5-trihydroxycyclohexanecarboxylic acid	(3-CQA- <i>p</i> -NH <sub>2</sub> )	21 or 22	7
(1 <i>S</i> ,3 <i>R</i> ,4 <i>R</i> ,5 <i>R</i> )-5-[[ <i>(2E)</i> -3-(3-amine-4-hydroxyphenyl)prop-2-enoyl]oxy]-1,3,4-trihydroxycyclohexanecarboxylic acid	(5-CQA- <i>m</i> -NH <sub>2</sub> )	23 or 24	8
(1 <i>S</i> ,3 <i>R</i> ,4 <i>R</i> ,5 <i>R</i> )-5-[[ <i>(2E)</i> -3-(4-amine-3-hydroxyphenyl)prop-2-enoyl]oxy]-1,3,4-trihydroxycyclohexanecarboxylic acid	(5-CQA- <i>p</i> -NH <sub>2</sub> )	23 or 24	9
(1 <i>S</i> ,3 <i>R</i> ,4 <i>R</i> ,5 <i>R</i> )-4-[[ <i>(2E)</i> -3-(3-amine-4-hydroxyphenyl)prop-2-enoyl]oxy]-1,3,5-trihydroxycyclohexanecarboxylic acid	(4-CQA- <i>m</i> -NH <sub>2</sub> )	25 or 26	10
(1 <i>S</i> ,3 <i>R</i> ,4 <i>R</i> ,5 <i>R</i> )-4-[[ <i>(2E)</i> -3-(4-amine-3-hydroxyphenyl)prop-2-enoyl]oxy]-1,3,5-trihydroxycyclohexanecarboxylic acid	(4-CQA- <i>p</i> -NH <sub>2</sub> )	25 or 26	11

<sup>a</sup>See Figure 1. <sup>b</sup>See Figure 2.

In each run, PDA spectra in the range 200–600 nm and MS spectra in the range of *m/z*100–1000 were collected continuously. SIM function was used to better visualize the chromatographic separation and to remove the signal connected with the buffer components. The time periods and monitored ions are collected in Table 1.

The column effluent was ionized by electrospray ionization (ESI). The ESI needle potential was 4.5 kV in the negative ionization mode. To identify the chlorogenic acid isomers and the chlorogenic acid transformation products, the functions of secondary (MS<sup>n</sup>) ion fragmentation were applied. The collision energy was chosen individually for each group of the examined compounds. The comparison of the obtained results with the literature data<sup>18</sup> was helpful for assignment of CQA region-isomers and their derivatives. To identify 5-*O*-caffeoylquinic acid derivatives in the examined samples, LC/ESI/TOF/MS analysis was additionally performed. LC/ESI/TOF/MS analysis was carried out on the Agilent (Agilent Technologies, Palo Alto, CA, USA) liquid chromatography system. MS analysis was performed on the orthogonal TOF/MS equipped with an electrospray interface (Agilent Technologies, Santa Clara, CA, USA). Negative mode using full scan mode in TOF/MS analysis was applied, and the mass range was set at 100–600 Da. Chromatographic separation was performed using the same chromatographic column and the same gradient elution as those described for the LC/ESI/IT/MS analysis.

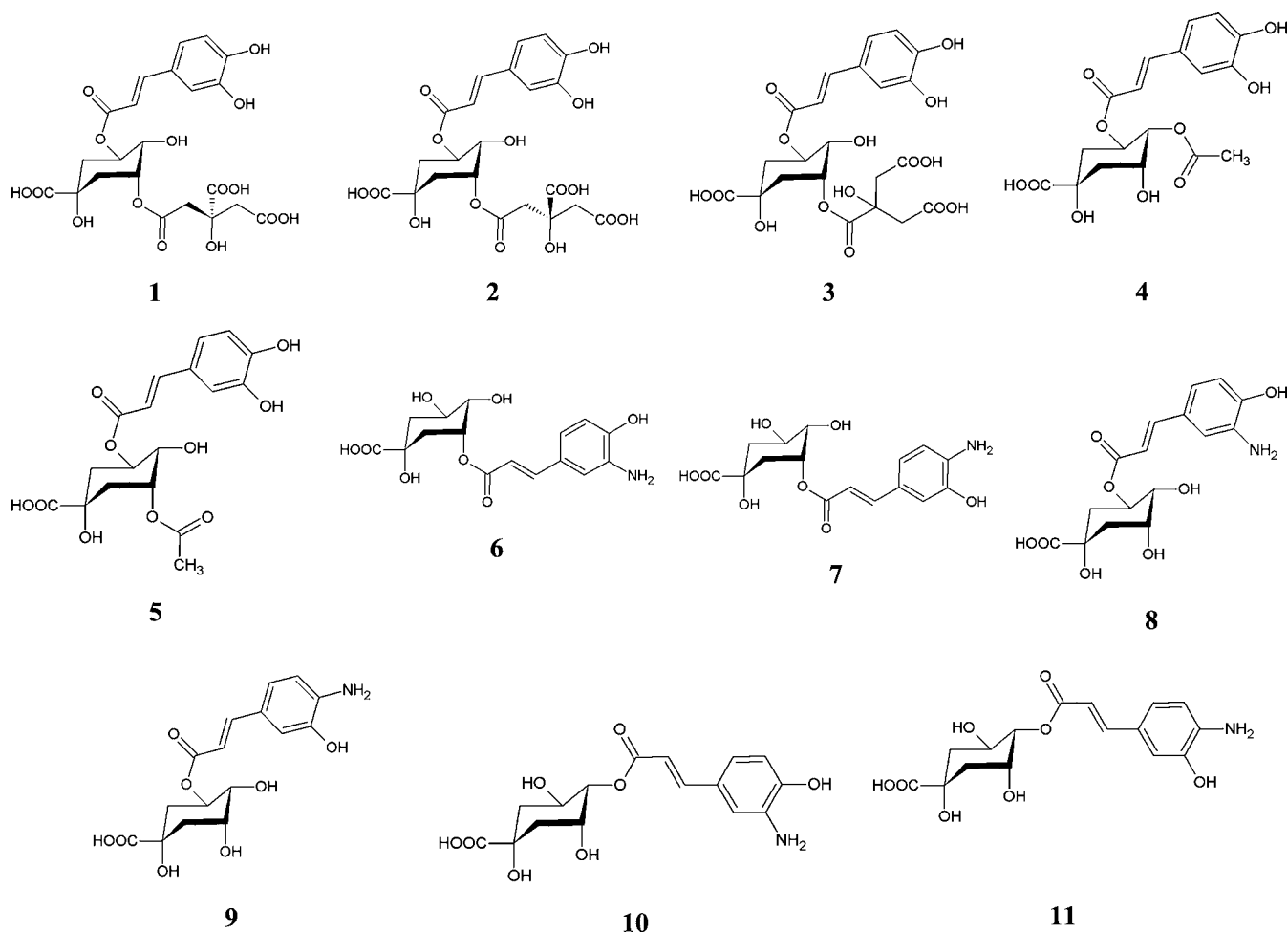
Because of the lack of standards of esters and amine derivatives, the amounts of these compound were calculated by relating their chromatographic responses to the calibration curve for 5-CQA.

## RESULTS AND DISCUSSION

Figure 1 presents the chromatograms of 5-CQA water solutions buffered at pH 7.0 using phosphoric, boric, citrate, and acetate buffers and the chromatogram of 5-CQA water solutions buffered at pH 9.0 using ammonia buffer, all heated under reflux for 5 h. The exemplary samples imitate the chlorogenic acid extract obtained for this compound during its hot buffered water extraction at pH 7.0 and/or 9.0.

As in results from ref 17, the heating of 5-CQA water solution in phosphoric buffer leads to the formation of 14 compounds, which are the transformation and isomerization products of the parent compound. The analysis of the results reported in this article (the experiments were performed in analogous way as in ref 17) shows that the same compounds appear in 5-CQA water solutions buffered by different buffers; see Table 2.

A more detailed consideration of the obtained chromatograms shows that additional compounds are formed. PDA and MS<sup>n</sup> data



**Figure 2.** Molecular structures of individual ester and amine derivatives formed during 5-CQA transformation in citric, acetic, and ammonium buffers. 1, (1*S*,3*R*,4*R*,5*R*)-3-[[4-carboxy-3*R*-hydroxybutanoyl]oxy]-5-[[*(2E)*-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,4-dihydroxycyclohexanecarboxylic acid; 2, (1*S*,3*R*,4*R*,5*R*)-3-[[4-carboxy-3*S*-hydroxybutanoyl]oxy]-5-[[*(2E)*-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,4-dihydroxycyclohexanecarboxylic acid; 3, (1*S*,3*R*,4*R*,5*R*)-3-hydroxypentanedioic-5-[[*(2E)*-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,4-dihydroxycyclohexanecarboxylic acid; 4, (1*S*,3*R*,4*R*,5*R*)-4-acetyloxy-5-[[*(2E)*-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,3-dihydroxycyclohexanecarboxylic acid; 5, (1*S*,3*R*,4*R*,5*R*)-3-acetyloxy-5-[[*(2E)*-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,4-dihydroxycyclohexanecarboxylic acid; 6, (1*S*,3*R*,4*R*,5*R*)-3-[[*(2E)*-3-(3-amine-4-hydroxyphenyl)prop-2-enoyl]oxy]-1,4,5-trihydroxycyclohexanecarboxylic acid; 7, (1*S*,3*R*,4*R*,5*R*)-3-[[*(2E)*-3-(4-amine-3-hydroxyphenyl)prop-2-enoyl]oxy]-1,3,4-trihydroxycyclohexanecarboxylic acid; 8, (1*S*,3*R*,4*R*,5*R*)-5-[[*(2E)*-3-(3-amine-4-hydroxyphenyl)prop-2-enoyl]oxy]-1,3,4-trihydroxycyclohexanecarboxylic acid; 9, (1*S*,3*R*,4*R*,5*R*)-5-[[*(2E)*-3-(4-amine-3-hydroxyphenyl)prop-2-enoyl]oxy]-1,3,4-trihydroxycyclohexanecarboxylic acid; 10, (1*S*,3*R*,4*R*,5*R*)-4-[[*(2E)*-3-(3-amine-4-hydroxyphenyl)prop-2-enoyl]oxy]-1,3,5-trihydroxycyclohexanecarboxylic acid; 11, (1*S*,3*R*,4*R*,5*R*)-4-[[*(2E)*-3-(4-amine-3-hydroxyphenyl)prop-2-enoyl]oxy]-1,3,5-trihydroxycyclohexanecarboxylic acid.

indicate that these compounds can be identified as ester and amine derivatives of CQA. In the case of citric buffer, three additional derivatives are formed during 5-CQA transformation (peaks 16–18 in Figure 1C): peaks 16 and 17, diastereoisomeric pair (1*S*,3*R*,4*R*,5*R*)-3-[[4-carboxy-3*R*/*S*-hydroxybutanoyl]oxy]-5-[[*(2E)*-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,4-dihydroxycyclohexanecarboxylic acid, (5-CQA-3-1-3*R*/*S*-Cit); peak 18, (1*S*,3*R*,4*R*,5*R*)-3-hydroxypentanedioic-5-[[*(2E)*-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,4-dihydroxycyclohexanecarboxylic acid, (5-CQA-3-3-Cit).

In the case of acetic buffer, two additional derivatives are formed (peaks 19 and 20 in Figure 1D): peak 19, (1*S*,3*R*,4*R*,5*R*)-4-acetyloxy-5-[[*(2E)*-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,3-dihydroxycyclohexanecarboxylic acid, (5-CQA-4-Ac); peak 20, (1*S*,3*R*,4*R*,5*R*)-3-acetyloxy-5-[[*(2E)*-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy]-1,4-dihydroxycyclohexanecarboxylic acid, (5-CQA-3-Ac);

In the case of ammonium buffer, six additional derivatives appear in the mixture after 5-CQA transformation (peaks 21–26 in Figure 1E): peaks 21 and 22, (1*S*,3*R*,4*R*,5*R*)-3-[[*(2E)*-3-(3/4-amine-4-hydroxyphenyl)prop-2-enoyl]oxy]-1,4,5-trihydroxycyclohexanecarboxylic acid, (3-CQA-*m/p*-NH<sub>2</sub>); peaks 23 and 24, (1*S*,3*R*,4*R*,5*R*)-5-[[*(2E)*-3-(3/4-amine-4-hydroxyphenyl)prop-2-enoyl]oxy]-1,3,4-trihydroxycyclohexanecarboxylic acid, (5-CQA-*m/p*-NH<sub>2</sub>); peaks 25 and 26, (1*S*,3*R*,4*R*,5*R*)-4-[[*(2E)*-3-(3/4-amine-4-hydroxyphenyl)prop-2-enoyl]oxy]-1,3,5-trihydroxycyclohexanecarboxylic acid, (4-CQA-*m/p*-NH<sub>2</sub>).

Such identification results from the following. The ion molecular weights of the compounds corresponding to peaks 16–18 are the same and exceed the 5-CQA ion molecular weight by the molecular weight of citric acid lessened by 18 Da (molecular weight of water, a byproduct of the esterification reaction). The performed MS<sup>2</sup> does not allow one to identify the position of the citric moiety substitution in 5-CQA citric derivatives. Because of the very low concentration of these derivatives and their low

stability in the ionization process, MS<sup>3</sup> spectra could not be carried out. The same relationship is observed in acetic esters of 5-CQA (peaks 19 and 20); however, the lower number of acetic in relation to citric 5-CQA derivatives is difficult to explain. Citric acid is able to react with hydroxyl groups in positions 1-, 3-, and 4-, and with the carboxyl group of quinic moiety due to the presence of carboxyl and hydroxyl groups in its molecule. Because of steric hindrance, its reaction with the 1- and 4- hydroxyl group and with the carboxyl group of quinic moiety is less likely. It is more probable that the 3-hydroxyl group reacts with the side carboxyl group of citric acid forming two diastereoisomers, and with the central carboxyl group of citric acid forming the third ester 5-CQA derivative. The last assumption is consistent with the obtained chromatograms (see Figure 1C). Peaks 16 and 17 have a close retention and a similar magnitude, which indicates a similarity of the chemical properties and amounts of corresponding 5-CQA citric derivatives (diastereoisomeric pair).

**Table 4. Negative Ion MS<sup>n</sup> Data for Product Transformation of 5-O-Caffeoylquinic Acid**

peak number	MS <sup>1</sup>		MS <sup>2</sup>			comps
	parent ion m/z	base peak m/z	secondary peak			
			m/z	intensity (%)		
16	527.1	191.2	179.1 365.3	22.1 9.1	(5-CQA-3-1-3S-Cit) or (5-CQA-3-1-3S-Cit)	
17	527.2	191.2	179.2 365.3	18.2 11.2	(5-CQA-3-1-3R-Cit) or (5-CQA-3-1-3R-Cit)	
18	527.1	191.2	179.1 365.2	31.2 27.2	(5-CQA-3-3-Cit)	
19	395.3	191.2	179.2 233.3	19.1 31.8	(5-CQA-4-Ac)	
20	395.4	191.1	179.1 233.3	11.3 39.8	(5-CQA-3-Ac)	
21	351.9	191.4	178.1	67.4	(3-CQA- <i>p</i> -NH <sub>2</sub> ) or (3-CQA- <i>m</i> -NH <sub>2</sub> )	
22	351.8	191.4	178.2	73.2	(3-CQA- <i>m</i> -NH <sub>2</sub> ) or (3-CQA- <i>p</i> -NH <sub>2</sub> )	
23	351.8	191.3	178.1	5.3	(5-CQA- <i>m</i> -NH <sub>2</sub> ) or (5-CQA- <i>p</i> -NH <sub>2</sub> )	
24	351.9	191.3	178.1	8.8	(5-CQA- <i>m</i> -NH <sub>2</sub> ) or (5-CQA- <i>p</i> -NH <sub>2</sub> )	
25	351.8	178.2	191.2	32.1	(4-CQA- <i>m</i> -NH <sub>2</sub> ) or (4-CQA- <i>p</i> -NH <sub>2</sub> )	
26	351.9	178.1	191.3	24.2	(4-CQA- <i>m</i> -NH <sub>2</sub> ) or (4-CQA- <i>p</i> -NH <sub>2</sub> )	

In the case of acetic acid, due to a smaller size of its molecule in relation to the citric acid molecule, the esterification of the 3- and 4-hydroxyl groups of quinic moiety is more probable; however, the formation of the 5-CQA acetic derivative in position 3 seems to be more likely. If so, the last derivative should correspond with peak 19.

The esterification of hydroxyl groups in the aromatic ring of 5-CQA requires the application of acid anhydride or acid chloride. Hence, the esterification of hydroxylic groups coupled to the aromatic ring looks impossible in the applied experimental conditions. Probably the employment of a very sensitive LC-NMR system would be more helpful for an unequivocal identification of citric and acetic moiety substitution in CQA.

The ion molecular weights of the compounds corresponding to peaks 21–26 are also the same and lower by 1 Da than the 5-CQA ion molecular weight. The observed difference in molecular weight of molecular ions indicates the substitution of the –OH group (alcoholic or phenolic group) in the quinic moiety or in the aromatic ring, or the substitution of –OH group in the carboxylic group by the –NH<sub>2</sub> group (the difference in the molecular weight of these groups equals 1 Da). It results from the MS<sup>2</sup> analysis that these CQA derivatives decompose into two ions –191 *m/z*, corresponding to the molecular weight of quinic ion, and 178 *m/z*, which is lower by 1 Da than the molecular weight of caffeic acid. Hence, the obtained results indicate the substitution of –OH groups by –NH<sub>2</sub> in the caffeic moiety. There are some additional arguments showing the correctness of this identification. Low temperature substitution in the aromatic ring is more privileged than in the aliphatic and/or cyclic structure; however, it requires the application of a catalyst (Lewis acid).<sup>19</sup> The used ammonium buffer was contaminated with Fe<sup>3+</sup> (5 ppm), which played the role of catalyst of the substitution reaction during aromatic amine synthesis from phenolic substrates. Moreover, hydroxyl groups in the caffeic moiety are in ortho position. The ortho position of hydroxyl groups favor the substitution of one of them. It is worth mentioning that a higher temperature than that used in these experiments is required for the substitution of the –OH group by –NH<sub>2</sub> in the –COOH group.

The formation of six amine CQA derivatives results from the substitution of the meta or para –OH group by –NH<sub>2</sub> in the most abundant CQA isomers present in heated 5-CQA water solution, 3-CQA, 4-CQA, and 5-CQA. From the results from the chromatograms (see the exemplary chromatogram in Figure 1E), three pairs of peaks differing in height can be distinguished among the peaks representing six amine CQA derivatives. Assuming that the amount of amine CQA derivatives is directly

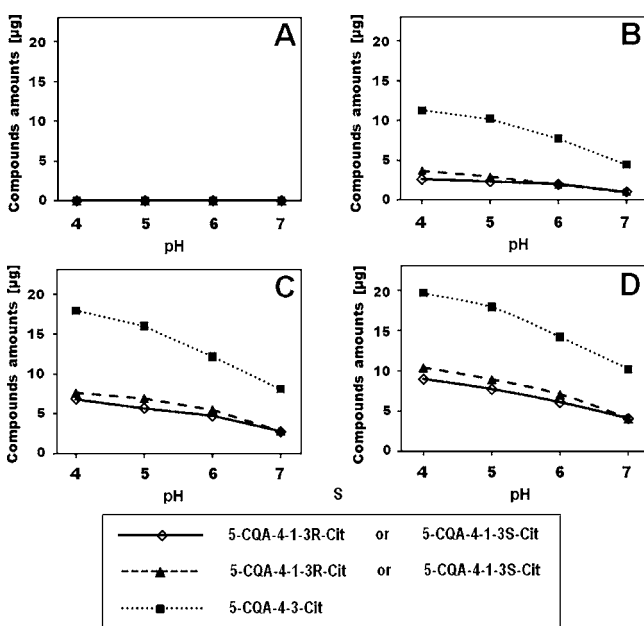
**Table 5. HRMS Data for Product Transformation of 5-O-Caffeoylquinic Acid**

peak number	elemental composition [M – H] <sup>–</sup>	[M – H] <sup>–</sup> (Da)		error		comps
		experimental	calcd	(ppm)	(mDa)	
17	C <sub>22</sub> H <sub>23</sub> O <sub>15</sub>	527.1039	527.1037	0.4	0.2	(5-CQA-3-1-3R-Cit) or (5-CQA-3-1-3S-Cit)
18	C <sub>22</sub> H <sub>23</sub> O <sub>15</sub>	527.1033	527.1037	0.8	–0.4	(5-CQA-3-3-Cit)
19	C <sub>18</sub> H <sub>19</sub> O <sub>10</sub>	395.0981	395.0978	0.8	0.3	(5-CQA-4-Ac)
20	C <sub>18</sub> H <sub>19</sub> O <sub>10</sub>	395.0975	395.0978	0.8	–0.3	(5-CQA-3-Ac)
21	C <sub>16</sub> H <sub>18</sub> O <sub>8</sub> N	352.1030	352.1032	0.6	–0.2	(3-CQA- <i>m</i> -NH <sub>2</sub> ) or (3-CQA- <i>p</i> -NH <sub>2</sub> )
22	C <sub>16</sub> H <sub>18</sub> O <sub>8</sub> N	352.1029	352.1032	0.9	–0.3	(3-CQA- <i>m</i> -NH <sub>2</sub> ) or (3-CQA- <i>p</i> -NH <sub>2</sub> )
23	C <sub>16</sub> H <sub>18</sub> O <sub>8</sub> N	352.1037	352.1032	1.4	0.5	(5-CQA- <i>m</i> -NH <sub>2</sub> ) or (5-CQA- <i>p</i> -NH <sub>2</sub> )
24	C <sub>16</sub> H <sub>18</sub> O <sub>8</sub> N	352.1036	352.1032	1.1	0.4	(5-CQA- <i>m</i> -NH <sub>2</sub> ) or (5-CQA- <i>p</i> -NH <sub>2</sub> )
25	C <sub>16</sub> H <sub>18</sub> O <sub>8</sub> N	352.1039	352.1032	2.0	0.7	(4-CQA- <i>m</i> -NH <sub>2</sub> ) or (4-CQA- <i>p</i> -NH <sub>2</sub> )
26	C <sub>16</sub> H <sub>18</sub> O <sub>8</sub> N	352.1027	352.1032	1.4	–0.5	(4-CQA- <i>m</i> -NH <sub>2</sub> ) or (4-CQA- <i>p</i> -NH <sub>2</sub> )

proportional to the amount of their precursors, the peak pairs 21/22, 23/24, and 25/26 correspond to the amine derivatives of 3-CQA, 5-CQA, and 4-CQA, respectively.

For better clarity, the names of all the identified new compounds, their peaks and structure numbers, shortcuts, and chemical structures are collected in Table 3 and graphically presented in Figure 2. The MS<sup>n</sup> and HRMS data for the mentioned compounds are collected in Tables 4 and 5.

The influence of pH and heating time on the amount of individual ester and amine derivatives formed during 5-CQA transformation and degradation in water solutions buffered by different buffer types is presented in Figures 3–5.

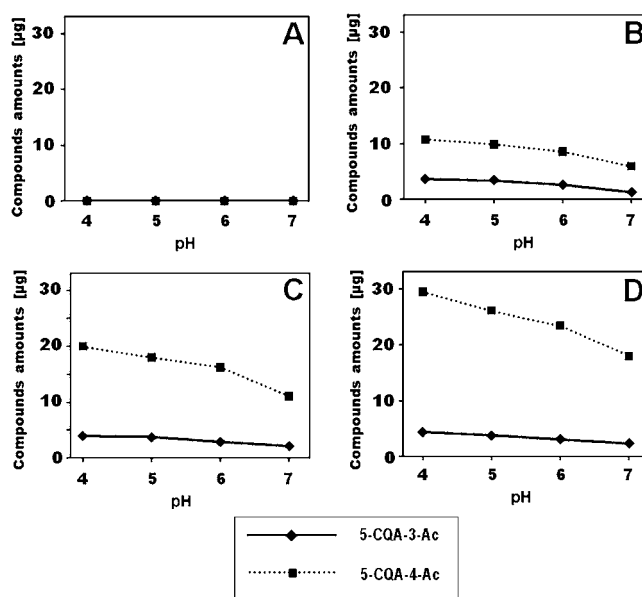


**Figure 3.** Influence of pH on the amount of citric esters formed during the heating of 5-CQA water solution buffered by citric buffer. Heating time of buffered 5-CQA water solution: 10 min (A); 1 h (B); 3 h (C); and 5 h (D).

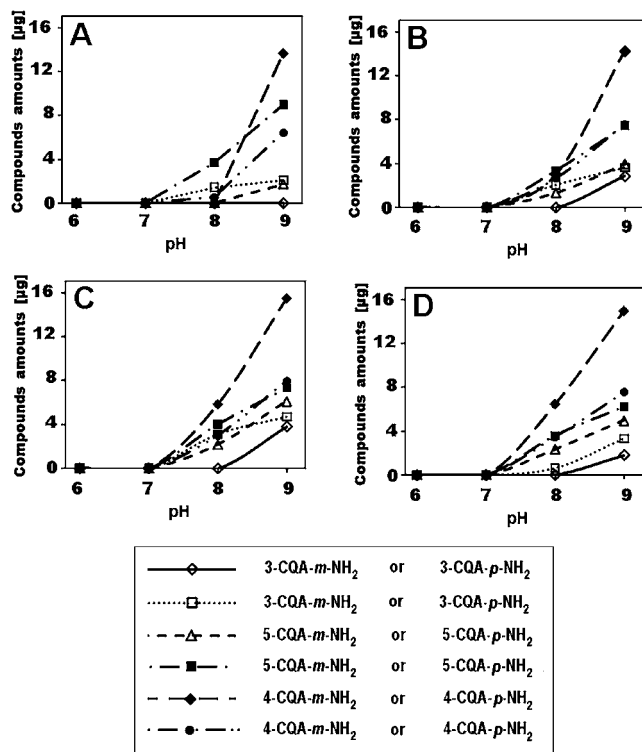
Figure 3 illustrates the influence of pH increase versus the amount of three citric esters of 5-CQA. As seen in the presented plots, the increase of heating time causes a small increase in the amount of the 5-CQA citric derivatives. Moreover, the elevated hydrogen ion concentration favors the formation of these derivatives, which agrees with literature. The same conclusion can be made for the plots in Figure 4, which presents the influence of pH increase on the amount of two acetic esters of 5-CQA. Quite a different situation is observed in the case of amine CQA derivatives; see Figure 5. The formation of CQA amines is favored in an alkali environment. Moreover, there is no significant influence of heating time on the amount of these derivatives, which can result from the gradual thermal decomposition of ammonium buffer.

The presented study proves that 5-*O*-caffeoylquinic acid, during its buffered water extraction, not only undergoes such transformations as isomerization, water molecule addition, hydrolysis and recombination of hydrolysis products but also can react with buffer components forming esters and amine derivatives, depending on buffer composition. The performed experiments show that the amount of each forming component is low and depends on the heating time, buffer pH, and buffer type.

Liquid extraction is applied most frequently today as a sample preparation procedure for plant analysis. To increase extraction



**Figure 4.** Influence of pH on the amount of acetic esters formed during the heating of 5-CQA water solution buffered by acetic buffer. Heating time of buffered 5-CQA water solution: 10 min (A); 1 h (B); 3 h (C); and 5 h (D).



**Figure 5.** Influence of pH on the amount of amine derivatives formed during the heating of 5-CQA water solution buffered by ammonium buffer. Heating time of buffered 5-CQA water solution: 10 min (A); 1 h (B); 3 h (C); and 5 h (D).

efficiency, buffering extractants of different pH are frequently used. The pH of the same plant extract can vary owing to the contents of plant components differing in their acidic–basic character, e.g., organic acids and basic, mineral, and organic salts. In light of these facts and the presented results, it is quite possible that the transformation and reaction products of 5-*O*-caffeoylquinic acid can be mistakenly treated as new components

not previously found in the examined plant. The presence of these products can also lead to erroneous quantitative estimations of plant composition when some or all components formed from 5-O-caffeoylquinic acid during its water or buffered water extraction really exist in the examined plant and the 5-O-caffeoylquinic acid transformation process only adds to their amount. This is why the presented results are important for researchers investigating plant metabolism and looking for new plant components.

## AUTHOR INFORMATION

### Corresponding Author

\*Tel: +48 81 537 55 45. Fax: +48 81 533 33 48. E-mail: dawid@poczta.umcs.lublin.pl.

### Notes

The authors declare no competing financial interest.

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