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LC-Orbitrap-HRMS Determination of Two Novel Plastochromanol Homologues

*Tim Hammerschick, Jana Graf, and Walter Vetter**

Scope: The antioxidant plastochromanol-8 (PC-8) is a tocochromanol which differs from γ-tocotrienol in having an unsaturated side chain of eight instead of three isoprene units. The recent isolation of PC-8 from flaxseed oil indicates the additional presence of lower shares of two previously unknown homologues, plastochromanol-7 (PC-7) and plastochromanol-9 (PC-9), which feature seven and nine isoprenoid units respectively on the γ-chromanol backbone. Here, a fast LC-Orbitrap-HRMS method is applied for the determination of PC-7 and PC-9 in seven plant oils and a plant extract.
Methods and results: The presence of PC-7, PC-8, and PC-9 is confirmed in all eight investigated samples by LC-Orbitrap-HRMS analysis after saponification. PC-8 amounts of ≈315–350 mg kg^{-1} in two flaxseed oils, ≈75 mg kg^{-1} in rapeseed oil, ≈38 mg kg^{-1} in camelina oil, ≈80–120 mg kg^{-1} in two mustard oils, ≈90 mg kg^{-1} in candle nut oil, and ≈900 mg kg^{-1} dry weight in *Cecropia* leaves are determined by quantification.
Semi-quantification of PC-7 and PC-9 indicated the presence of ≈0.1–1% of PC-7 and PC-9 in varied relative ratios.
Conclusion: The novel plastochromanol homologues are of particular interest to researchers with focus on vitamin E and other tocochromanols because of their unexplored bioactivity.

1. Introduction

Plastochromanol-8 (PC-8) is a naturally occurring tocochromanol that has been discovered in the mid-1960s in leaves of the rubber tree.[1,2] PC-8 shares the chromanol backbone with γ-configuration (7,8-dimethyl-substitution) as found in γ-tocopherol and γ-tocotrienol. However, it distinguishes from these classic tocochromanols by its very long side chain that resembles the constitution of tocotrienols but features eight isoprenoid units instead of three (**Figure 1**). Specifically, the molecular formula of $C_{53}H_{82}O_2$ of PC-8 exceeds that of most other non-saponifiable lipid compounds. Because of the 7,8-dimethyl pattern on the chromanol backbone, PC-8 is known as an excellent antioxidant.[3–6]

Recently, we reported the isolation of PC-8 by countercurrent chromatography (CCC) and centrifugal partition chromatography (CPC) from flaxseed oil.[7] In this context we noted the additional presence of two hitherto unreported plastochromanol homologues. One homologue featured seven (plastochromanol-7, PC-7) and the other one nine isoprenoid units in the side chain (plastochromanol-9, PC-9) (Figure 1).[7] Like PC-8, PC-7 and PC-9 are structurally related to the well-known γ-tocotrienol (γ-T3, PC-3). Bioactivity of the novel plastochromanols PC-7 and PC-9 is evitable given the structural similarity with PC-8 and γ-T_3. Although the direct detection of PC-7 and PC-9 in flaxseed oil by high-performance liquid chromatography with fluorescence detection (HPLC/FLD) failed in the initial study,[7] the discovery of these two tocochromanols exactly 100 years after the first report of tocopherols was surprising, also in the light of almost 60 years of research on PC-8 and its wide distribution in the plant kingdom.[8–10]

Here, we aimed to verify the presence of PC-7 and PC-9 as two previously overlooked minor plastochromanol homologues. Instead of the sophisticated enrichment procedure of Hammerschick et al.,[7] which included sample saponification, gel permeation chromatography, and countercurrent separation methods, we established a fast method for the direct detection of PC-7 and PC-9. Specifically, samples were saponified, hexane-extracted, and, after solvent reduction, directly measured by LC-Orbitrap-HRMS. Due to the high PC-8 content in flaxseed oil (≈250 mg kg^{-1} oil[11]) and rapeseed oil (up to ≈90 mg kg^{-1} oil[11]), these two matrices were primarily selected by us along with other oils pressed from botanically related species. In addition, a review article of Kruk et al.[12] reported the presence of considerable amounts of PC-8 in the leaves of *Cecropia sp.* (species not specified), a tree naturally occurring in the Neotropics.[13] The growing of a tree of this family, *Cecropia peltata L.*, in a greenhouse of the arboretum on the campus of our university, enabled us to add fresh leaves from this tree to the sample portfolio. Additional HPLC/FLD measurements

T. Hammerschick, J. Graf, W. Vetter
Department of Food Chemistry (170b)
Institute of Food Chemistry
University of Hohenheim
D-70593 Stuttgart, Germany
E-mail: walter.vetter@uni-hohenheim.de

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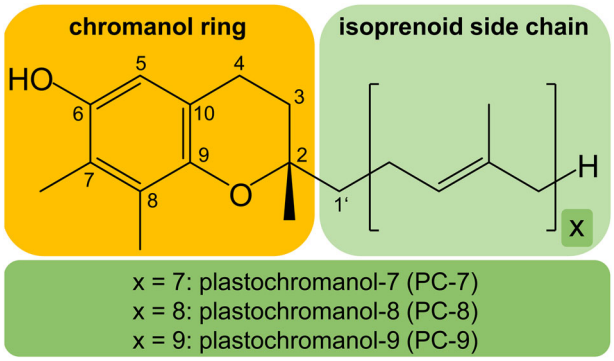

Figure 1. Chemical structure of plastochromanol-7 (PC-7), plastochromanol-8 (PC-8), and plastochromanol-9 (PC-9).

were carried out to determine the amount of PC-8 in the samples.

2. Results and Discussion

2.1. Detection and Quantification of PC-8 as Well as Identification of Plastochromanol-7 (PC-7) and Plastochromanol-9 (PC-9) in Flaxseed Oil

LC-Orbitrap-HRMS analysis of the unsaponifiable matter of both flaxseed samples enabled the detection of PC-8 (t_R, 17.02 min) by extracting the theoretical *m/z* value of the quasi molecular ion $[M+H]^+$ (*m/z* 751.6388) from the full scan chromatogram. Subsequent screening of the corresponding *m/z* values of the homologous series of PC-4 to PC-10 ($\Delta m = 68.0626$ Da between two subsequent pairs) verified the presence of two further minor peaks which were flanking PC-8. Namely, PC-7 eluted at 16.03 min (*m/z* 683.5762) and PC-9 at 17.82 min (*m/z* 819.7014) (**Figure 2**a). The LC elution order of PC-7 < PC-8 < PC-9 not only agreed with an increased retardation with longer isoprenoid chain in RP-HPLC (Figure 2a); specifically, each additional isoprene unit increased the LC retention time by ≈1 min. Interestingly, this was also valid for γ-tocotrienol (γ-T_3; PC-3) which eluted after 11.9 min from the LC column, so that the retention times of all plastochromanol members linearly increased with the length of the isoprene unit (Figure 2b). However, no further plastochromanols with different chain lengths (PC-4–PC-6; >PC-9) could be detected although the exact masses and LC retention times could be well-predicted. This was in agreement with Hammerschick et al.[7] (Figure S1, Supporting Information).

Next to $[M+H]^+$ ions, the LC/MS spectra of the three plastochromanols also featured the diagnostic fragment ions of γ-substituted tocochromanols, i.e., *m/z* 151.0754 and *m/z* 191.1065, respectively.[14,15] Also, not only the monoisotopic peaks of the $[M+H]^+$ ions of PC-7, PC-8, and PC-9 could be determined with high accuracy (mass deviations <4 ppm between theoretical and measured *m/z* values), but also those of the ^{13}C isotope peaks, if present (Table S1, Supporting Information). For instance, four isotope peaks were detected for PC-8 which corresponded with 0–3 ^{13}C atoms, respectively, and the abundance ratios fitted well with the theoretical values of 100.0%:57.3%:16.1%:3.0%. Due to the lower total amounts, only three or two ^{13}C isotope peaks could be detected for PC-7

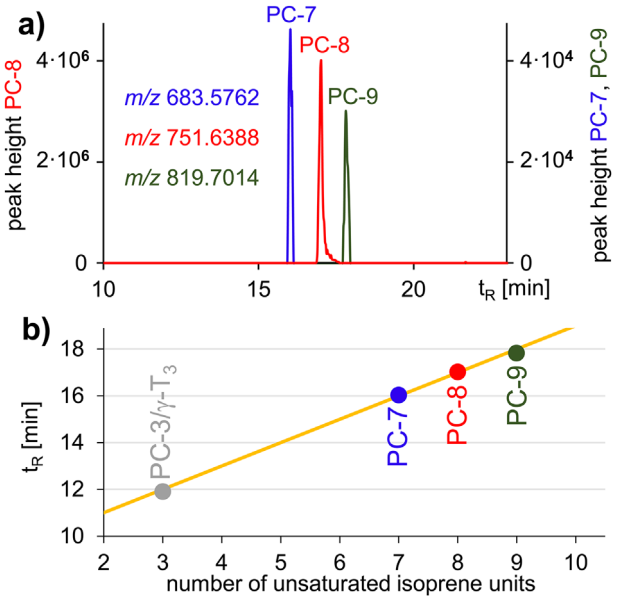

Figure 2. **a**) LC-HRMS chromatograms after extraction of the ion traces of the quasi molecular ions ($[M+H]^+$) of PC-7 (*m/z* 683.5762, blue line), PC-8 (*m/z* 751.6388, red line), and PC-9 (*m/z* 819.7014, green line) of the saponified flaxseed oil 1. **b**) Predicted (orange line) and measured LC retention times (coloured dots) of the homologous series of plastochromanol-8.

and PC-9 in the flaxseed oil samples by LC-Orbitrap-HRMS (Table S1, Supporting Information). Still, the deviations from the theoretical abundance ratio of 100.0%:51.9%:13.3% (PC-7) and 100.0%:62.7%:19.3% (PC-9) were in a small and acceptable range (Table S1, Supporting Information). However, background noise was not observed in the LC-Orbitrap-HRMS chromatograms when the exact masses of the $[M+H]^+$ ions of PC-7, PC-8, and PC-9 were extracted from the full scan chromatogram. Also, the peak heights were higher than the default peak height value of $>10^4$ recommended for LC-Orbitrap-HRMS measurements.[16–18]

Additional extraction of the exact masses of (potential and hitherto unknown) "plastochromanols with α-configuration" (5,7,8-trimethyl-substitution in the aromatic part) did not indicate their presence. Namely, an additional methyl group would shift $[M+H]^+$ by 14.0157 u to higher mass (e.g., α-PC-8 *m/z* 765.6544). In the same way, absence of "plastochromanols with δ-configuration" (8-methyl-substitution in the aromatic part) could be excluded by screening $[M+H]^+$ at 14.0157 u below the one of PC-8 (δ-PC-8 *m/z* 737.6231). However, the mass spectrum of PC-8 featured ions corresponding with $[M+H]^+$ of "δ-PC-8" configuration with only up to seven isoprene units similar to the quasi molecular ions $[M+H]^+$ of PC-8 homologues with up to six isoprene units (Figure S1, Supporting Information). Yet, these were all fragment ions of the conventional PC-8.

Subsequent quantification of PC-8 could be carried out by means of an accurately weighed PC-8 standard recently isolated by countercurrent chromatographic techniques.[7] LC-Orbitrap-HRMS determinations resulted in virtually the same PC-8 amounts of ≈315 and ≈350 mg kg^{-1} oil in the two flaxseed oil samples (**Table 1**). These amounts were slightly higher than the upper range of PC-8 previously

Table 1. Amounts of plastochromanol-8 (PC-8) and ratios of plastochromanol-7 to plastochromanol-8 (PC-7/PC-8) and plastochromanol-9 to plastochromanol-8 (PC-9/PC-8) in flaxseed oils ($n = 2$), rapeseed oil, mustard oil ($n = 2$), camelina oil, candle nut oil, and *Cecropia* leaves.

	HPLC/FLD		LC-Orbitrap-HRMS					
Sample[a)]	PC-8 [mg kg^{-1}]	Mean value of PC-8 [mg kg^{-1}]	PC-8 [mg kg^{-1}]	Mean value of PC-8 [mg kg^{-1}]	PC-7/PC-8	Mean value PC-7/PC-8	PC-9/PC-8	Mean value of PC-9/PC-8
Flaxseed oil 1 A	290	288	337	315	1.01%	0.97%	0.68%	0.65%
Flaxseed oil 1B	286		293		0.94%		0.63%	
Flaxseed oil 2 A	330	339	368	352	0.39%	0.37%	1.12%	1.01%
Flaxseed oil 2 B	347		336		0.35%		0.90%	
Rapeseed oil A	80	78	77	74	0.51%	0.56%	0.26%	0.24%
Rapeseed oil B	76		71		0.60%		0.22%	
Camelina oil A	38	39	35	37	0.37%	0.44%	0.12%	0.09%
Camelina oil B	39		39		0.51%		0.06%	
Mustard 1 oil A	112	115	119	118	0.34%	0.38%	0.36%	0.37%
Mustard oil 1 B	117		117		0.42%		0.38%	
Mustard oil 2 A	79	81	76	80	0.31%	0.34%	0.34%	0.36%
Mustard oil 2 B	82		84		0.37%		0.39%	
Candle nut oil A	87	89	95	91	0.51%	0.56%	0.97%	0.94%
Candle nut oil B	90		87		0.61%		0.91%	
Cecropia leaves A	925	915	893	888	0.60%	0.55%	0.50%	0.52%
Cecropia leaves B	905		883		0.51%		0.55%	

[a)] PC-8 determined by HPLC/FLD and LC-Orbitrap-HRMS, PC-7 and PC-9 determined by LC-Orbitrap-HRMS.

reported in flaxseed oil samples (≈170–300 mg PC-8 kg^{-1} oil[19]). These authors also had used a semi-synthesized PC-8 reference standard.[19] Also other colleagues reporting PC-8 levels in the past had isolated an PC-8 standard[4,5,20,21] or used a structurally related compound like γ-tocopherol as a reference standard.[10] In addition to isolating a PC-8 standard by countercurrent chromatographic techniques,[7] isolation by analytical supercritical fluid chromatography (SFC),[22] preparative column chromatography followed by thin layer chromatography (TLC)[4] or semi-preparative HPLC is also possible.[21] These studies brought afore that the PC-8 content of seeds was dependent of genetic factors[12,23] and the degree of ripeness.[24] In the present LC-Orbitrap-HRMS chromatograms of the flaxseed oils, PC-8 was accompanied by two small peaks originating from PC-7 and PC-9 which confirmed the recent indication in a different flaxseed oil sample by omitting the cumbersome procedure of Hammerschick et al.[7]

In void of a reference standard, PC-7 and PC-9 were determined by the peak areas of the extracted m/z values of $[M+H]^+$ ions relative to one of the quantitative standard of PC-8. With this approach PC-7 and PC-9 were present at ≈1.0% and ≈0.65%, respectively, of PC-8 in flaxseed oil 1. Levels were the same in flaxseed oil 2 but the relevance of the homologues was reversed, namely ≈0.4% PC-7 and ≈1.0% PC-9 (Table 1). The semi-quantification as outlines above indicated the presence of ≈1.3–3.5 mg kg^{-1} oil PC-7 and PC-9 in the two flaxseed oil samples (Table 1).

Remarkably, the contributions of PC-7 and PC-9 were similar to those observed during their discovery during the isolation of PC-8 (both <1% of PC-8[7]). However, with focus on the isolation of PC-8, a partial loss of PC-7 and PC-9 could not have been excluded in the previous study. Yet, the comparable abundance ratios of PC-7 and PC-9 relative to PC-8 indicated little—if any—loss of the novel minor plastochromanols. Accordingly, it seemed that PC-7 and PC-9 were generally present in flaxseed oils at the reported percentage of PC-8. After the verification of their presence in flaxseed oil, we aimed to detect PC-7 and PC-9 in further matrices previously known to feature PC-8 (Sections 2.2 and 2.3).

2.2. Detection and Quantification of PC-8, PC-7, and PC-9 in Further Oils (Rapeseed Oil, Camelina Oil, Mustard Oil, and Candle Nut Oil)

The PC-8 content of ≈75 mg kg^{-1} rapeseed oil was lower than in the flaxseed oil samples (Table 1). Still, this amount was in the upper end of the reported literature range of ≈8–90 mg PC-8 kg^{-1} in rapeseed oil.[19,25] However, due to the overall lower amount of PC-8 of 1/4th or less compared to the flaxseed oils, only three instead of four ^{13}C isotope peaks could be detected for PC-8 (Table S1, Supporting Information). Similarly, only a very small intensity of the monoisotopic peak of PC-7 and PC-9, respectively, and no ^{13}C isotope peaks could be detected at the retention times known from the flaxseed oil samples (Table S1, Supporting Information). Under these unfavorable circumstances with very low peak heights (<10^4) and the lack of verifying isotope peaks suitable as confirmation ions, the presence of PC-7 and PC-9 could be unequivocally confirmed but exact (semi-)quantitative levels could not be reported this time. Cautiously spoken, PC-7 was estimated to amount to ≈0.56% of PC-8 and PC-9 about ≈0.24% of PC-8 (Table 1).

Similar observations were made with camelina oil, a species related to rape within the *Brassicaceae* family. The present camelina seed oil contained ≈38 mg kg^{-1} oil PC-8 (Table 1), which was within the range of 15–43 mg kg^{-1} oil reported in the literature.[19] Although the PC-8 amount was even lower than in the flaxseed oil samples discussed afore, the presence of PC-7 and PC-9 could be confirmed in the camelina oil by mass-selective peaks at the correct retention times. However, the peaks in the LC-Orbitrap-HRMS chromatogram were very small (<10^4 counts) and verifying isotope peaks could not be detected. A careful estimation indicated PC-7 and PC-9 amounts of ≈0.44% and ≈0.09% of PC-8 (Table 1).

White mustard (*Sinapis alba*) seed oils had not been analyzed before on PC-8 but a brown mustard (*Brassica juncea*) oil was found to contain 46 mg PC-8 kg^{-1} oil.[26] The two white mustard oils showed even higher PC-8 amounts of ≈117 and ≈80 mg kg^{-1} oil (Table 1). Next to different origins (Brandenburg, Eastern Germany vs Swabian Alb, Southern Germany), genetic factors, cultivation conditions, but also differences in production process and storage could have been the reason for differences in the PC-8 content.[12,23,24] Both investigated white mustard oils also showed a similar plastochromanol pattern. The PC-7 and PC-9 content of ≈0.37% of the PC-8 content was the same in both samples and they amounted to ≈0.4 mg kg^{-1} in mustard oil 1 and ≈0.3 mg kg^{-1} in mustard oil 2, respectively (Table 1).

Oil from *Euphorbiaceae* candle nuts (*Aleurites moluccanus*), also known as the kukui nut tree, was investigated because of its botanical relationship to the *Euphorbiaceae* rubber tree (*Hevea brasiliensis*), in which PC-8 was first detected almost 60 years ago.[1] In agreement with these predictions, PC-8 was present at ≈90 mg kg^{-1} oil, and this amount was similar to those of the mustard oils (Table 1). In addition to the first identification of PC-8 in this matrix, PC-7 and PC-9 could also be detected at ≈0.56% (PC-7) and ≈0.94% (PC-9) of PC-8. Accordingly, the PC-7 and PC-9 amounts relative to PC-8 were higher than in the studied rapeseed, camelina, and mustard oils (Table 1). Semi-quantification revealed a PC-7 content of ≈0.5 mg kg^{-1} and a PC-9 content of ≈0.8 mg kg^{-1} in the candle nut oil.

Measured *m/z* values of the isotopic pattern of PC-7, PC-8, and PC-9 could be determined with high accuracy for rapeseed, camelina, mustard, and candle nut oil (mass deviations <4 ppm between theoretical and measured *m/z* values, Table S1, Supporting Information).

2.3. Detection and Quantification of PC-8, PC-7, and PC-9 in *Cecropia* Leaves

In the same way, PC-8 could be quantified by LC-Orbitrap-HRMS at 900 mg kg^{-1} dry weight in *Cecropia* leaves from a tree grown in a greenhouse at our university (Table 1). This value was ≈50% of the amount determined in the leaves of an unspecified member of *Cecropia* sp.[12] Based on the lipid weight, the PC-8 amount in the present *Cecropia* leaves was ≈100 fold higher than in flaxseed oils. This high PC-8 content in the present sample from the greenhouse in Hohenheim (Germany) confirmed that neither the habitat nor the life-long growth in the greenhouse were crucial for the presence of high amounts of PC-8 in *C. peltata L.* The reasons for the different PC-8 contents in this and the literature sample of *Cecropia* sp. are manifold and could be due to genetic factors including the species, climate and growing conditions, leaf age and developmental stage, and harvest time.[12,23] In particular, the effect of the growing conditions of the *Cecropia* leaves in the greenhouse is difficult to predict as no other comparable examples are known in the literature. On the one hand, only leaves from the top of the tree were used this time as samples, but on the other hand, the leaves were collected during the winter months without high sun exposure.

Despite the differences, the very high content of PC-8 in *Cecropia* leaves detected by Kruk et al.[12] could be confirmed in the present study. It is worth recalling that presence of PC-8 in leaves has been associated with predominant or exclusive presence in chloroplasts.[12] In this context, it has been suggested that PC-8 could be a scavenger of singlet oxygen in hydrophobic environments.[5,12] On the first glance, this sounds surprising since quenchers usually are rich in multiple conjugated double bonds which are not found in PC-8. However, PC-8 is known to be very instable and isomers with conjugated double bonds may be formed in contact with light. Hence, it would be very likely that the scavenger power increases with increasing lengths of the isoprenoid side chain.

Similarly, to the other samples, the peak of PC-8 in the LC-Orbitrap-HRMS chromatogram was flanked by low abundant peaks of PC-7 and PC-9 at a distance of ≈1 min (Section 2.1). Also, ratios of ≈0.55% PC-7 and PC-9 of PC-8 were determined in the *Cecropia* leaves (Table 1). Accordingly, the amount of PC-7 and PC-9 was ≈5 mg kg^{-1} dry weight in *Cecropia* leaves. Measured *m/z* values of the isotopic pattern of PC-7, PC-8, and PC-9 with the deviations between measured and theoretical values (all <4 ppm) were similar to those determined in flaxseed oil (Table S1, Supporting Information).

3. Concluding Remarks

LC-Orbitrap-HRMS analysis confirmed the presence of two novel members of the tocochromanol family, namely PC-7 and PC-9 in the oil of flaxseed, rapeseed, camelina seed, and white mustard seed as well as in the leaves of *Cecropia*. By means of a quantitative PC-8 standard, shared amounts of ≈0.3–5 mg PC-7 and PC-9 per kg flaxseed oil, mustard oil, candle nut oil, or *Cecropia* leaves (dry weight) were semi-quantified being about two orders of magnitude lower than the sum of tocochromanols in the samples. However, these amounts were still in the range of PC-8 in many other matrices (e.g., sunflower oil ≈2 mg PC-8 kg^{-1} oil, rice bran oil ≈1 mg PC-8 kg^{-1} oil)[12,19] and also of (minor) tocochromanols in food (e.g., carrot: ≈5 mg α-tocopherol kg^{-1}, tomato ≈3.8 mg γ-tocopherol kg^{-1}).[27] Moreover, the present LC-Orbitrap-HRMS method can be used to screen other matrices on PC-7, PC-8, and PC-9 in this concentration range.

The biosynthesis of PC-8 in plants involves several steps with the side chain being introduced at a very early stage.[12] Details of the biosynthesis of PC-7 and PC-9 are unknown. At this point, it is unclear whether PC-7 and PC-9 were willingly formed by the organisms themselves or whether they were artefacts of the PC-8 biosynthesis. PC-8 is formed by solanesylation of homogenistic acid with nonaprenyl diphosphate by all-*trans*-nonaprenyl diphosphate synthase.[12] Hence, one possibility could be that PC-7 is biosynthesized in the same manner by

octaprenyl diphosphate (one isoprenoid unit less) and PC-9 with decaprenyl diphosphate (one isoprenoid unit more). However, this needs to be verified in the future. The LC-Orbitrap-HRMS method reported in this study could help to solve some of these open questions and to investigate related topics of the plastochromanol family in future. For future quantification of these novel compounds and investigation of their bioactivity, an isolation from *Cecropia* leaves, which showed the highest levels with ≈5 mg PC-7 or PC-9 kg^{-1} dry matter, would be conceivable. The isolation of PC-7 and PC-9 could be carried out in a similar way as described for PC-8.[7]

4. Experimental Section

Chemicals: Acetic acid (96%), *iso*-propanol (HPLC grade), potassium hydroxide (KOH, >85%), and pyrogallol (>99%) were purchased from Carl Roth (Karlsruhe, Germany). Ethyl acetate (>99%), formic acid (LC-MS grade), and sodium chloride (NaCl, >99%) were ordered from Sigma–Aldrich (Steinheim, Germany), whereas methanol (HPLC grade), and *n*-hexane (HPLC grade) were from Th. Geyer (Renningen, Germany). Fisher Scientific (Schwerte, Germany) delivered ammonium formiate (LC-MS grade), while acetonitrile was from Bernd Kraft (Duisburg, Germany). Technical grade ethanol from BASF (Ludwigshafen, Germany) was distilled before use. Westfalen Company (Münster, Germany) supplied nitrogen (99.95%) and demineralized water was produced in-house. PC-8 (>99%) was isolated from flaxseed oil as previously described.[7] In brief, the oil was carefully saponified with ethanolic KOH using pyrogallol and a nitrogen atmosphere to prevent PC-8 oxidation. Then, PC-8 was collected with unsaponifiable matter and subsequently enriched by gel permeation chromatography followed by countercurrent chromatography using the solvent system *n*-hexane/benzotrifluoride/acetonitrile (20:7:13, v/v/v). Pure PC-8 (>99%) was obtained in a final purification step by centrifugal partition chromatography using the solvent system hexamethyldisiloxane/acetonitrile (1:1, v/v).[7]

Samples: One cold-pressed flaxseed oil (flaxseed oil 1), one native cold-pressed flaxseed oil (flaxseed oil 2), and one native cold-pressed rapeseed oil (rapeseed oil) were purchased from commercial suppliers in Stuttgart, Germany. Two native cold-pressed white mustard oils (*S. alba*, cultivation areas: Brandenburg Saxony, Germany [mustard oil 1] and Swabian Alb, Germany [mustard oil 2]), one native cold-pressed camelina oil, and one refined candle nut (kukui nut) oil were bought from several internet stores. Leaves of *C. peltata L.* (*Cecropia* leaves), grown in a greenhouse on campus of the University of Hohenheim, Stuttgart, were collected on the January 16, 2023. Collected leaves were immediately separated from their stems, cut to pieces, and directly frozen. After 2 days, the deep-frozen samples were lyophilized in a LYOVAC GT 2 system (Leybold-Heraeus, Hürth, Germany). Afterwards, the freeze-dried leaves were homogenized using a laboratory mill (IKA A11, IKA, Staufen, Germany) and stored at −80 °C until analysis.

Analytical Saponification: In duplicate, ≈30–40 mg of flaxseed oil, ≈40–60 mg of rapeseed oil, mustard oil, camelina oil, candle nut oil, and ≈200 mg of freeze-dried and homogenized *Cecropia* leaves were placed in a brown glass tube, respectively. After adding 2 mL 6% pyrogallol in ethanol w/v, 0.9 mL demineralized water, and 0.9 mL 50% KOH in water w/w, the tube was flushed with nitrogen.[28] The sealed tubes were heated to 75 °C with occasional shaking and after 1 h, the sample solutions were cooled in an ice bath. After the addition of 1 mL demineralized water, 0.3 mL acetic acid, and 3 mL *n*-hexane/ethyl acetate (9:1, v/v), the sealed tubes were shaken vigorously and after 5 min the upper organic phases were transferred into new tubes, respectively. The extraction of the lower aqueous phase with 3 mL *n*-hexane/ethyl acetate (9:1, v/v) was repeated twice. The solvent of the combined organic phases was removed by a gentle stream of nitrogen at 30 °C. The residues were dissolved in 3 mL *n*-hexane/ethyl acetate (9:1, v/v) and washed three times with 2 mL KOH in water (pH 9). Aliquots of 2 mL each of the upper phases were transferred to pre-weighed tubes. After evaporation of the solvent, the residue masses were determined gravimetrically. Samples were re-dissolved in 1 mL *n*-hexane/ethyl acetate (9:1, v/v) and stored at −80 °C until analysis.

High Performance Liquid Chromatography with a Fluorescence Detector (HPLC/FLD): HPLC/FLD analysis were performed as previously described in details.[7,19] Briefly, 3, 5, or 10 µL of aliquots (≈0.1 mg mL^{-1}) of the saponified samples, and 3, 5, 10, 20 µL of PC-8 standard (2 µg mL^{-1}) were injected onto a Nucleosil C_{18} column (250 mm length × 4.6 mm internal diameter, 5 µm particle size [Macherey-Nagel, Düren, Germany]). Separations were performed isocratically with *n*-hexane/methanol (15:85, v/v) at a flow rate of 1.5 mL min^{-1}. The eluent was measured with an FP-920 fluorescence detector (λ_{ex} = 290 nm, λ_{em} = 330 nm, Jasco, Tokyo, Japan) during the run time of 15 min.

High Performance Liquid Chromatography with High Resolution Mass Spectrometer (LC-HRMS): LC-Orbitrap-HRMS analyses were performed with an Agilent 1290 system (Waldbronn, Germany) connected with a Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific, Waltham, MA, USA) according to Hammerschick et al.[7] In brief, 5–20 µL of aliquots (≈1 mg mL^{-1}) of the saponified samples, and 3 µL of PC-8 standard (0.2 mg mL^{-1}) were injected and chromatographed on a Kinetex 1.7 µm PFP (pentafluorophenylpropyl) column (150 mm length × 2.1 mm internal diameter, Phenomenex, Aschaffenburg, Germany) at 40 °C. ACN/water (6:4, v/v) with 5 mM ammonium formiate + 0.2% formic acid (solvent A) and *iso*-propanol/ACN (9:1, v/v) with 5 mM ammonium formiate + 0.2% formic acid (solvent B) was used in a gradient elution at 0.3 mL min^{-1} as follows: 0 min (100% A)—25 min (100% B)—29 min (100% A)—35 min (100% A). Analytes were ionized by atmospheric pressure chemical ionization (APCI) and *m/z* 110–1250 was recorded at a resolution (R) of 70,000 (FWHM) using a maximum ion injection time of 100 ms and an inclusion list with the quasi molecular ions $[M+H]^+$ of PC-8 (*m/z* 751.6388) and its potential homologues PC-10 (*m/z* 887.7640), PC-9 (*m/z* 819.7014), PC-7 (*m/z* 683.5762), PC-6 (*m/z* 615.5136), PC-5 (*m/z* 547.4510), and PC-4 (*m/z* 479.3884).[7]

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

T.H.: investigation, conceptualization, visualization, supervision, writing – original draft. J.G.: investigation, writing – review & editing. W.V.: conceptualization, resources, supervision, writing – review & editing.

Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article.

Keywords

plastochromanol-7, plastochromanol-8, plastochromanol-9, tocochromanol, vitamin E

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