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Edited by

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INRA/ AgroParisTech and College de France
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75005 Paris
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PB 8149 Dep
0033 Oslo
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Ferulate Trimers and Tetramers in Cereal Grains

Mirko Bunzel[1,2,*], Diane Dobberstein[1], Birgit Heuermann[1], Philipp Brüning[1], Carola Funk[1], Hans Steinhart[2], Hoon Kim[3], John Ralph[3,4]

[1]*University of Minnesota, Department of Food Science and Nutrition, 1334 Eckles Avenue, St. Paul, MN 55108, USA. * Corresponding author: mbunzel@umn.edu*
[2]*University of Hamburg, Institute of Biochemistry and Food Chemistry, Grindelallee 117, 20146 Hamburg, Germany.*
[3]*U.S. Dairy Forage Research Center, Agricultural Research Service, U.S. Department of Agriculture, 1925 Linden Drive West, Madison, WI 53706, USA.*
[4]*Department of Biological Systems Engineering, University of Wisconsin, Madison, WI 53706, USA.*

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Summary

Due to their ability to cross-link polysaccharides with each other and with lignin in the plant cell wall, ferulate and its dehydrodimers have been extensively studied in the past. The occurrence of higher ferulate oligomers has been recently demonstrated in *in vitro* reactions, and *in vivo* by isolation of ferulate dehydrotrimers from maize bran fiber and maize straw. *In vitro* reactions with ethyl ferulate and peroxidase/H_2O_2 gave 8-*O*-4/8-5(cyclic)-dehydrotriferulate as the main trimer and 8-5(cyclic)/4-*O*-5/8-5(cyclic)-dehydrotetraferulate as the main tetramer. Seven different ferulic acid dehydrotrimers have been isolated from maize bran and identified as 5-5/8-*O*-4-, 5-5/8-*O*-4(H_2O)-, 8-8(cyclic)/8-*O*-4-, 8-*O*-4/8-*O*-4-, 8-*O*-4/8-5(non-cyclic), 8-5(non-cyclic)/5-5-, and 8-8(tetrahydrofuran)/5-5-dehydrotriferulates. In addition, two ferulate tetramers, 4-O-8/5-5/8-*O*-4- and 4-*O*-8/5-5/8-5(non-cyclic)-dehydrotetraferulates, were detected in maize bran fiber.

Introduction

Plants belonging to the family of Gramineae contain hydroxycinnamates, principally ferulate, bound to cell wall polysaccharides. In graminaceous plants ferulates are bound to arabinoxylans where they acylate the *O*-5-positions of the arabinosyl side-chains [1].
Despite being minor compounds in plant cell walls, ferulates and ferulate oligomers have a significant role in cross-linking wall polymers. Polysaccharides are cross-linked to each other but also to lignin and probably to proteins. Cross-linking of plant cell wall polymers strengthens the wall [2] and also influences the properties of wall components in food ingredients. In cereal grains, cross-linking of polysaccharides with each other or lignin may influence the physiological effects of dietary fiber due to changes of its physico-chemical properties [3]. In addition cross-linking of polysaccharides with each other or with proteins may affect the quality of dough and bread [4]. Furthermore, hydroxycinnamates show antioxidant [5, 6] and possibly chemoprotective effects [7]. In ruminant nutrition, hydroxycinnamate cross-links are an issue due to the negative impacts of cell wall cross-links on forage digestibility [8].
Ferulate dehydrodimerization of hydroxycinnamate esters is possible via oxidative coupling mechanisms [9, 10], but photochemical dimerization processes also occur to a lesser degree. Several ferulate dehydrodimers (DFA) were found in a variety of graminaceous plants, for

example in different cereal grains (maize, wheat, spelt, rice, wild rice, barley, rye, oats, millet) [3].

The aim of our studies was to demonstrate possible trimeric and tetrameric ferulate cross-links in *in vitro* reactions and to detect ferulate trimers and tetramers in maize and other cereals.

Material and Methods

***In vitro* reactions.** Ethyl ferulate was used as substrate and horseradish peroxidase/H_2O_2 (molar ferulate:H_2O_2 ratio 1:1) as the oxidizing system. Oligomerization was performed in a sodium phosphate buffer at pH 6.0. Addition of 30% ethanol to this solution significantly enhanced the proportion of higher ferulate oligomers (*unpublished results*). Size exclusion chromatography of the reaction product mixtures using Bio-Beads S-X3 as the stationary phase and tetrahydrofuran as eluent led to fractions separated by molecular weight. The fractions containing ferulate trimers and tetramers were further separated by using RP-HPLC. The major ferulate trimer and tetramer were structurally elucidated by using mass spectrometry and one- and two-dimensional NMR.

Ferulate trimers and tetramers form maize bran and maize straw. Maize bran insoluble dietary fiber was isolated by sequential treatment of maize bran with a-amylase, protease and amylogucosidase. Maize straw was exhaustively extracted with ethanol and acetone. Maize insoluble fiber or maize straw was hydrolyzed by using 2 M NaOH for 18 h at room temperature. After acidification of the hydrolyzate the liberated ferulate mono- and oligomers were extracted into diethyl ether. Following a liquid-liquid separation clean-up, the ferulate mono- and oligomers were fractionated using size exclusion chromatography on BioBeads S-X3 and/or Sephadex LH-20 chromatography. Further separation or purification was carried out by semi-preparative phenylhexyl-RP-HPLC. Structural elucidation of pure ferulate trimers and tetramers was performed using UV-spectroscopy, mass spectrometry, and one- and two-dimensional NMR experiments. Experimental details are described in references [11-14].

Results and Discussion

***In vitro* reactions.** The main trimer (estimated by UV-detection) from the *in vitro* system was identified as the triethylester of 8-*O*-4/8-5(cyclic)-dehydrotriferulic acid (8-*O*-4/8-5c-TriFA, **1**, Fig. 1) and the main tetramer as the tetraethylester of 8-5(cyclic)/4-*O*-5/8-5(cyclic)-dehydrotetraferulic acid (8-5c/4-*O*-5/8-5c-TetraFA, **2**) (*unpublished results*). The isolation of 8-*O*-4/8-5c-TriFA **1** as the main product from our model system was surprising since the 8-5(cyclic)-DFA-diethylester dominates the dimer fraction in comparable *in vitro* reactions [15] whereas 8-*O*-4-DFA-diethylester constitutes only a small portion of the dimers. Therefore, it was supposed that the main trimers would be formed by addition of a third ferulate to a pre-formed 8-5(cyclic)-diferulate. However, the formation of 8-*O*-4/8-5c-TriFA **1** requires that the 8-*O*-4-diferulate is formed first, and the 8-5(cyclic)-linkage is formed by addition of the third ferulate; a pre-formed 8-5(cyclic)-diferulate is not able to couple via

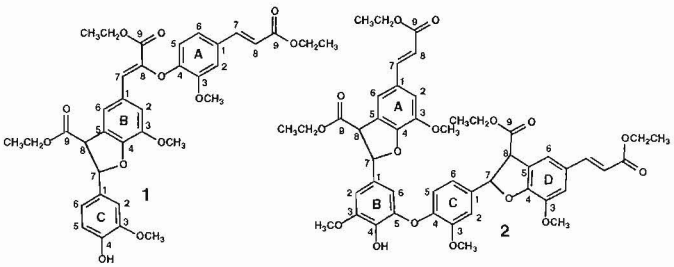

Figure 1. Dehydrotriferulate and dehydrotetraferulate ethyl esters isolated from model reactions (compounds 1 and 2)

the propenylic 8-position because the moiety's phenolic hydroxyl group is blocked precluding the formation of a radical with single-electron density at the required 8-position.
The main tetramer is likely formed by 4-*O*-5-coupling of two pre-formed 8-5(cyclic)-DFA-diethylesters. In analogy to the biosynthesis of lignin, formation of a 4-*O*-5-linkage is logical since 5-5- and 4-*O*-5-linkages in lignin predominantly stem from oligomer-oligomer coupling [16].
Ferulate trimers and tetramers form maize bran and maize straw. Seven ferulate trimers were isolated and identified from maize bran insoluble fiber (**3-9**, Fig. 2). Com-

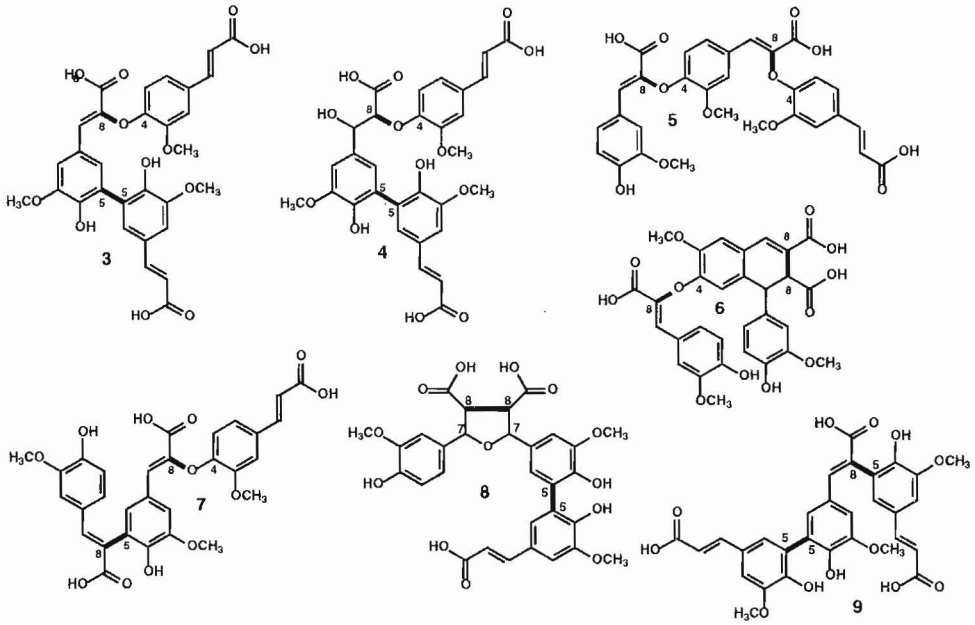

Figure 2. Dehydrotriferulates isolated from maize bran insoluble fiber.

pounds **7** and **9** likely do not exist in this form *in planta*: compounds containing an 8–5(non-cyclic)-coupled dimeric unit are probably formed from their phenylcoumaran precursors containing an 8–5(cyclic)-coupled dimeric unit during saponification. As shown in Figure 3, opening of the 2,3-dihydrobenzofuran ring occurs following deprotonation of the phenolic

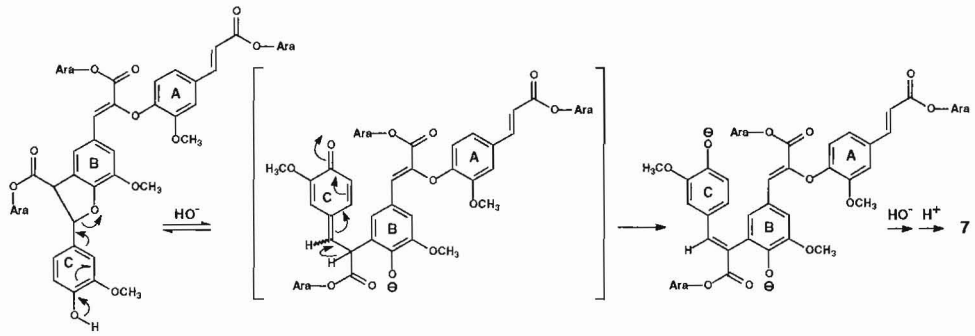

Figure 3. Formation of trimer 7 from its supposed precursor in the plant, the arabinoxylan ester of 8-O-4/8-5c-triferulate.

hydroxyl group. Re-aromatization of ring C is achieved by elimination of the acidic 8-proton.

For some of these trimers the reaction sequence is determined as more detailed in the original papers [11-14]. Ferulate trimers **3** and **6** were also identified in alkaline hydrolyzates of the insoluble fibers of wheat and rye by RP-HPLC with diode-array detection using the isolated trimers from maize bran as standard compounds. Up to four trimers, e.g. trimers **3** and **5**, were also isolated from maize straw and identified by mass spectrometry and NMR, demonstrating that the formation of higher ferulate oligomers is not only restricted to cereal grains.

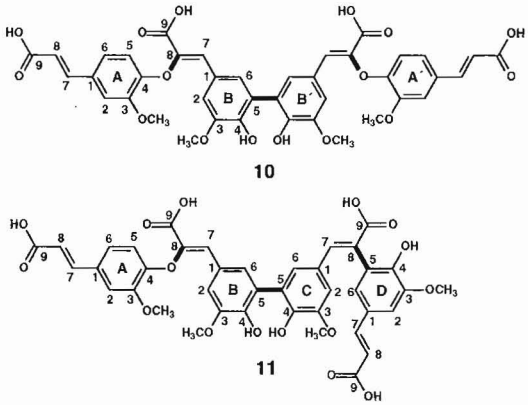

Figure 4. Dehydrotetraferulates isolated from maize bran insoluble fiber.

Two ferulate tetramers were isolated from maize bran and identified as 4-*O*-8/5-5/8-*O*-4- and 4-*O*-8/5-5/8-5(non-cyclic)-dehydrotetraferulates (**10**, **11**, Fig. 4). Since both tetramers contain a 5-5/8-*O*-4-dehydrotriferulate moiety, the predominant dehydrotrimer in maize bran, it is not possible to deduce whether tetramers are formed by coupling of a fourth unit to a preformed dehydrotriferulate or by 5-5-coupling of preformed 8-*O*-4- and 8-5-dehydrodiferulates.

The findings of such a diverse array of trimers and tetramers emphasize the significance of ferulate oligomers as cross-links in the plant-cell wall and in cell-wall related food and feed.

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