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DIFERULIC ACID AS A POSSIBLE CROSSLINK IN HEMICELLULOSES FROM WHEAT GERM

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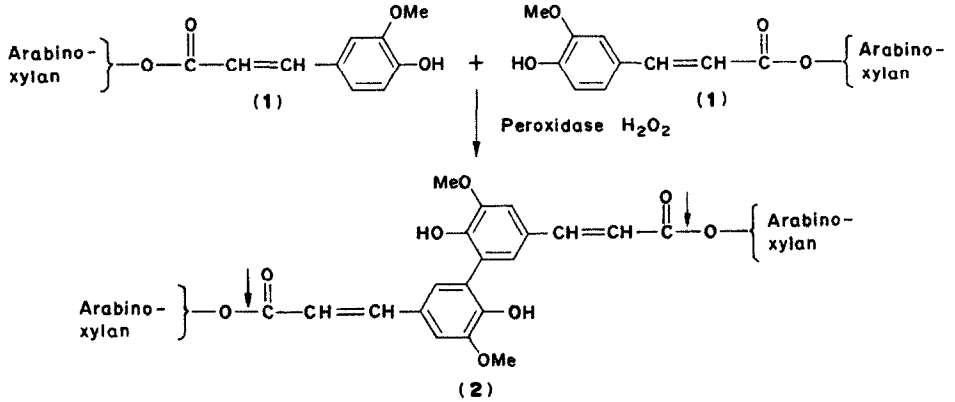
Abstract—Bound diferulic acid has been identified in small amounts in the water-insoluble pentosans of wheat endosperm. Evidence is presented suggesting that diferulic acid crosslinks adjacent polysaccharide molecules and reduces their solubility.

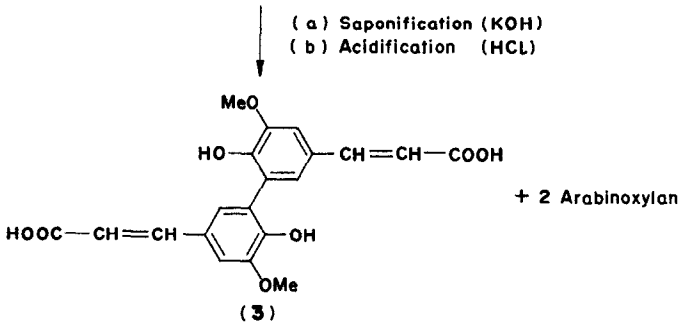
INTRODUCTION

White wheat flour (*Triticum aestivum*) contains thin cell walls of endosperm which consist predominantly of pentosans (arabinoxylans) the greater part of which are water-insoluble[1]. Smaller quantities of hexosans (β-glucan and glucomannans) [1, 2] and a soluble arabinogalactan-peptide have also been identified [3, 4]. The soluble [5] and insoluble [6] arabinoxylans contain small amounts of ferulic acid bound by ester linkages to the pentosans. Both arabinoxylans are similar in composition; the reasons for the insolubility of the larger part of these pentosans are not known [2, 6]. It has been suggested that ferulic acid residues are dimerized by oxidative phenolic coupling to form diferulic crosslinks which would insolubilize the pentosans [6, 7]. To test this hypothesis the insoluble wheat flour pentosans were investigated for the possible presence of bound diferulic acid.

RESULTS AND DISCUSSION

The water-insoluble pentosans were isolated with a yield of 4.3% from the ground endosperm of wheat by water extraction, wet sieving and enzymatic degradation of starch. The preparation contained about 60% polysaccharides (2/3 arabinoxylans and 1/3 β-glucans); the remainder consisted of protein and lipid material and





(3)

very small amounts of bound phenols (1.8 mg/g) [8]. The polyphenols were liberated by alkali treatment. Ferulic and diferulic acid (3) could be identified by TLC, the ratio of the two acids being 5:1. The presence of both acids could be confirmed by GLC, the peak with RR_t = 20.8 min. was identical with synthetic trimethylated diferulic acid. The detection of diferulic acid by this GLC technique proved to be difficult because side reactions during methylation reduced the yield of methylated product.

Neither free nor bound diferulic acid have so far been reported in plants. However, (3) was shown to be formed when synthetic ferulic acid esters of polysaccharides were oxidized with peroxidase-H_2O_2 [9]. Similarly, (3) is produced when solutions of wheat flour arabino-xylans containing small amounts of bound ferulic acid are treated by this enzyme system; the reaction is readily visible by gelation of the solution [7]. The formation of (3) can be explained by oxidative phenolic coupling of ferulic acid side groups (1) attached to arabino-xylan chains (see Scheme). (3) Is liberated by alkali saponification of (2) without degradation of the polysaccharides. The bulky polysaccharide chains prevent side reactions during oxidation which extensively occur when free ferulic acid is oxidized [10]. The detection of small amounts of diferulic acid in hemicelluloses from wheat endosperm points to a similar coupling reaction during development of the wheat grain; it could also explain the insolubility of some of the arabinoxylans.

EXPERIMENTAL

Preparation of water-insoluble pentosans. 250 g of white wheat flour (milled from No. 2 Manitoba Northern, hard red spring wheat) was first extracted with 500 ml ice cold H_2O and centrifuged; the extraction was repeated 4 times with 300 ml ice cold H_2O. Residue was separated into starch and wheat gluten by wet sieving on silk bolting cloth (XX double extra, 0.1 mm aperture); the gluten proteins were retained on the cloth. The starch suspension was centrifuged at 1300 *g* for 15 min and at 7000 *g* for 15 min , the insoluble pentosans were associated with the light starch fraction ("starch tailings") which appeared as a swollen, yellowish layer on top of the pure, white starch. This upper layer was scraped off, heated for 3 min to 80°C in Pi buffer of pH 7.2, cooled and treated with crystalline α-amylase (*Bac. subtilis*) in a dialysing bag [4, 8]. The starch free pentosan preparation was lyophilized and analyzed for lipids, proteins and carbohydrate composition by conventional methods [8].

Saponification and extraction of phenolic compounds. Ferulic and diferulic acid were liberated from 0.5–1 g of the pentosan preparation by treatment with 50 ml 0.5 N KOH at 60° under N_2 for 100 min; for the detection of diferulic acid by GLC about 20 g of pentosans had to be saponified. The slightly brown solutions were acidified to pH 3.5 by addition of ice cold 6 N HCl. The phenolic compounds were extracted with EtOAc, the extracts washed with H_2O, dried and evaporated to dryness. The oily residues were dissolved in MeOH or DMF for TLC and GLC analyses. Total phenols were measured with the Folin–Ciocalteau reagent using ferulic acid as standard.

Detection of ferulic and diferulic acid. For TLC, Si gel (Merck Kieselgel F_{254}) and the solvent system C_6H_6–dioxane–AcOH, 90:25:4 was used. Synthetic diferulic acid (3) [10, 11] and ferulic acid were used as reference compounds, the spots being located by diazotized benzidine and Folin-reagent; values were 0.18 and 0.41 respectively. GLC was carried out on a Fractovap 2200 with dual FID. A 2 m x 2 mm glass column packed with 1% OV-17 on Chromosorb G 80/100 DMCS, AW at isothermal temperatures of 145°C (ferulic acid) and 270°C (diferulic acid) and N_2 at 52 ml/min was used. Detector and injector temperatures were 220° for ferulic and 320° for diferulic acid, respectively. The phenolic acids were methylated prior to GLC with CH_2N_2 for 15 hr at room temp in the dark in DMF. The following reference compounds were prepared by partial and complete methylation with CH_2N_2: trimethylated diferulic acid and ferulic acid methyl ester by partial methylation in DMF–H_2O, 1:1 and Et_2O respectively, completely methylated diferulic and ferulic acid by methylation in DMF [10].

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