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Note

Separation of 5-N-alkylresorcinols by reversed-phase high-performance liquid chromatography

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5-*n*-Alkylresorcinols appear in various plants[1–7] as a mixture of homologs which differ in the length of the aliphatic chain. The analysis of 5-*n*-alkylresorcinols is usually performed either by gas chromatography[3–6,8] or by reversed-phase thin-layer chromatography[5]. The gas chromatographic method requires esterification of the compounds into more volatile derivatives. In case of higher homologs this process is incomplete[5] and can cause errors in quantitative determination of the homologs. Another disadvantage of this method is, that for isolation of the individual compounds de-esterification is necessary. Application of high-performance liquid chromatography (HPLC) on reversed-phase columns is more attractive for the separation of 5-*n*-alkylresorcinols because of rapidity and simplicity. In the past, this method has been applied successfully for the separation of phenols[9], alkylphenols[10], isomers of hydroxybenzoic acid[11], hydroxyphenylacetic acids[12] and catechins[13]. Both the analysis of the individual homologs of 5-*n*-alkylresorcinols isolated from grains and, the separation on preparative scale are important to understand the biological role of these compounds.

MATERIALS AND METHODS

Instrumentation

The HPLC separations were performed with a Pye Unicam (Cambridge, Great Britain) solvent delivery system (LC 20 separator). The chromatographic column was of stainless steel (25 × 0.4 cm I.D.) (Knauer, Berlin, G.F.R.) packed with bonded phase silica gel (LiChrosorb Si 60, RP-2, particle diameter 10 μm, E. Merck, Darmstadt, G.F.R.). Absorption measurements were done at 282 nm (the absorption maximum of 5-*n*-alkylresorcinols) with a variable wavelength spectrophotometer, model SP6-400 (Pye Unicam, Cambridge, Great Britain). The measuring volume of the flow-cell was 100 μl. A multi-range pen recorder (Hitachi, CPD 53, Japan) was used and the deflection due to the initial absorbance of the eluent was compensated electrically. The samples were injected with a Model 7105 universal septumless injector (Rheodyne, Berkeley, Calif., U.S.A.).

Reagents

For column elution different mixtures of methyl acetate, methanol and water were used. Methyl acetate and methanol (both HPLC quality) were purchased from J. T. Baker (Phillipsburgh, N.J., U.S.A.). Prior to use, the air from the eluting solvents was removed by sonication for 5 min with a bath sonicator (Branson, Danburg, Conn., U.S.A.).

Orcinol (5-*n*-methylresorcinol) and 5-*n*-pentadecylresorcinol were obtained from Aldrich (Milwaukee, Wisc., U.S.A.). 5-*n*-Alkylresorcinol samples from rye grains were prepared from acetone extracts of whole grains, according to the method of Mejbaum-Katzenellenbogen *et al.*[14]. 5-*n*-Heptadecylresorcinol, 5-*n*-nonadecylresorcinol, 5-*n*-heneicosoresorcinol, 5-*n*-tricosoresorcinol and 5-*n*-pentacosoresorcinol were obtained by reversed-phase thin-layer chromatography according to the method of Briggs[5] and analysed by gas chromatography. Samples were dissolved in the same solvent as used for elution, the concentration of the samples was 5% (w/v).

RESULTS AND DISCUSSION

Based on the results of the experiments of Briggs[5] it was suggested that liquid chromatography on reversed-phase columns, could offer a good possibility for separation of 5-*n*-alkylresorcinols. Methyl acetate–methanol–water mixtures were chosen because of the good solubility of 5-*n*-alkylresorcinols. These solvent mixtures are nearly transparent at 282 nm, rather volatile and have a high solvent polarity, ϵ° value. Dioxane was not suitable due to the rapid formation of peroxides. The polarity of the solvent system was varied by changing the amount of water, whereas the methyl acetate–methanol ratio was kept at 2:1 (w/v). The polarity of the eluent system strongly influences the retention time of the individual 5-*n*-alkylresorcinols and is indicated in the figure legends. Before sample analysis, the column was first washed with methyl acetate–methanol (2:1, v/v). Then the adsorbent was equilibrated with the eluent system until a stable baseline was obtained. Most of the experiments were performed at an average flow-rate of 2.5 ml/min. The experiments were done at room temperature, since no influence on the retention time was found between 15 and 35°. First, the retention times of orcinol and 5-*n*-pentadecylresorcinol and of each of the six 5-*n*-alkylresorcinols individually or in a mixture were determined. The optimal separation of a mixture of 5-*n*-alkylresorcinol homologs is illustrated in Fig. 1. The retention times increased with increasing aliphatic chain length. The separation of higher homologs, 15–25 carbon atoms in the aliphatic chain, is strongly influenced by the polarity of the eluent. The effect of reducing the water content on the separation of 5-*n*-alkylresorcinols is shown in Fig. 2. Small changes ($\pm$5%, v/v) in the percentage of the water content of the eluent induce significant changes in the retention times and separation capacity of the column. It was pointed out that the optimal water concentration of the methyl acetate–methanol mixture for the separation of 5-*n*-alkylresorcinols on a RP-2-silica column is 27% (v/v). The complete resolution by liquid chromatography of 5-*n*-alkylresorcinols isolated from grains, allows not only analyses of mixtures of these compounds but also isolation of the pure molecular species on a milligram scale as shown in Fig. 3. The main species in rye is the C_{19} derivative (5-*n*-nonadecylresorcinol). In wheat, essentially the same species are found with an increase in the percentage of the C_{21} derivative (5-*n*-

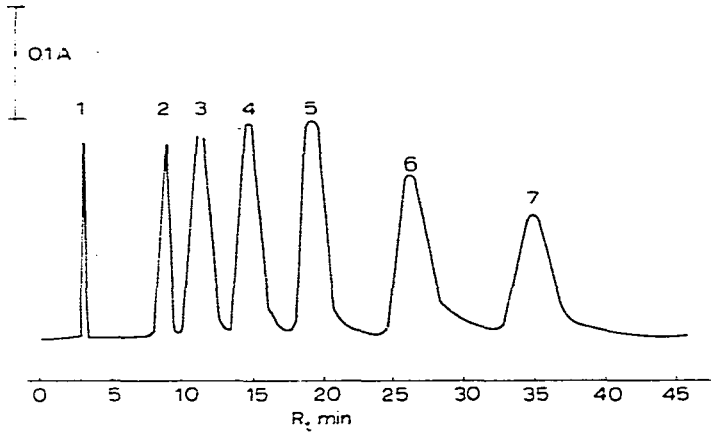

Fig. 1. Separation of 5-*n*-alkylresorcinols. Solvent system: methyl acetate–methanol (2:1, v/v), containing 27% (v/v) of water; flow-rate 2.5 ml/min; detection at 282 nm; sample load: 2.5 mg in 50 μl solvent; pressure 145 bar. 1 = 5-*n*-Methylresorcinol; 2 = 5-*n*-pentadecylresorcinol; 3 = 5-*n*-heptadecylresorcinol; 4 = 5-*n*-nonadecylresorcinol; 5 = 5-*n*-heneicosoresorcinol; 6 = 5-*n*-tricosoresorcinol; 7 = 5-*n*-pentacosoresorcinol.

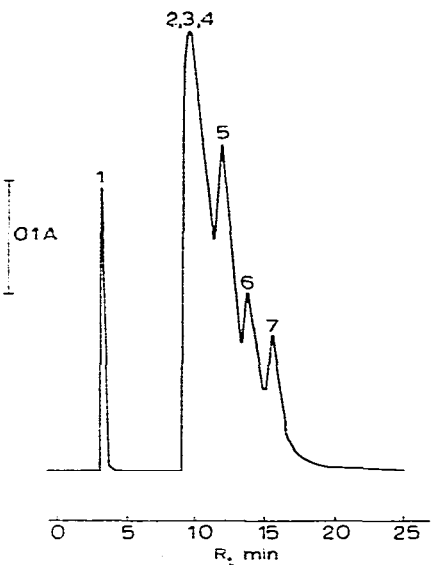

Fig. 2. Effect of reduced polarity on the separation of 5-*n*-alkylresorcinol homologs. Solvent system: methyl acetate–methanol (2:1, v/v), containing 22% (v/v) of water; flow-rate 2.5 ml/min; detection at 282 nm; sample load: 2.5 mg in 50-μl solvent; pressure 130 bar; peaks as in Fig. 1.

heneicosoresorcinol) and a decrease of the C_{17} derivative (5-*n*-heptadecylresorcinol). The separation and retention times proved to be extremely reproducible in this system. The application of HPLC provides a simple way for analyses and separation on a semi preparative scale of 5-*n*-alkylresorcinol homologs isolated from grains, without chemical modification of the compounds. Proton magnetic resonance measurements of the single species and of the total grain extracts of alkylresorcinols in C^2HCl_3 did not show the presence of methoxy groups.

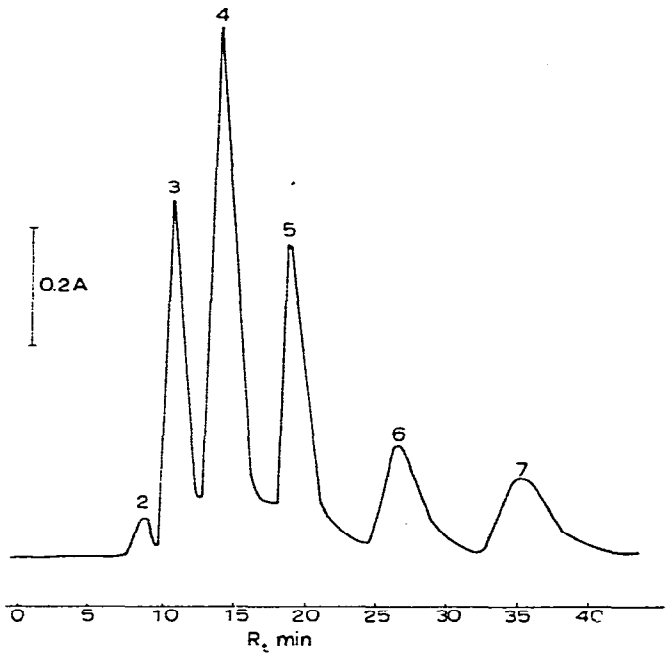

Fig. 3. Separation of 5-*n*-alkylresorcinols, isolated from rye grains. Solvent system: methyl acetate–methanol (2:1, v/v), containing 27% (v/v) of water; flow-rate 2.5 ml/min; detection at 282 nm; sample load: 7.5 mg in 150-μl solvent; pressure 145 bar; peaks as in Fig. 1.

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