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## Note

# Thin-layer chromatographic mapping of 5-*n*-alk(en)ylresorcinol homologues from cereal grains

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Alkyl and alkenyl derivatives of resorcinol are responsible for the deleterious effect of rye grains in animal breeding[1–3]. For the determination of the composition of 5-*n*-alk(en)ylresorcinol homologues in different samples of cereal grain materials, gas chromatographic (GC)[4,5] or high-performance liquid chromatographic (HPLC)[6] methods are usually used. GC analysis is sometimes unsatisfactory owing to incomplete derivatization of the 5-*n*-alk(en)ylresorcinols[4], and both methods need special instrumentation that is not always available at plant breeding laboratories.

For rapid screening programmes for the determination and comparison of the compositions of homologues, thin-layer chromatographic (TLC) methods are to be preferred. Previously described TLC techniques[4,7] for the quantitative determination of separated fractions[8,9] made possible only the evaluation of the amounts of total saturated or unsaturated homologues or the determination of the aliphatic chain length of the whole 5-*n*-alk(en)ylresorcinol mixture. In addition, these techniques need two different chromatographic separations on separate TLC plates.

In this paper, a procedure for the analysis of the composition of 5-*n*-alk(en)-ylresorcinol homologues by two-dimensional TLC on pre-treated silica gel plates is presented.

## EXPERIMENTAL

5-*n*-Alk(en)ylresorcinol homologue standards were isolated from acetone extracts of rye grains by an HPLC procedure to be described elsewhere[10].

Acetone oils were obtained from cereal grains with the method described earlier[11]. The alk(en)ylresorcinols and acetone oils were dissolved in *n*-propanol and about 2% solutions were used for the analysis. Other reagents were of analytical-reagent grade.

Plastic-backed plates (20 × 20 cm), precoated with a 0.2 mm layer of silica gel (Merck, Darmstadt, F.R.G., No. 5748), were used for TLC.

### *Thin-layer chromatography: general procedure*

The original plate was cut to give two 10 × 15 cm plates. A 2.5 cm strip from the shorter edge was impregnated with a 20% solution of silver nitrate in 50% methanol (Fig. 1) and dried. The samples were applied to the impregnated part of the

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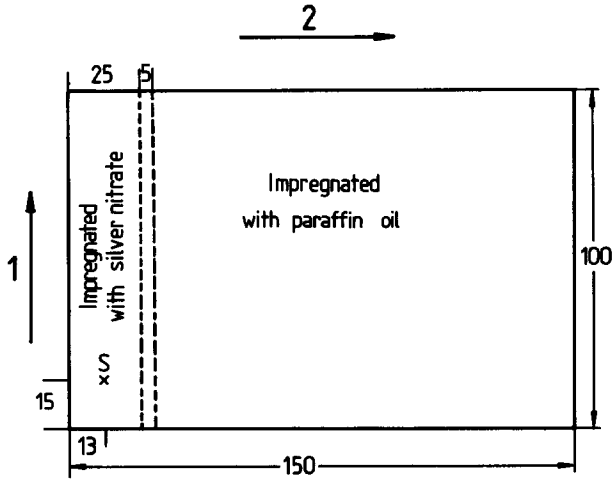


Fig. 1. Preparation of silica gel-coated plates for 5-*n*-alk(en)ylresorcinol mapping. Dimensions in millimetres. S = start point.

plate as a 2 mm spot, 15 mm from the bottom and 13 mm from the left-hand edge of the plate (Fig. 1). Disposable a 1- and 2-$\mu$l capillaries are particularly suitable for this purpose.

The chromatogram was run in the direction indicated by arrow 1 (Fig. 1) in benzene–ethyl acetate (85:15) to a distance of 8 cm. After the development the plate was air-dried and the excess of silver nitrate removed by washing with water (three times with 250 ml for 15 min). The dried plate was then impregnated with 5% paraffin oil solution in *n*-hexane, as shown in Fig. 1, for 15–20 min. The solvent was evaporated from the plate with air and the plate was developed in the direction of arrow 2 (Fig. 1) in acetone–methanol–water (60:15:25) to a distance of 12 cm.

For the detection of 5-*n*-alk(en)ylresorcinols the dried plates were immersed in 0.2% Fast Blue B in 0.05 *N* HCl solution for 30 min.

## RESULTS AND DISCUSSION

An example of the application of the described TLC procedure to the analysis of standard 5-*n*-alk(en)ylresorcinol homologues is shown in Fig. 2. The spots obtained are arranged in three rows and seven columns, the rows representing homologues with different aliphatic chain unsaturation and the columns homologues with different chain lengths. This separation provides information about the contribution of each homologue to the 5-*n*-alk(en)ylresorcinol mixture.

Fig. 3 illustrates the differences in the 5-*n*-alk(en)ylresorcinol homologue composition for rye and wheat grains. Comparing the alkylresorcinol maps obtained, significant differences can be seen between rye and wheat not only with respect to aliphatic chain length but also to the composition and amounts of unsaturated homologues. Relatively large amounts of homologues with one double bond in their aliphatic chain and the presence of diunsaturated species with relatively short chains are observed with rye 5-*n*-alk(en)ylresorcinols. Small changes in the $R_F$ values de-

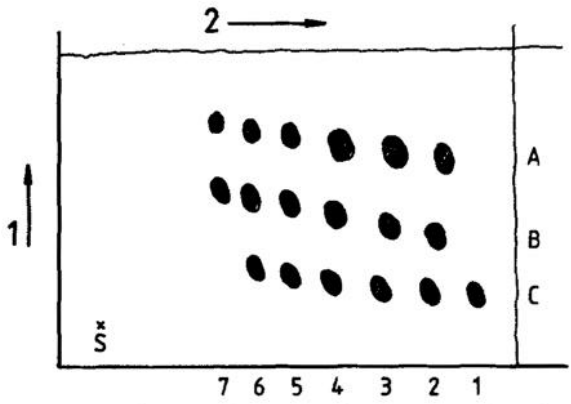


Fig. 2. Two-dimensional thin-layer chromatogram of standard 5-*n*-alk(en)ylresorcinol homologues. Sample size: 35 μg of standard mixture in 2 μl of *n*-propanol. Development: 1 = double development (first to a distance of 5 cm, then to 8 cm) in benzene–ethyl acetate (85:15); 2 = acetone–methanol–water (60:15:25). Detection: Fast Blue B. Columns 1–7: spots represent aliphatic chain length with the carbon atoms numbers of 1, 13; 2, 15; 3, 17; 4, 19; 5, 21; 6, 23; and 7, 25. Rows: A, saturated derivatives; B, monoolefinic derivatives; C, diolefinic derivatives.

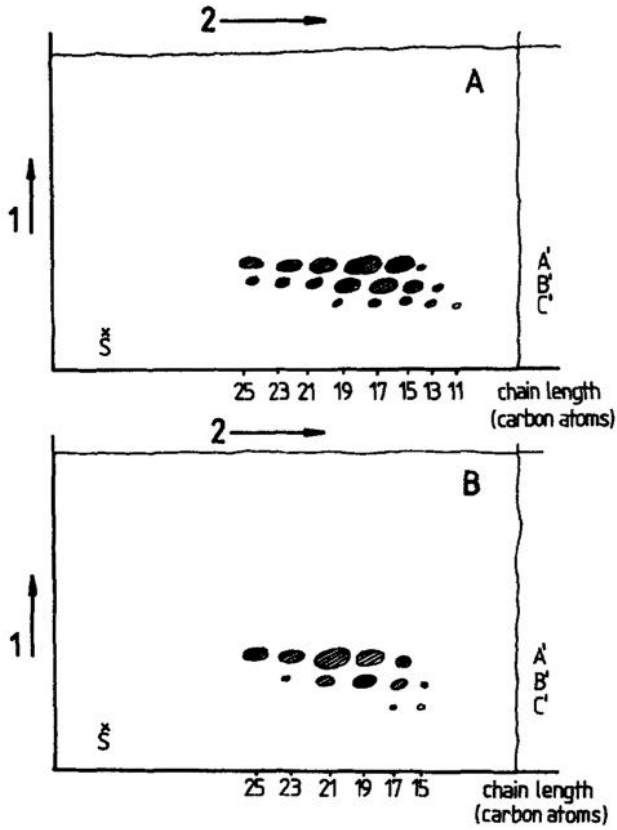


Fig. 3. Maps of 5-*n*-alk(en)ylresorcinol homologues obtained for acetone extracts of (A) rye and (B) wheat grains. Sample size: 40 μg of acetone oils in 2 μl of *n*-propanol. A', Saturated derivatives; B', monoolefinic derivatives; C', diolofinic derivatives.

pending on the degree of unsaturation of the homologues were also observed. These results are in good agreement with those obtained previously[10].

Previously published TLC methods for the analysis of 5-*n*-alk(en)ylresorcinol homologues composition were based on two separate chromatographic steps[8,9] and the determination of the aliphatic chain length of unsaturated species was possible only after their prior isolation. The method proposed in this paper makes possible the rapid analysis of 5-*n*-alk(en)ylresorcinol homologues even in extracts obtained from one grain sample. It could be used, for example, in preliminary studies when information about broad changes in resorcinol derivatives is required or, alternatively, to monitor the composition of homologues during the plant selection procedure.

Plant selection is important for both animal breeding and plant resistance programmes[12]. The observed high biological activity of unsaturated derivatives of 5-*n*-alk(en)ylresorcinols[13–15] indicates the need for the analysis of the composition of these derivatives in selected plant lines. The unsaturated derivatives of 5-*n*-alkylresorcinols could be responsible for the previously proposed role of alkylresorcinols in cereal grain resistance, *e.g.*, to fungi[4,12].

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