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# Alkylresorcinols in rye (*Secale cereale* L.) grains. V. Chromatographic analysis of 5-n-alk(en)ylresorcinols during their preparation

ARKADIUSZ KOZUBEK, FRANCISZEK TŁUSCIK, WANDA MEJBAUM-KATZENELLENBOGEN

Institute of Biochemistry, Wrocław University, Tamka 2,
50-137 Wrocław, Poland

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Abstract

Analysis of 5-n-alk(en)ylresorcinols during the course of their preparation from rye grains showed that changes of the composition of 5-n-alkylresorcinol homologues in acetone oil, raw preparation, and alkylresorcinol fraction obtained after chromatography on silica gel, were insignificant. About 20% of alk(en)-ylresorcinols were washed out from acetone oil with pentane. Over 50% of alk(en)ylresorcinols eluted by pentane constituted homologues with unsaturated hydrocarbon chains (5-n-alkenylresorcinols). Preparation obtained after chromatographic separation constituted a mixture of 5-n-alk(en)ylresorcinols with saturated hydrocarbon chains (5-n-alkylresorcinols).

## INTRODUCTION

5-n-alk(en)ylresorcinols (higher homologues of 1,3-dihydroxy-5-alk(en)ylbenzene) with an odd number of carbon atoms in the aliphatic chain, and of different degree of unsaturation, occur in many plants of the families *Anacardiaceae* (Kawamura 1928, Backer and Haack 1941a, b), *Ginkgoaceae* (Furukawa 1934a, b), *Proteaceae* (Occolowitz and Wright 1962, Ritchie et al. 1965, Cirigottis et al. 1974), *Myrsinaceae* (Madrigal et al. 1977), *Gramineae* (Wenkert et al. 1964, Wieringa 1967, Briggs 1974). Amount of these compounds in cereal grains is the highest in rye (up to 3000 ppm of dry weight), and the lowest in oat (below 300 ppm) (Evans et al. 1973, Verdeal and Lopez 1976, Becker et al. 1977, Kozubek and Białkowska 1976 — unpubl. data). Biological role of these compounds in grain remains still not clear. Some authors suggest (Wieringa and Pol

1965, Wieringa 1967, Pawlik et al. 1976) that inhibition of growth and diseases, noted in animals fed rye (North 1933, Halpin et al. 1936, Moran et al. 1969), are caused by high content of alkylresorcinols in rye grains. Keil et al. (1945) showed that toxicity of pentadec(en)ylresorcinols in poison ivy (*Rhus toxicodendron*) increased along with unsaturation of aliphatic chain. These data suggest the necessity of determining the content of unsaturated alk(en)ylresorcinols in cereal grains, as also studying their effect upon domestic animals.

In order to assess toxicity of 5-n-alk(en)ylresorcinols isolated from rye it is necessary to possess pure preparation of both saturated and unsaturated homologues. Simple three-step method of alkylresorcinols isolation (Mejbaum-Katzenellenbogen et al. 1978) allows to obtain chromatographically pure preparations from intact whole rye grains with the yield 64%.

In this work quantitative chromatographic analysis of particular homologues of rye alk(en)ylresorcinols during isolation and purification procedure was performed.

## MATERIAL AND METHODS

Rye of "Dańkowskie Złote" variety, seed generation — elita, from 1978 harvest, obtained from the Plant Breeding Station Rogaczewo. Pure alkylresorcinols were obtained from whole rye grains by the method of Mejbaum-Katzenellenbogen et al. (1978). Intact whole grains were extracted twice with equal volume of acetone at room temperature. After evaporation of the solvent acetone oils (OA) were combined and washed out several times with small portions of n-pentane. Thus a material soluble in n-pentane (OP) and a sediment of raw preparation (S) were obtained. The sediment was purified by column chromatography on silica gel, obtaining pure alkylresorcinols in one fraction (F2).

Standard mixture of 5-n-alkylresorcinols was obtained by a separation of acetone oil by preparative thin layer chromatography on silica gel, and then on silica gel impregnated with silver nitrate in the chloroform-acetone mixture (95 : 5).

## ARGENTATION CHROMATOGRAPHY

Plastic sheets covered with silica gel (4 × 10 cm) were impregnated for 5 min. in 10% silver nitrate in 50% methanol and dried. Samples of 0.01% acetone solutions (10-20 µl) were applied on the plates. Chromatograms were developed in chloroform-acetone (95 : 5) on 8 cm distance and stained with 0.5% Fast Blue B in 5% acetic acid.

## GAS CHROMATOGRAPHY

Before analysis samples were converted into trimethylsilyl derivatives (TMSi). The material (5-10 mg) was dissolved in 1 ml of TMSi-Universal Reagent and heated at 70° C for 3 h in tightly closed test tubes. The samples of 1-10 µl were injected on the column. Separation was carried out with the apparatus type GCHF 18.3, produced by VEB Chromatron, Berlin (GDR) in the following conditions: stainless steel column 3 m long, internal diameter 4 mm, packed with 3% SE-30 on Gas Chrom Q; nitrogen as a carrier gas. Flame-ionization detector (FID), sensitivity $1 \times 10^{10}$. Gas flows: $N_2$ — 40 ml/min., $H_2$ — 20 ml/min., air — 200 ml/min. Temperature of injector and of detector — 300° C. Separation isothermic, at 285° C.

**Reagents:** plastic sheets covered with silica gel — Merck (Nr 5748), Darmstadt (GFR); 3% SE-30 on 125-150 µm Gas Chrom Q; TMSi-Universal Reagent (mixture of N, O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) with trimethylchlorosilane (TMCS) and trimethylsilylimidazole (TSIM) in the ratio 3 : 2 : 3) — Serva, Heidelberg (GFR); 5-n-pentadecylresorcinol — Aldrich, Milwaukee Wisc. (USA); the other reagents — POCh, Gliwice, Poland.

## RESULTS AND DISCUSSION

Retention time of particular 5-n-alk(en)ylresorcinol homologues was defined using 5-n-alkylresorcinol and 5-n-alkenylresorcinol standards, obtained by preparative thin-layer chromatography of acetone oil and pure 5-n-pentadecylresorcinol. The readings of the logarithms of retention time (log $R_t$) against the number of carbon atoms in the alk(en)ylresorcinol hydrocarbon chain gives a straight line. Retention times of particular 5-n-alk(en)ylresorcinol homologues from rye grains are presented in Table 1.

Table 1

Retention times of particular homologues of alkyl- and alkenylresorcinols

| Homologue | Saturated $R_t$ (min) | Unsaturated $R_t$ (min) |
|---|---|---|
| Pentadecyl | 4.2 | 4.0 |
| Heptadecyl | 6.8 | 6.4 |
| Nonadecyl | 10.8 | 10.3 |
| Heneicosyl | 17.3 | 15.8 |
| Tricosyl | 27.0 | 25.3 |
| Pentacosyl | 42.3 | 39.5 |
| Heptacosyl | 66.0 | 63.1 |

Figure 1 shows the chromatograms obtained for trimethylsilyl derivatives of 5-n-alk(en)ylresorcinols in successive stages of 5-n-alkylresorcinols preparation from rye grains. In acetone oil particular saturated and unsaturated homologues of 5-n-alk(en)ylresorcinols with the same carbon atoms number have been shown as well by gas chromatographic analysis (Fig. 1a) as by argentation chromatography (Fig. 2a). The homologues with unsaturated hydrocarbon chain represent about 20% of total alk(en)ylresorcinols present in rye. On the other hand the alk(en)ylresorcinols occurring in the material soluble in n-pentane (OP) consist

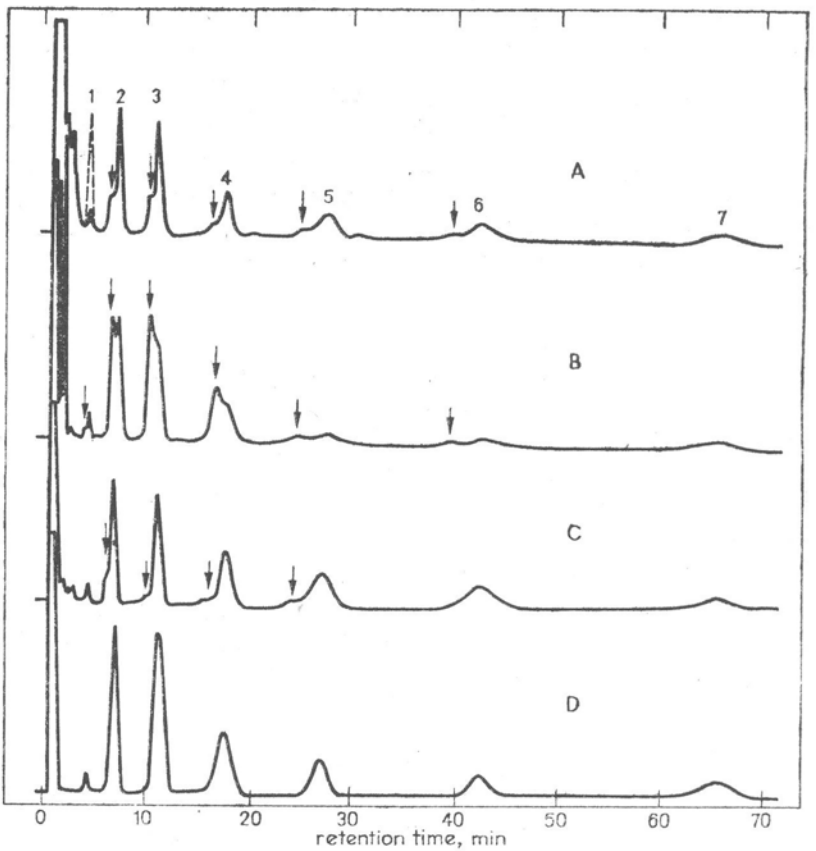


Fig. 1. Chromatograms obtained during separation of trimethylsilyl derivatives of alk(en)ylresorcinols present in the material during 5-n-alkylresorcinol preparation from rye grains

Numbers denote particular homologues (as in Table 1); arrows indicate respective alkenylresorcinols. Dashed line represents fraction of standard 5-n-pentadecylresorcinol. Conditions of separation are presented in the "Material and methods" section. A — acetone oil (OA); B — pentane oil (OP); C — raw alkylresorcinol preparation (S); D — 5-n-alkylresorcinols purified by column chromatography (fraction F2)

of compounds containing over 50% of unsaturated alkylresorcinols (Fig. 1b, 2b). The raw preparation (S) contained small amounts of alkenylresorcinols (Figs. 1c, 2c). Pure alkylresorcinols obtained after chromatographic purification of raw preparation (F2 fraction) are composed only of saturated homologues (Figs. 1d, 2d). Percentage of saturated and unsaturated homologues in acetone oil, material soluble in n-pentane, raw preparation and pure 5-n-alkylresorcinols (F2) are presented in Table 2. Heptadecyl, nonadecyl and heneicosyl resorcinols were the main components of these materials. They constituted over 55% of all alk(en)ylresorcinols. In pentane oil percentage of homologues with shorter chain was distinctly higher.

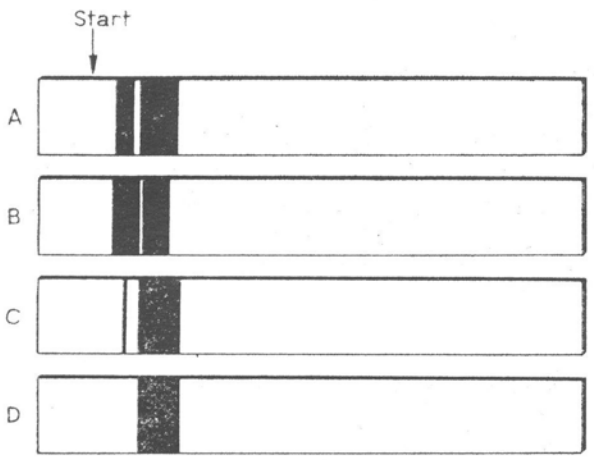


Fig. 2. Schemes of separation in thin-layer argentation chromatography during 5-n-alkylresorcinol preparation from rye grains
Conditions of separations are presented in the "Material and methods" section. A — acetone oil (OA); B — pentane oil (OP); C — raw alkylresorcinol preparation (S); D — 5-n-alkylresorcinols purified by column chromatography (fraction F2)

Amount of alkenylresorcinols changed significantly during the procedure. The concentration of alkenylresorcinols in acetone oil was 23% of total amount of resorcinols, whereas in pentane oil increased over two-fold. Raw preparation, obtained after washing out of acetone oil with pentane, contained only 8.7% of alkenylresorcinols. Further purification on silica gel column practically eliminated all remaining alkenylresorcinols.

Consequently, use of pentane gives a homogenous mixture of 5-n-alkylresorcinol homologues, with similar composition as in acetone extract. This mixture is totally devoid of alkenylresorcinols. According to the literature data (K e i l et al. 1945, O g i u et al. 1950a, b, D a e S u p H a n 1964) alk(en)ylresorcinol derivatives with double bonds in the side chain

Table 2

Percentage content of alkyl- and alkenylresorcinol homologues during 5-n-alkylresorcinol preparation from rye grains

| Homologue | Acetone oil (OA) | | Pentane oil (OP) | | Sediment (S) | | Fraction (F2) | |
|---|---|---|---|---|---|---|---|---|
| | sat. | unsat. | sat. | unsat. | sat. | unsat. | sat. | unsat. |
| Pentadecyl | 1.6 | 0.3 | 2.1 | 0.5 | 1.5 | | 2.8 | — |
| Heptadecyl | 19.4 | 5.3 | 15.2 | 15.2 | 22.3 | 3.2 | 23.7 | — |
| Nonadecyl | 23.5 | 9.0 | 15.0 | 19.7 | 29.2 | 2.7 | 32.3 | — |
| Heneicosyl | 15.8 | 6.7 | 11.1 | 13.4 | 19.5 | 1.9 | 21.5 | — |
| Tricosyl | 7.9 | 1.0 | 2.3 | 3.0 | 8.7 | 0.9 | 8.9 | — |
| Pentacosyl | 7.4 | 0.8 | 1.1 | 1.4 | 8.4 | | 8.7 | — |
| Heptacosyl | 1.3 | — | — | — | 1.7 | — | 2.1 | — |
| Total | 76.9 | 23.1 | 46.8 | 53.2 | 91.3 | 8.7 | 100.0 | |

sat. — saturated, unsat. — unsaturated

show a higher biological activity. Alkylresorcinol preparations rich of alkenylresorcinols can be isolated from pentane oil. This type of preparation obtained from rye grains could be used in feeding experiments, aimed at an assessment of the "toxicity" of unsaturated derivatives of long chain 1,3-dihydroxy-5-alkyl-benzenes.

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## *Alkilorezorcynole w ziarniakach żyta (Secale cereale L.) V. Analiza 5-n-alkilorezorcynoli w poszczególnych etapach ich preparacji z ziarniaków żyta*

Streszczenie

Analiza poszczególnych etapów preparacji rezorcynoli z żyta wykazała, że skład homologów 5-n-alkilorezorcynoli w oleju acetonowym, surowym preparacie oraz frakcji alkilorezorcynoli uzyskanej po chromatografii na żelu krzemionkowym zmienia się w niewielkim stopniu. Pentan, używany do wstępnego oczyszczania alkilorezorynoli wymywa z oleju acetonowego około 20% alkilorezorcynoli, głównie zawierających homologi o krótszych łańcuchach węglowodorowych. Ponad 50% alkilorezorcynoli wymywanych przez pentan stanowią homologi z nienasyconymi łańcuchami węglowodorowymi (5-n-alkenylorezorcynole). Preparat po rozdziale chromatograficznym jest mieszaniną tylko 5-n-alkilorezorcynoli z nasyconym łańcuchem węglowodorowym.