

Alkylresorcinols of rye (*Secale cereale* L.) caryopses

IV. Three-step preparation of 5-n-alkylresorcinols

W. MEJBAUM-KATZENELLENBOGEN, F. TŁUŚCIK, A. KOZUBEK

Institute of Biochemistry, Wrocław University, Wrocław

(Received: July 6, 1978)

Abstract

Whole rye or wheat grains were extracted with acetone at room temperature. The extracts were pooled and acetone was evaporated. The remaining acetone oil was washed out with n-pentane until a colourless filtrate was obtained. The residue was dried, then dissolved in chloroform-acetone (95:5) mixture and separated by column chromatography on silica gel. The fractions containing 5-n-alkylresorcinols were pooled, condensed and crystallised from n-hexane. The preparation obtained was homogeneous in thin-layer chromatography and gave the UV spectrum characteristic for 5-n-alkyl derivatives of resorcinol.

INTRODUCTION

Alkyl derivatives of dihydroxybenzene are common in fruits of some species of the family *Anacardiaceae*, *Proteaceae*, *Gramineae* and in *Ginkgo biloba*. The earliest known representative of this type of compounds is bilobol described as early as 1928 by Kawamura. Wieringa (1967) obtained from rye grain a mixture of 5-n-alkylresorcinol homologues containing 15-25 carbons in the aliphatic chain, 15 per cent of them had unsaturated chains.

Tłuścik (1978) demonstrated that practically all 5-n-alkylresorcinols are present in the outer cuticle of the caryopsis seed coat constituting in rye 40 and in wheat 20 per cent of its weight.

Formerly methanol was used for purification of alkylresorcinols extracted from the caryopses (Mejbaum-Katzenellenbogen et al., 1975). The oil obtained after evaporation of acetone was dissolved in methanol and the alkylresorcinols were precipitated by addition of

an equal volume of water. The alkylresorcinol sediment was separated by filtration or centrifugation. Since separation of the sediment is rather difficult and greatly prolongs the time of preparation, in the present work an attempt was made to omit this step. N-pentane was used for removal of contaminations from the acetone oil, and the crude alkylresorcinol preparation was additionally purified on a silica gel column. A more detailed spectrophotometric (UV) and chromatographic characteristic of the alkylresorcinol preparation and of the lipid contaminations removed in the particular steps is given.

MATERIAL AND METHODS

Material. Rye of the Dańkowskie Złote variety, seed generation — original, harvested in 1976 at the Plant Breeding Station Rogaczewo and wheat Grana, seed generation — elite, harvested in 1976 at the same Station, Division Choryń. The investigations were performed after storing the seeds for one year.

Reagents: Fast Blue B (Chemapol, Czechoslovakia), p-nitroanilin (Loba-Chemie, Austria). The remaining reagents were Polish products (POCh, Gliwice).

Alkylresorcinols were determined by the p-nitroaniline method of Bray (1952) in the modification of Mejbaum-Katzenellenbogen et al. (1975) and spectrophotometrically at $A_{276}^{1\text{ cm}}$ and $A_{282.5}^{1\text{ cm}}$ ($A_{276}/A_{282.5} = 1.02$). Thin-layer chromatography was run on foil sheets coated with silica gel. (Merck, no. 5748 or Silufol, Kavalier). The chromatograms were developed in the following systems: benzene, chloroform, chloroform-acetone (95:5), chloroform-acetone (85:15), petroleum-ether-ethyl ether-acetic acid (90:10:2), n-hexane-ethyl acetate (95:5). The chromatograms were developed by spraying with (a) 20 per cent ethanol solution of phosphomolybdic acid at 110°C for 5 min, (b) 1 per cent vanillin in 50 per cent orthophosphoric acid at 110°C for 5 min, (c) 1 per cent aqueous Fast Blue B solution at room temperature. The drop test for alkylresorcinol detection consisted in placing on a piece of foil coated with silica gel (Silufol) a drop of the examined solution and treating it with a drop of 1 per cent Fast Blue B. The sensitivity of alkyl derivatives of resorcinol was 0.05 µg.

Column chromatography. The crude alkylresorcinol preparation (ca. 10 g) was dissolved in 80 ml of a chloroform-acetone (95:5) mixture warmed to about 35°C and placed on a 4 × 50 cm column filled with 250 g silica gel suspended in a chloroform-acetone mixture (95:5). Elution was run with the same mixture at a flow rate of 180 ml/hour. About 50-ml fractions were collected in which the alkylresorcinol content was checked by the drop test.

Fractions 1-13, 14-32, 33-42 and 43-50 were combined to give 4 fractions — F₁, F₂, F₃ and F₄.

Spectrophotometric measurements were done in a Specord UV-Vis, Zeiss Jena (East Germany) apparatus.

RESULTS

The here described method of isolation and purification of 5-n-alkylresorcinols from rye or wheat grain consists of 3 steps: (1) extraction of the grain with acetone and concentration of the extract to an oil consistency; (2) washing of the acetone oil with n-pentane to obtain the crude alkylresorcinol preparation (S) and pentane oil (P) containing most of the impurities, (3) purification of the crude alkylresorcinol preparation on a column filled with silica gel. Table 1 gives an example of preparation in which from 20 kg of rye grain 14.5 g of alkylresorcinols were obtained with a 64 per cent yield.

Step. 1. Extraction of the grain with acetone, concentration of the extracts to obtain acetone oils

The grain was placed in glass flasks and flooded with acetone in a 1:0.5 proportion by weight, that is the minimal quantity necessary to immerse the grain. The whole was left to stand at room temperature and after 24 h filtered through paper. The filtered off grain was once more flooded with acetone in the same proportion and filtered after 24 h. The dried grain was ground and flooded again with acetone in the proportion 1:5 and filtered after 24 h. The volume of the separately collected extracts (E-1, E-2, E-3) was measured, the concentration was determined and the alkylresorcinol content was calculated in the particular extracts. The first one (E1) contained the most alkylresorcinols (0.18 g/100 ml), and the third one the least (0.0008 g/100 ml). The particular extracts were concentrated in a rotating vacuum evaporator at 55°C to constant weight. The brownish-yellow oils obtained were weighed, the concentration of alkylresorcinols was determined in them and the percentage of impurities was calculated. The oil material obtained from whole grains (A-1 and A-2) constituted 0.2 per cent and that from ground grain (A-3) as much as 1.4 per cent of the grain weight. The first oil contained 40 per cent, the second 65 per cent and the third as much as 99.7 per cent of impurities and trace amounts of alkylresorcinols (ca. 0.3%). As little as 12 per cent of material soluble in acetone is extracted from whole grain, and it contains practically all the alkylresorcinols.

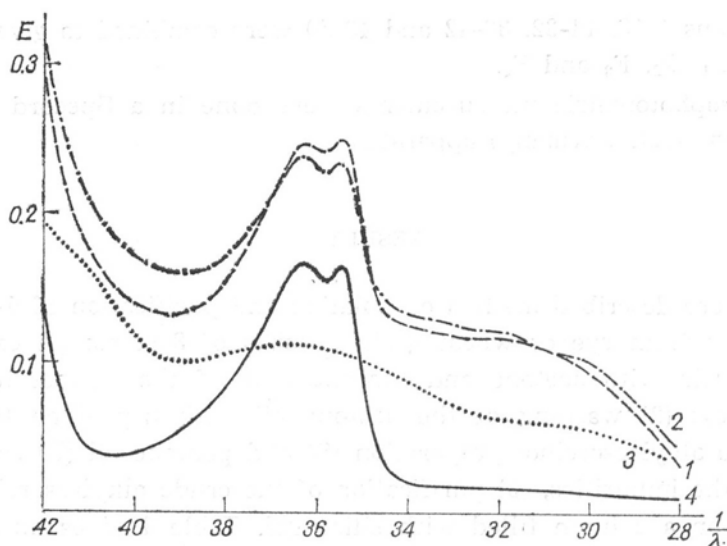


Fig. 1. UV spectra of acetone oils and orcinol in methanol solutions. Concentration in samples: oil — 125 $\mu\text{g/ml}$, orcinol — 25 $\mu\text{g/ml}$. ---- oil A-1, - · - · - oil A-2, oil A-3, — orcinol

The UV spectra of methanol solutions of the oil obtained and the spectrum of 5-n-methylresorcinol (orcinol) are shown in Fig. 1. The A-3 spectrum differs markedly from that of orcinol and of both the remaining oils (A-1 and A-2). Both the latter give two closely lying maxima characteristic for orcinol. The contaminations greatly increase absorption at wave number 39 and slightly at 34 and 32.

Thin-layer chromatography on plates coated with silica gel in the system chloroform-acetone (95:5) supplied further information concern-

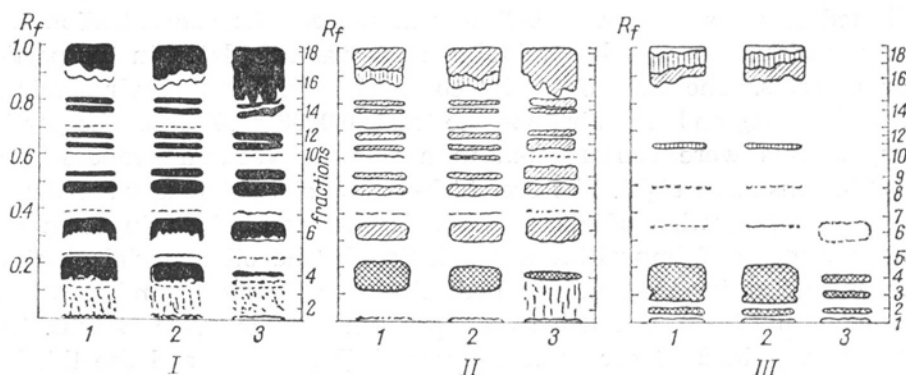


Fig. 2. Separation by thin-layer chromatography on silica gel (Merck 5748 plates, chloroform-acetone (95:5) system, 2.5 mg samples) of acetone oils from rye. Development: I — phosphoromolybdic acid, II — vanillin, III — Fast Blue B. 1 — A-1 oil, 2 — A-2 oil, 3 — A-3 oil

ing the lipid material extracted with acetone from whole grains and ground ones. The chromatograms were developed with phosphomolybdic acid (Fig. 2, 1), vanillin (Fig. 2, 2) and Fast Blue B solution (Fig. 2, 3). Phosphomolybdic acid stains all fractions dark blue. Vanillin gives a red colouring only with alkylresorcinols, and with the remaining lipid fractions it gives blue or yellow spots. Fast Blue B stains the phenol derivatives violet. Chromatograms A-1, A-2 and A-3 developed with phosphomolybdic acid did not differ significantly from one another, except that in A-3 the bands corresponding to alkylresorcinols are less pronounced. Vanillin distinguishes distinctly the two chromatograms A-1 and A-2 from A-3. The widest differences appear in the chromatograms developed with Fast Blue B solution.

It appears from the comparison of the chromatograms obtained that the best developer for assay of the degree of contamination of the examined lipid material is phosphomolybdic acid, whereas Fast Blue B is the most sensitive developer for determination and detection of trace amounts of alkylresorcinols (ca. 0.05 μ g). Vanillin, on the other hand, is the most specific developer for alkylresorcinols.

Since it was found that oil A-3 contains practically no alkylresorcinol derivatives of resorcin, in the further procedure both oils (A-1 and A-2) were combined.

Step 2. Washing of acetone oil with n-pentane. Characteristic of the crude alkylresorcinol (S) preparation and of pentane oil (P)

The combined oils A-1 and A-2 were suspended in a small amount of n-pentane, transferred to a filter in a Buchner funnel and washed with small n-pentane portions until a colourless filtrate was obtained. The white sediment remaining on the filter was air-dried. The yellow pentane filtrates were combined and dried in a stream of air. Both fractions were dried to constant weight, weighed, alkylresorcinol concentration was determined and their content in the whole material obtained was calculated. Fraction S contained 90 per cent alkylresorcinols and only 10 per cent impurities. The dark brown thick pentane oil P contained 20 per cent alkylresorcinols and 80 per cent of contaminations. The yield of the alkylresorcinol preparations was at this stage 72 per cent.

Fig. 3 shows the UV spectra of methanol solutions of fractions S and P. In both spectra the absorption ratio at wave number 36.5/35.5 differs from that in the orcinol spectrum. This ratio for the crude alkylresorcinol preparation is too low (0.94), and for pentane oil it is too high (1.09). The crude preparation contains impurities which enhance absorption at higher wave numbers.

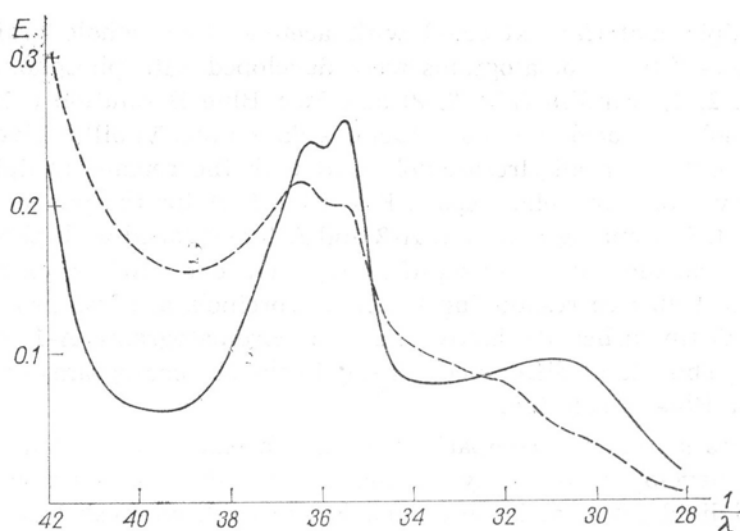


Fig. 3. UV spectra of methanol solution (125 µg/ml) of crude alkylresorcinol preparation (S) — and of pentane oil (P) - - - -

In thin-layer chromatography of the crude alkylresorcinol preparation contaminations with lower R_f than that of alkylresorcinols are noticeable (Fig. 4). Pentane oil contains most of impurities present in the acetone oil (A-1 and A-2).

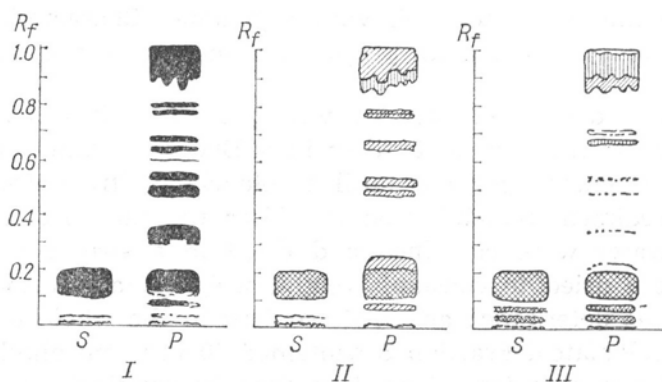


Fig. 4. Separation by thin-layer chromatography on silica gel of crude alkylresorcinol preparation (S) and of pentane oil (P). Samples 1.5 mg. Chromatography and development as described in fig. 2

Step 3. Column chromatography on silica gel of the crude alkylresorcinol preparation and analysis of the particular eluate fractions

The outflowing eluates were combined to give 4 fractions: F_1 , F_2 , F_3 and F_4 (see Material and methods). After evaporation of the solvents the material was weighed and the 5-n-alkylresorcinol content was de-

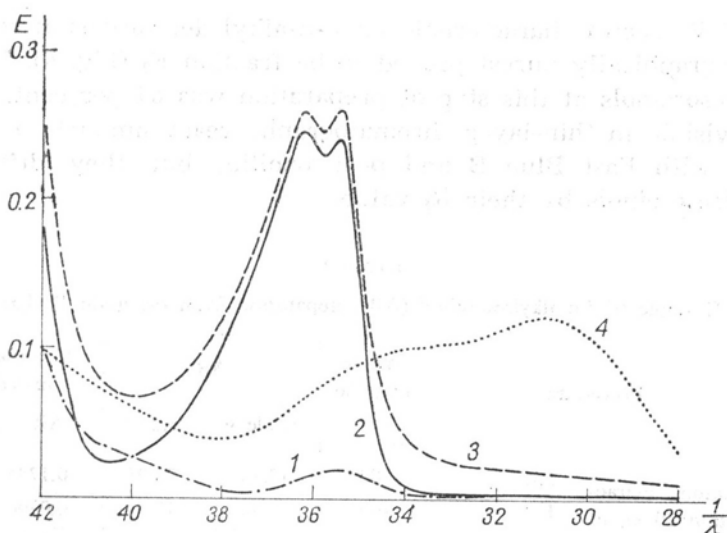


Fig. 5. UV spectra of methanol solution (125 µg/ml) of fractions obtained in column-chromatographic separation of crude alkylresorcinols preparation (S).----- fraction 1, — fraction 2, - - - - fraction 3, fraction 4

terminated in it by the p-nitroanilin method. Ninety five per cent of the material placed on the column was recovered. Fractions F_1 and F_4 gave a negative reaction with Fast Blue B and responded negatively to p-nitroanilin. They only contained impurities. On the other hand, fractions F_2 and F_3 contained all the material reacting with p-nitroaniline (100% alkylresorcinols). Analysis of the methanol solutions in the UV showed that fraction F_1 contains compounds enhancing absorption in wave number range 38 to 41, whereas fraction 4 absorbs at wavelengths 30-34 and 39-41 (Fig. 5). Methanol solution of fraction F_2 and

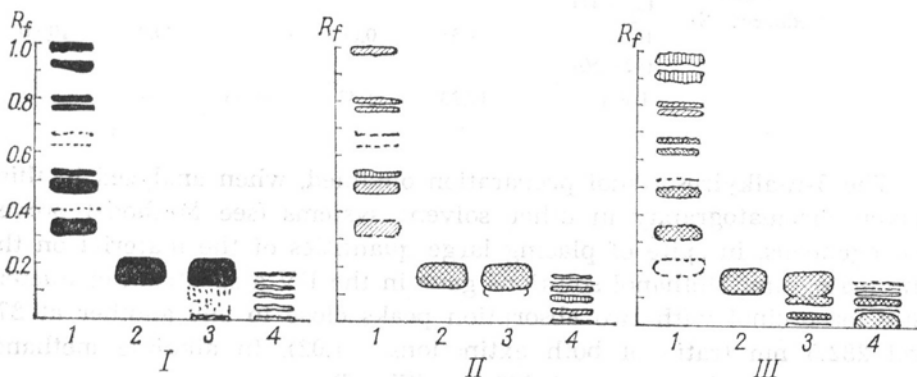


Fig. 6. Separation by thin-layer chromatography on silica gel of fractions obtained from column separation on silica gel of alkylresorcinol crude preparation (S). Samples 1.2 mg. Conditions of chromatography and development as in fig. 2
1 — fraction 1, 2 — fraction 2, 3 — fraction 3, 4 — fraction 4

F₃ gave UV spectra characteristic for 5-n-alkyl derivatives of resorcinol, Chromatographically purest proved to be fraction F₂ (Fig. 6). The yield of alkylresorcinols at this step of preparation was 64 per cent. The impurities visible in thin-layer chromatography react similarly as alkylresorcinols with Fast Blue B and p-nitroanilin, but they differ from 5-n-alkylresorcinols by their *R_f* value.

Table 1

Example of 5-n-alkylresorcinol (AR) preparation from rye grain (20 kg)

| Step | Procedure | | Whole material g | AR | | Composition of material, % | |
|------|---|------------------------|---------------------|---------|---------|----------------------------|------------|
| | | | | whole g | yield % | AR | impurities |
| I | Acetone extraction 20 kg of grain ground grain | E-1 | 101 | 17.85 | 78.91 | 0.1785 | — |
| | | E-2 | 101 | 3.96 | 17.51 | 0.0396 | — |
| | | E-3 | 1001 | 0.81 | 3.58 | 0.0008 | — |
| | | Total | 1201 | 22.62 | 100.0 | — | — |
| | Concentration in evaporator 55°C to constant weight | A-1 | 29.24 | 17.53 | 77.49 | 59.95 | 40.05 |
| | | A-2 | 10.04 | 3.58 | 15.82 | 35.65 | 64.35 |
| | | A-3 | 285.80 | 0.78 | 3.44 | 0.27 | 99.73 |
| | | Total | 325.08 | 21.89 | 96.89 | — | 93.27 |
| | | A-1 + A-2 | 39.28 | 21.11 | 93.32 | 53.74 | 46.26 |
| II | Purification with pentane | sediment S | 18.20 | 16.41 | 72.54 | 90.16 | 9.84 |
| | | pentane oil P | 20.76 | 4.52 | 19.98 | 21.77 | 78.23 |
| | | | | | | | |
| III | Chromatography of crude preparation (sediment—S) | F ₁ (1—13) | 0.21 | 0.00 | 0.0 | 0.0 | 100.0 |
| | | F ₂ (14—32) | 17.51 | 14.50 | 64.10 | 100 | 0.0 |
| | | F ₃ (33—41) | 0.96 | 0.97 | 4.28 | 100 | 0.0 |
| | | F ₄ (42—50) | 1.55 | 0.00 | | 0.0 | 100.0 |
| | | Total | 17.23 | 15.47 | 68.38 | — | — |

The 5-n-alkylresorcinol preparation obtained, when analysed in thin-layer chromatography in other solvent systems (see Methods) proved homogeneous, in spite of placing large quantities of the material on the chromatogram. Methanol solutions gave in the UV a spectrum characteristic for orcinol with two absorption peaks close to one another at 276 and 282.5 nm (ratio of both extinctions = 1.02). In alkaline methanol solution one peak appears at 290 nm (Fig. 7).

Application of the above described procedure to whole unground wheat grains gives a preparation with the same spectrophotometric and chromatographic characteristics.

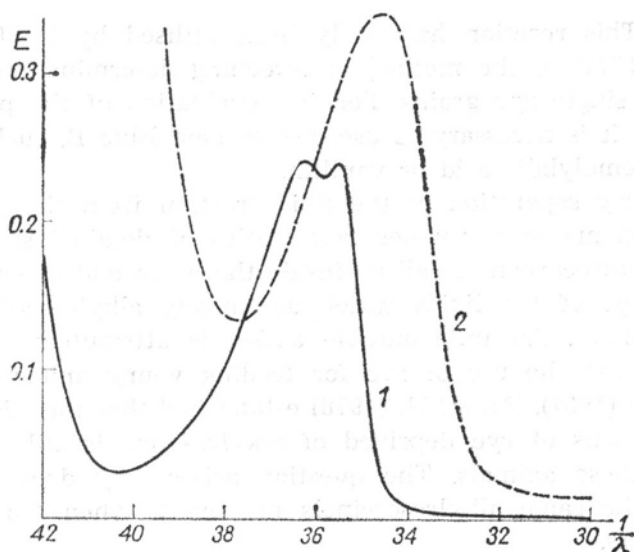


Fig. 7. UV spectra of 5-n-alkyl-resorcinol preparation solutions 1 — in methanol (125 $\mu\text{g/ml}$), 2 — in methanol and 0.1 N NaOH

DISCUSSION

Twofold extraction of whole grains with acetone at room temperature yields practically all the 5-n-alkyl-resorcinol derivatives both from rye and wheat caryopses. The extracts obtained are already highly purified since they do not contain the lipid and hydrocarbon fractions of the endosperm and aleurone layer. The amounts of oil obtained in this way are 10 times less than those yielded from ground grains. It results from the here presented investigations that grinding of grain before extraction with acetone is not only unnecessary but even unfavourable since it drastically raises the content of contaminant substances difficult to separate by means of organic solvents both polar and nonpolar. Petroleum ether used for purification of the acetone extracts not only extracts the nonpolar components but also large quantities of alkylresorcinols. More suitable for this purpose proved n-pentane in which alkylresorcinols do not dissolve readily, whereas lipids and hydrocarbons present in the extracts of whole rye and wheat grains are almost completely dissolved. This solvent removes the fraction migrating in thin-layer chromatography with the front of the alkylresorcinol fraction. Column chromatography on silica gel was particularly useful for removing compounds with properties similar to those of alkylresorcinols, reacting with Fast Blue B and p-nitroanilin.

Of importance was the finding that Fast Blue B is one of the most sensitive reagents for determination of trace amounts of alkyl derivatives

of phenol. This reaction has lately been utilised by Hoffman and Wenzel (1977) in the method of screening determination of alkylresorcinols in single rye grains. For full evaluation of the purity of the preparations it is necessary to use, beside Fast Blue B, such developers as phosphoromolybdic acid or vanillin.

Preliminary separation of the lipid fraction from the pericarp and seed integuments open further possibilities of detailed studies on the lipids and hydrocarbons localised inside the grain and of gaining a better knowledge of the lipids which accompany alkylresorcinols in the seed integument. An unfavourable action is attributed to these substances, so that the use of rye for feeding young animals is limited. Wieringa (1967), Pawlik (1978) established that the addition to the feed of chickens of rye deprived of alkylresorcinols still restricts the growth of these animals. The question arises why does wheat grain containing the same alkylresorcinols not exert, when fed to animals, similar effects.

For a full knowledge of the biology of rye and wheat grain a closer characteristic of the lipid fractions and others, other phenol derivatives beside alkylresorcinols will be necessary. Up till now but little attention was devoted to these compounds, particularly to the endosperm lipids.

REFERENCES

- Bra'y H. G., Humphris B. G., Thorpe W. V., White K., Wood P. B., 1952. *Biochem. J.* 52: 416-421.
Hoffmann F., Wenzel G., 1977. *Theor. Appl. Genet.* 50: 1-2.
Kawamura J., 1928. *Japan J. Chem.* 3: 89 from Backer H. J., Haack N. H., 1941. *Rec. Trav. Chim. Pay-Bas.* 60: 656-677.
Mejbaum-Katzenellenbogen W., Tłuścik F., Kozubek A., Sikorski A., Maresz Z., 1975. *Acta Soc. Bot. Polon.* 44 (4): 479-489.
Pawlik J., 1978. *Zeszyty Naukowe Zootechniki. Monografie i rozprawy.* — in press.
Tłuścik F., 1978. *Acta Soc. Bot. Polon.* 47 (3): 211-218.
Wieringa G. W., 1967. On the occurrence of growth inhibiting substances in rye. H. Veenman en Zonen N. V., Wageningen.

Author's addresses:

W. Mejbaum-Katzenellenbogen, F. Tłuścik,
A. Kozubek, Institute of Biochemistry,
Wrocław University,
Tamka 2; 50-137 Wrocław; Poland

Alkilorezorcynole ziarniaków żyta (Secale cereale L.)
IV. Trzyetapowa preparacja 5-n-alkilorezorcynoli

Streszczenie

Całe ziarna żyta lub pszenicy ekstrahowano acetonem w temperaturze pokojowej. Wyciągi łączono i zagęszczano do konsystencji oleju. Olej acetonowy prze-mywano n-pentanem aż do uzyskania bezbarwnych popłuczyn. Pozostałość suszono, rozpuszczano w mieszaninie chloroformu z acetonem (95:5) i poddawano chroma-tografii kolumnowej na żelu krzemionkowym. Frakcje zawierające czyste 5-n-al-kiłorezorcynole łączono, zagęszczano i krystalizowano z n-heksanu. Uzyskany pre-parat jest jednorodny w chromatografii cienkowsarstwowej i daje charakterystyczne dla 5-n-alkilowych pochodnych rezorcyny widmo w UV.