ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLV, nr 1—2
1976

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

III. Application of detergents for extraction of proteins and alkylresorcinols

W. MEJBAUM-KATZENELLENBOGEN, J. ŁOMAKO, W. ŁOMAKO

Institute of Biochemistry, University of Wrocław

(Received: July 14, 1975)

Abstract

In the work here described sodium dodecyl sulphate solutions (SDS), tween 80 and triton X 100 were used for isolation of proteins and 5-n-alkylresorcinols from ground rye grain. It was found that the above named detergents extract different protein and various amounts of alkylresorcin derivatives. The results indicate that 5-n-alkylresorcinols are localized in the membraneous structures of rye caryopses.

INTRODUCTION

The present paper is a continuation of investigations carried on in this Department concerning the chemical and physiological characteristic of alkyl derivatives of 1,3-dihydroxybenzene from rye caryopses. Interest in these compounds was aroused by the investigations of Wieringa (1967) who found that they are toxic for farm animals.

In a previous study Mejbaum-Katzenellenbogen et al. (1975, 1975) established, applying their own method of colorimetric determination of total alkylresorcinols in single rye caryopses, that these compounds are localized in the outer layers of the caryopsis. These authors used for alkylresorcinol extraction as organic solvent — acetone.

In the present study, basing on the suggestion concerning the localization of alkylresorcinols in the outer parts of the caryopsis, we decided to

* The study was supported by Institute of Plant Breeding and Aclimatization within the project No 09-11, Research grant No 06-16.

use for extraction of these compounds detergents instead of organic solvents. This made possible a simultaneous electrophoretic characterization of the proteins accompanying alkylresorcinols.

MATERIAL AND METHODS

Material: cv. Dańkowskie Złote rye from the Breeding Station in Jeleniec from the 1974 harvest were used.

Methods

1. Extraction of proteins and 5-n-derivatives of alkylresorcin from ground rye grain:

Rye caryopses were comminuted in a laboratory grinding mill (S.P. MEDOSS — Warszawa). The ground grain portions were infused with a fivefold volume of the solvent. Extracting mixtures: 0.9 per cent NaCl solution and solution of the following detergents in 0.9 per cent NaCl : 0.5 per cent triton X 100 (Serva Feinbiochemica GmbH Co), 1 per cent dodecyl sulphate sodium salt (BDH Chemicals Ltd. Poole England) and 1 per cent tween 80 (Schuchardt, München). The suspension after 1 h shaking at room temperature was centrifuged at 16 000 rpm/min for 40 min. Part of the extracts was purified on a Sephadex G 25 column (Pharmacia Fine Chemicals) of 3 × 75 cm dimensions. Effluent fractions in volume V_0 were collected and lyophilized. To the second part of the extracts 6 volumes of acetone were added and after 1 h they were centrifuged for 5 min at 4000 rpm. From the clear supernatant 1-ml samples were taken for 5-n-alkylresorcin determination by the p-nitroanilin method according to Mejbaum-Katzenellenbogen et al. (1975) and the remaining supernatant was concentrated to the volume of about 0.5 ml in an air flux.

2. Protein was determined by Lowry's (1951) method with bovine serum albumin(Koch-light Lab. Ldt.) as standard.

3. Protein electrophoresis in 7.5 per cent polyarylamide gel was performed at pH 8.9 after Davis (1962) and at pH 4.3 after Reisfeld et al. (1962). The gels were stained for protein with Coomassie Brilliant Blue (Dunker, Rueckert, 1969) and Remasol Brilliant Blue (Datyner, Finnimore, 1973) and then subjected to densitometry in an ERJ Carl Zeiss, Jena apparatus. Sugars were stained with alkaline fuchsin (Zacharius, Zell, 1969).

4. Lipoprotein electrophoresis was run at pH 8.3 in a discontinuous gel gradient according to Grajner et al. (1972). About 2.4 mg of lyophilized protein preparation was dissolved in 0.15 ml of tris-glycin buffer, pH 8.3 and 0.05 ml of saturated Sudan B solution in ethylene glycol

was added. The whole was left to stand for 20 h at 4°C, and afterwards subjected to electrophoretic separation in 0.05 ml samples.

5. Thin-layer chromatography of concentrated acetone supernatants was run on plates coated with silica gel. The chromatograms were developed in a chlorophorm-acetone system (85 : 15) and after spraying with 1 per cent vanillin solution in 50 per cent o-phosphoric acid colour was developed at 105°C. 5-n-alkylresorcinol prepared by the method of Mejbaum-Katzenellenbogen et al. (1975) was used as standard.

RESULTS

The detergent solutions used were found to extract from ground rye grain more protein than did NaCl solution (Table 1). The largest amounts

Table 1

The amount of protein extracted from rye caryopses with detergent solutions

Solution	Protein mg/g meal
0.9% NaCl	13.9
1.0% Tween 80 in 0.9% NaCl	16.6
0.5% Triton X-100 in 0.9% NaCl	16.3
1.0% SDS in 0.9% NaCl	24.6

of protein, about 24 mg from 1 g ground grain was extracted by the SDS solution. By triton X 100 or tween 80 from the same amount of grain about 16 mg of protein were extracted.

1. Protein electrophoresis at pH 8.9

Proteins extracted from ground rye with 0.9 per cent NaCl solution give in polyacrylamide gel 13 fractions (Fig. 1a) incuding eight of glycoprotein character (Fig. 3a). Proteins extracted with triton X 100 give 14 fractions (Fig. 1b). Both proteinograms differ solely by the pattern of the fraction migrating slowly towards the cathode in which in extractions with triton 2 new glycoprotein fractions appear (Fig. 3b).

Proteins extracted with tween 80 solution similarly as the proteins extracted with NaCl give 13 fractions (Fig. 1d). Differences were only observed in the fraction slowly migrating to the cathode. Here also a new strong glycoprotein fraction appeared (Fig. 3c) absent in triton X 100 extraction.

Proteins extracted into SDS solution give quite different electrophoretic pattern. The proteins separate into 11 fractions (Fig. 1c). It results from comparison of the electropherograms that as many as 4 glycoprotein fractions pass into SDS (Fig. 3d), the presence of which was not revealed in extraction with NaCl, triton X 100 and tween 80.

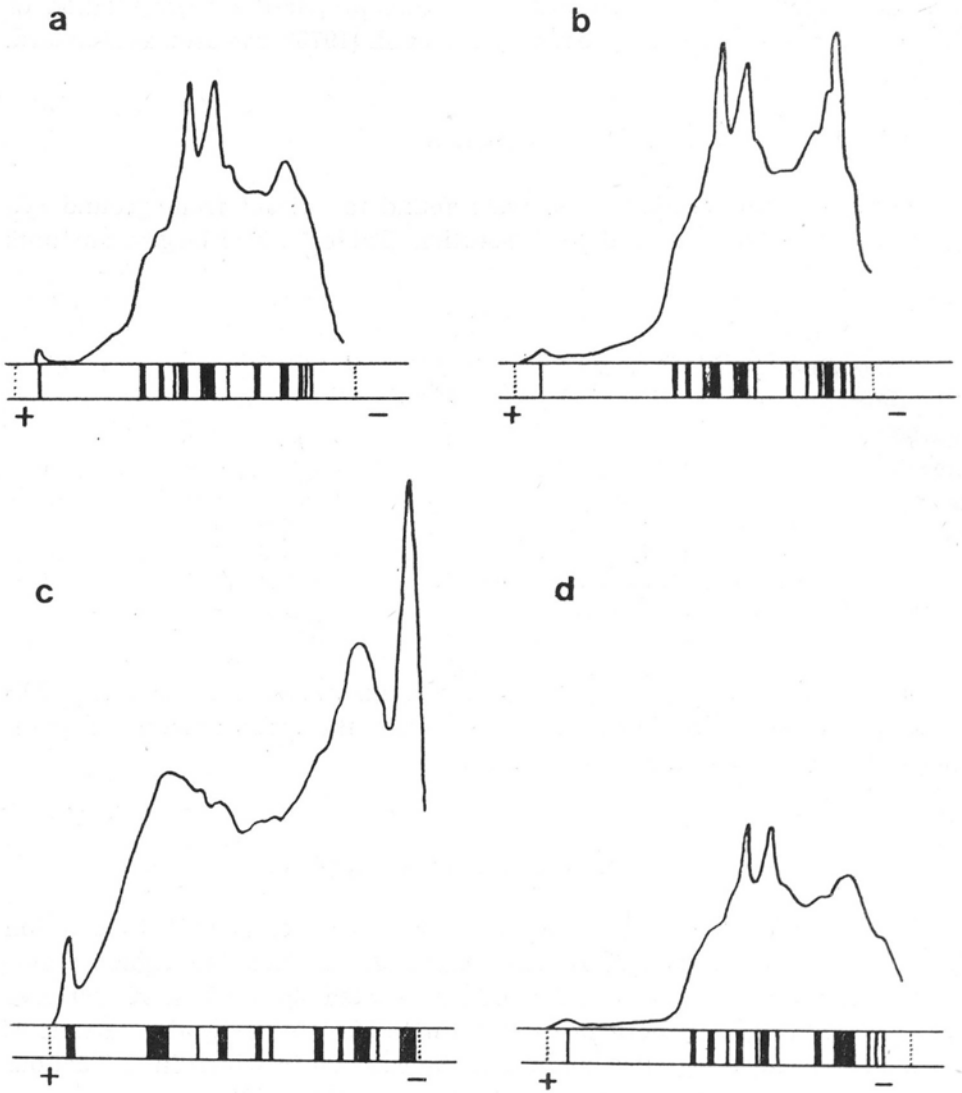

Fig. 1. Electrophoresis in polyacrylamide gel, pH 8.9, of rye grain proteins run in 7.5% polyacrylamide gel of 6.5 cm length at 3 mA per tube

About 0.3 mg of protein was subjected to separation. Electropherograms stained with Remasol Brilliant Blue.

a — Proteins extracted with 0.9% NaCl buffer, *b* — proteins extracted with 0.5% triton X 100 solution in 0.9% NaCl, *c* — proteins extracted with 1% SDS in 0.9% NaCl, *d* — proteins extracted with 1% tween 80 solution in 0.9% NaCl

2. Protein electrophoresis at pH 4.3

Proteins extracted into NaCl give 10 fractions (Fig. 2a), three of which are of the character of glycoproteins (Fig. 4a). At this pH the presence

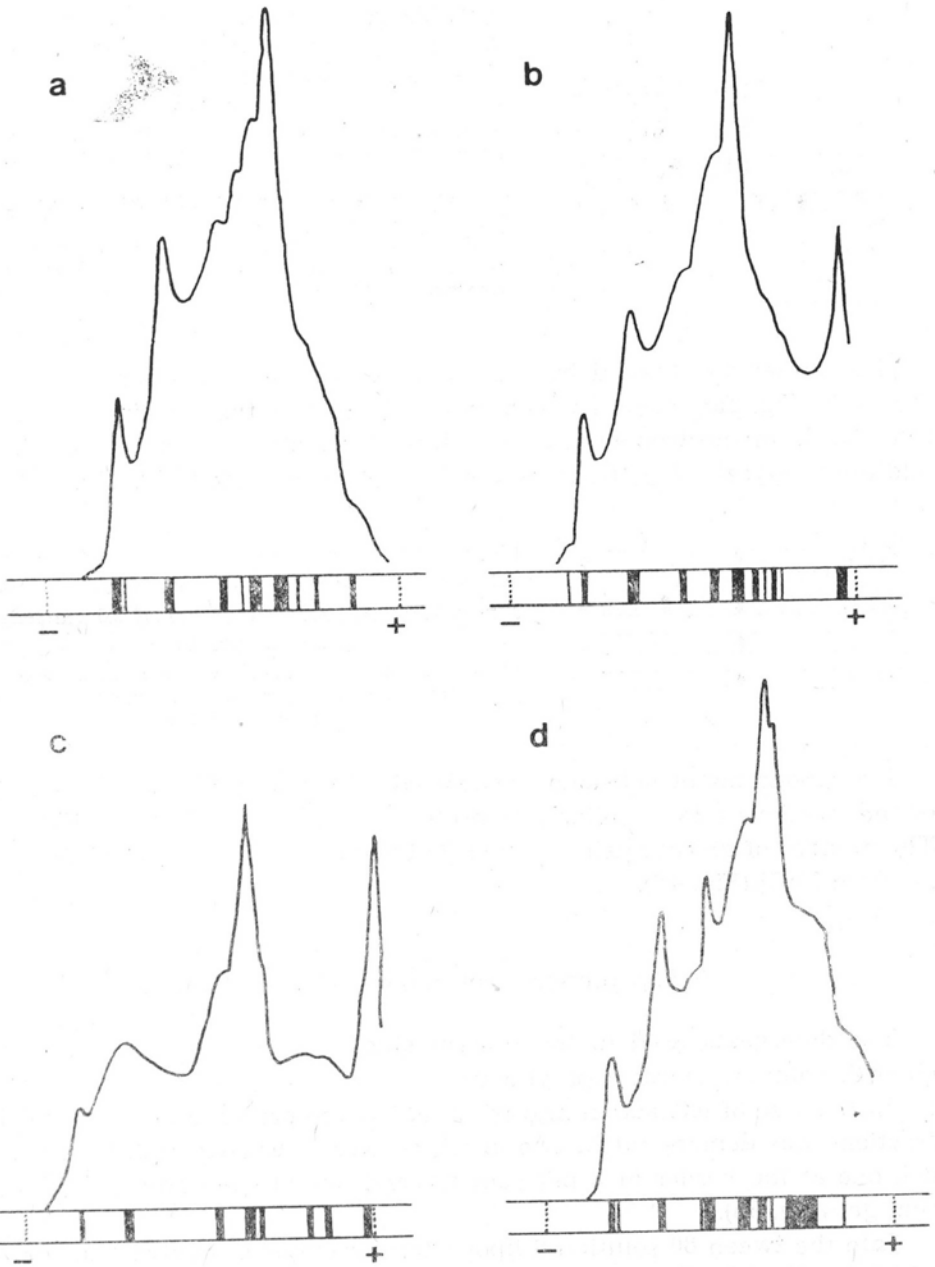

Fig. 2. Electrophoresis in polyacrylamide gel, pH 4.3, of proteins from rye caryopses run in 7.5% polyacrylamide gel of 6.5 cm length at 3 mA per tube

About 0.3 mg of protein was subjected to separation. Electropherograms stained with Coomassie Brilliant Blue. Further notations as in Fig. 1.

of 3 fractions was moreover demonstrated, migrating rapidly to the cathode and exhibiting sugars in fuchsin staining. They did not show protein either in Remasol or Coomassie staining.

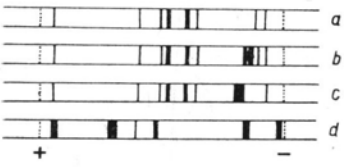

Fig. 3. Electrophoresis in polyacrylamide gel, pH 8.9, of glycoproteins from rye caryopses, run in 7.5% polyascrylamide gel of 6.5 cm length at 3 mA per tube

About 0.3 mg of proteins was subjected to separation. Electropherograms stained with alkaline fuchsin.

a — glycoproteins extracted with 0.9% NaCl, *b* — glycoproteids extracted with 0.5% triton X 100 solution in 0.9% NaCl, *c* — glycoproteins extracted with 1% SDS solution in 0.9% NaCl, *d* — glycoproteins extracted with 1% tween 80 solution in 0.9% NaCl

The proteins extracted by the triton X 100 solution separate into 11 fractions (Fig. 2b), eight of which move similarly as the proteins extracted into NaCl. In electropherograms stained for sugars the presence of an additional glycoprotein fraction has been demonstrated (Fig. 4b).

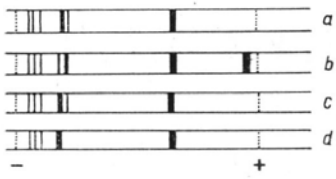

Fig. 4. Electrophoresis in polyacrylamide gel, pH 4.3, of glycoproteins from rye caryopses, run in 7.5% polyacrylamide gel of 6.5 cm length at 5 mA per tube

About 0.3 mg of protein was separated. Electropherograms stained with alkaline fuchsin. Further notations as in Fig. 3

Proteinograms of substances extracted with tween 80 show that most of the fractions move similarly to proteins extracted with NaCl (Fig. 2c). The number of glycoprotein fractions is identical as in the case of extraction with NaCl (Fig. 4d).

3. Lipoprotein electrophoresis at pH 8.3

The detergents used in the present study, in contrast to the sodium chloride solution, extract lipoproteins.

In the case of extraction into triton X 100 the presence of 5 lipoprotein fractions was demonstrated, two of which were localized in 2.75 per cent gel, one at the border of 3 per cent gel and one at the border of 7.5 per cent gel (Fig. 5b).

Into the tween 80 solution 6 lipoprotein fractions are extracted, one of which localized at the border of 3 per cent gel, one at the border of 3.5 per cent gel, one at the border of 7.5 per cent gel and two in 7.5 per cent gel (Fig. 5c).

The smallest number of lipoprotein fractions — only three — were found in SDS extracts (Fig. 5d).

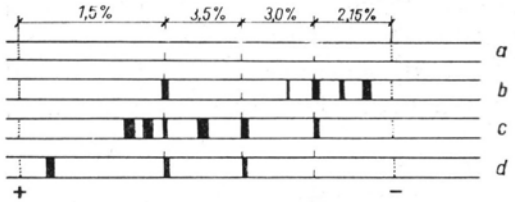

Fig. 5. Electrophoresis in polyacrylamide gel of lipoproteins from rye caryopses, run in discontinuous polyacrylamide gel gradient at pH 8.3

Gels 2.75%, 3.0 and 3.5% were all of 2 cm length, gel 7.5% gel was 4 cm long. Protein samples were incubated with Sudan B solution in ethylene glycol for 20 h at 4°C and subjected to electrophoretic separation at 3 mA per tube. About 0.6 mg of protein was subjected to electrophoresis.

a — proteins extracted with 0.9% NaCl solution; *b* — lipoproteins extracted with 0.5 triton X 100 solution in 0.9% NaCl; *c* — lipoproteins extracted with 1.0% tween 80 solution in 0.9% NaCl; *d* — lipoproteins extracted with 1.0% SDS solution in 0.9% NaCl

The characteristic fraction appearing in all lipoproteinograms is the fraction reaching the border of 7.5 per cent gel. In the case of extraction with triton X 100 and tween 80 similar fractions occur stopping in 3 per cent gel, whereas the fraction migrating to the end of 3 per cent gels is characteristic for SDS and tween 80 extracts.

4. Characteristic of phenol derivatives soluble in SDS, triton X 100 and tween 80 solution

It results from the data in Table 2 that application of detergents allows the extraction of 5-n-alkylresorcinols from ground rye grain. Most of these compounds, as much as 0.75 to 0.95 mg per 1 g of ground grain is obtained in extraction with triton X 100 solution. The action of tween 80 releases from the ground grain these substances in the amount of 0.35

Table 2

The amount of 5-n-alkylresorcinols extracted from rye caryopses with detergent solutions

Solution	5-n-alkylresorcinols mg/g meal
0.9 NaCl	0.00
1.0% Tween 80 in 0.9% NaCl	0.35—0,50
0.5% Triton X-100 in 0.9% NaCl	0.75—0.95
1.0% SDS in 0.9% NaCl	0.40—0.60

to 0.50 mg. Intermediate values were obtained when SDS solutions were used for extraction (0.40 to 0.60 mg/g ground grain).

Quantitative studies confirmed by thin-layer chromatography in the chloroform-acetone system showed the presence of 4 spots staining with vanillin phosphate. The largest of these corresponded in Rf to the 5-n-alkylresorcin standard. The number of spots in thin-layer chromatography of triton X 100, tween 80 and SDS extracts was the same (Fig. 6).

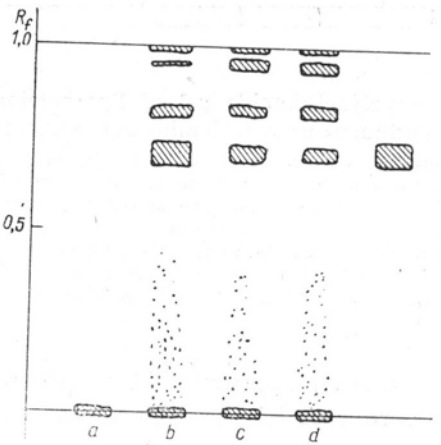

Fig. 6. Thin-layer chromatography of phenol derivatives extracted from rye caryopses

On glass plates coated with silica gel G, 0.03 ml of solutions obtained as described in Methods was placed. The chromatograms were developed along 10 cm in the system chloroform—acetone (85 : 15). After drying the plates were sprayed with 1% vanillin solution in 50% acetic acid and colour was developed at 105°C.

a — Extract into 0.9% NaCl; *b* — extract into 0.5% triton X 100 solution in 0.9% NaCl; *c* — extract into 1.0% tween 80 solution in 0.9% NaCl; *d* — extract into 1.0% SDS solution in 0.9% NaCl; *e* — 5-n-alkylresorcinol — standard

In the case of extracts obtained with triton X 100 solution the spot with Rf 0.95 was much less intensive than the corresponding spots from SDS and tween 80 extracts.

It was found that if detergents act on whole rye caryopses 5-n-alkylresorcinol does not appear in the extract.

The detergents used for 5-n-alkylresorcional extraction from ground rye grain previously treated with acetone for 1 h at 55° C released these compounds in the amount of about 0.23 to 0.28 mg/g ground grain. The quantity of substance remained the same, notwithstanding the detergent used. The chromatographic pattern of the compounds obtained is identical as that of grain extracts not treated with acetone.

In the next investigations, part of the ground grain after extraction with SDS or tween 80 was once more treated with the same detergents and a corresponding portion with triton X 100. The results of the experiments are shown in Table 3. Repeated extraction with DSS gives

Table 3

The amount of 5-n-alkylresorcinols extracted with Triton X-100 from ground rye grains treated previously with other detergents

Procedure	SDS	Triton X-100	Tween 80	Triton X-100
Extraction of meal (supernatant I	0.610		0.500	
Residue reextracted (supernatant II)	0.230	0.290	0.155	0.385
Total	0.840	0.900	0.655	0,885

0.84 mg and with tween 80 0.65 mg of 5-n-alkylresorcinols from 1 g of ground grain. The application of triton X 100 after the first extraction with SDS yields jointly 0.90 mg and after tween 0.88 mg of these compounds per 1 g gruond grain. It should be noted that, as far as the difference in the amount of 5-n-alkylresorcinols extracted in twofold extractions, with SDS adn tween 80, is between these detergents about 0.2 mg, the use of triton for the second extraction practically eliminates that difference.

DISCUSSION

The present experiments demonstrated that solutions of ionic detergents (SDS) and nonionic ones (triton X 100 and tween 80) extract certain protein, glycoprotein and lipoprotein fractions from ground rye grain, insoluble in sodium chloride solution.

Detergents are used for studying the structure and protein composition of membranes. So far the mechanism of their action on membranes and the mechanism of solubilization of their particular components is but little known. This phenomenon was most clearly revealed in the case of dodecyl sulphate. It is supposed that DSS interacts with positively charged hydrophilic protein groups (Burkhard, Stolzenberg, 1972) and specific hydrophobic receptors (Tanford, 1972). Choules et al. (1972) demonstrated that SDS in the first step loosens the membrane structure by introducing an aliphatic chain into its hydrophobic layer. If the SDS molecules are sufficiently numerous there occurs a violent breakdown of the structural components of the membrane and this may lead to the formation of saturated protein-detergent complex (Nelson, 1971; Reynolds, Tanford, 1972).

In the present investigation SDS was found to solubilize 4 glycoprotein fractions. It also appeared that this detergent extracts the fewest lipoprotein fractions. This fact may be due on the one hand, to the fact that certain noncovalent bonds in the membrane are resistant to the hydrophobic attack of this detergent (C h o u l e s et al., 1973), or else that SDS in high concentrations removes lipids from proteins (B o n s a l l, H u n t, 1971; T r i p l e t et al.

Other glycoprotein fractions are solubilized by triton X 100.

In extracts obtained with this detergent the presence of 4 new fractions of acid proteins were demonstrated, two of which were of the character of glycoproteins, and 3 new alkaline protein fractions including one glycoprotein one. The number of lipoprotein fractions was much higher than in DSS extract. The action of triton X 100 on membranes is less known than that of SDS. It is known that triton X 100 removes partly lipids from membranes (H e l e n i u s, S i m o n s, 1972) does not affect at all or only very slightly the conformation of solubilized proteins (B o n s a l l, H u n t, 1971; K i r k p a t r i c k, J a c o b s 1970).

Not much is known on the action of another nonionic detergent — tween 80. The present studies showed that it solubilizes a glycoprotein of acid character different from those soluble in SDS and triton, and at the same time it solubilizes the largest amount of lipoprotein fractions.

If we compare the electrophoretic pattern of proteins soluble in the detergents applied, it is seen that their action on the membraneous structure of rye caryopses is different, since they solubilize different proteins. Similar results were obtained by K i r k p a t r i c k et al. (1974) in investigations on the solubilization of proteins and phospholipids from erythrocyte membranes.

Another interesting finding is the fact that detergents solubilize phenol derivatives from rye caryopses. Among these, one was identified as 5-n--alkylresorcin by way of thin-layer chromatography. Triton X 100 was found to extract most alkylresorcinols, and tween 80 the least. The action with triton X 100 after SDS or tween 80 leads to the recovery of the total amount of alkylresorcinols. After treatment of whole grain with acetone (delipidation) the differences in the amounts of alkylresorcinols extracted by the particular detergents disappear.

On the basis of the results here presented it may be assumed that 5-n--alkylresorcin is localized in the membraneous structures of the rye caryopses and is bind noncovalently to the components of these membranes through the hydrophobic receptors of the aliphatic chain present in this compound. The different amounts of 5-n-alkylresorcinol solubilized by the particular detergents may be due to the observed differences in the action of the latter on the membraneous structure of rye caryopses.

REFERENCES

Bonsall R. W., Hunt S., 1971, Biochem. Biophys. Acta, 249—266.
Burkhard R. K., Stolzenberg G. E., 1972, Biochemistry, 11.
Choules G. L., Sandberg R. S., Steggli M., Eyrhig M., 1973, Biochemistry 12; 4544.
Datyner A., Finnimore E., 1973, Anal. Biochem., 52: 45.
Davis B. J., 1962, "Disc Electrophoresis", Distillation Prod. Div. Eastman Kodak Co., Rochester N. Y.
Dunker A. K., Rueckert R. R., 1969, J. Biol. Chem., 244: 5074.
Grajner K., Drewnowska I., Krawczyński J., 1972, Diagnostyka Lab., 8: 277.
Helenius A., Simons K., 1972, J. Biol. Chem., 247: 3656.
Kirkpatrick F. H., Gordesky S. E., Marinetti G. V., 1974, Biochem. Biophys. Acta 345: 154.
Kirkpatrick F. H., Jacobs E. E., 1970, J. Bioenerg., 1: 413.
Kirkpatrick F. H., Sandberg H. E., 1973, Biochem. Biophys. Acta 298: 209.
Lowry J. O. U., Rosebrough N. J., Farr A. L., Randall R. J., 1951, J. Biol. Chem. 193: 265.
Mejbaum-Katzenellenbogen W., Sikorski A., Tłuścik F., 1975, Acta Soc. Bot. Pol. 44: 479—459.
Mejbaum-Katzenellenbogen W., Tłuścik F., Kozubek A., Sikorski A., Maresz Z., 1975, Acta Soc. Bot. Pol. 44: 597—606.
Nelson C. A., 1971, J. Biol. Chem. 246: 3895.
Reisfeld R. A., Lewis U. J., Williams D. E., 1962, Nature 195: 281.
Reynolds J. A., Tanford C. 1970, Proc. Natl. Acad. Sci. USA 66: 1002.
Tanford C., 1972, J. Mol. Biol. 67: 59.
Triplet R. B., Summers J., Ellis D. E., Caraway K. L., 1972, Biochem. Biophys. Acta., 266: 484.
Wieringa G. W., 1967, On the occurence of growth inhibiting substances in rye. H. Veenman en Zonen N. V. Wageningen.
Zacharius Z. M., Zell T. E., 1969, Anal. Biochem., 30: 148.

Authors' address:
Prof. dr Wanda Mejbaum-Katzenellenbogen et co-authors
Institute of Biochemistry, University of Wrocław
Tamka 2; 50-137 Wrocław; Poland

Alkilorezorcynole w ziarniakach żyta (Secale cereale L.)

III. Zastosowanie detergentów do ekstrakcji białek i alkilorezorcynoli

Streszczenie

W przedstawionej pracy zastosowano roztwory siadczanu dodecylu (SDS), tweenu 80 oraz tritonu X 100 do izolacji białek i 5-n-alkilorezorcyny ze śruty ziarniaków żyta. Stwierdzono, że wymienione detergenty ekstrahują inne frakcje białkowe, glikoproteidowe i lipoproteidowe, a także różne ilości alkilowych pochodnych rezorcyny. Uzyskane wyniki tej pracy wskazują na zlokalizowanie 5-n-alkilorezorcynoli w strukturach błoniastych ziarniaków żyta.