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Alkyl resorcinols in grains from plants from the family *Gramineae*

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Abstract

5-n-alkylresorcinols were found in 22 of the 27 studied species of grasses. In *Agropyron caninum* and *Bromus macrostachys* only the content of alkyl resorcinols was determined, in *Agropyron repens, Bromus mollis* and *Elymus arenarius* the composition of alkyl resorcinol homologues was also established. When calculated per gram of dry mass of air-dried grains, the content of alkyl resorcinols was found to be: in the genus *Agropyron* — approximately 715 μg, in the genus *Bromus* approximately 60 μg and in *Elymus arenarius*, 272 μg. The homologues of alkyl resorcinols in the studied genera of grasses differ from the homologues found in wheat or rye by their greater content of long-chain fractions.

Key words: alkylresorcinols, Gramineae, grass.

INTRODUCTION

Among the many phenols found in plants, 5-n-alkylresorcinols are also present. These compounds are characterized by an alkyl substituent with an odd number of carbon atoms attached to carbon C-5 of resorcinol. The distribution of 5-n-alkylresorcinols in the plant kingdom has not been fully recognized yet. They were discovered for the first time in the *Anacardiaceae* family (Kawamura 1928 cit. after Backer and Haack 1941). Alkyl resorcinols have also been found in the following families: *Protaceae* (Occolowitz and Wright 1962 cit. after Madrigal et al. 1977), *Myristicaceae* (Madrigal et al. 1977) and *Ginkgoaceae* (Furukawa 1934 cit. after Backer and Haack 1941).

From among the cereals, 5-n-alkylresorcinols were first isolated and identified in wheat bran (Wenkert et al. 1964), later they were found

in rye (Wieringa 1967) and barley (Briggs 1974). Alkyl resorcinols are present in cereals in a combination of homologues having saturated and unsaturated alkyl chains, 15 to 29 carbon atoms long. In cereals, alkyl resorcinols are found only in grains. They are localized in the outer cuticle of wheat and rye grains, whereas their presence is not found in other layers of the pericarp, embryo or endosperm (Tłuścik 1978). In the family *Gramineae*, alkyl resorcinols were also found in the genera *Agropyron* and *Elymus* (Anioł 1974). Interest in these compounds increased when Wieringa (1967) found that they limit the growth and development of young animals.

MATERIALS AND METHODS

Rye (*Secale cereale* L.), Dańkowskie Złote variety, was obtained from Stacja Hodowli Roślin (Plant Breeding Station) at Rogaczewo. Grains from species belonging to the genus *Agropyron* (*A. intermedium*, *A. trichophorum*, *A. litorale* and *A. trachycaulon*), to the genus *Bromus* (*B. intermis*, *B. ramosus*, *B. sterilis*, *B. racemosus*, *B. commutatus* and *B. tectorum*), to the genus *Lolium* (*L. perenne*, *L. multiflorum*, *L. temulentum*, *L. remotum* and *L. rigidum*) and from species belonging to the genera *Elymus* (*E. glaucus*) and *Avenastrium* (*A.* sp.) were from the Botanical Garden of the Botany Institute at the Wrocław University. Grains from the species *Festuca rubra*, *Dactylis glomerata*, *Bromus mollis*, *Arrhenatherum elatius*, *Alopecurus pratensis*, *Agropyron repens* and *A. caninum* were gathered in the vicinity of Kamienna Góra (Lower Silesia). The species *Bromus macrostachys* and *Hordeum murinum* were from the vicinity of Wrocław, *Elymus arenarius* was gathered near Rewal (on the Baltic coast).

Determination of alkyl resorcinols. Alkyl resorcinols were determined after a few months to one year after harvest. Pure 5-n-alkylresorcinols from rye were obtained by a three-step preparation method according to Mejbaum-Katzenellenbogen et al. (1978). The preparation was used as a standard in further studies.

Thin-layer chromatography was carried out on plates covered with silica gel. The chromatograms were run in a solvent system of chloroform: acetone (95:5, 90:10, 85:15) with the solvent front allowed to advance for a distance of 10 cm. They were then stained with either a 1% aqueous solution of Fast Blue B or 1% vanillin in 50% orthophosphoric acid at a temperature of 110°C for 5-10 min.

Preparative thin-layer chromatography was carried out on glass plates (20×20 cm) covered with a 2 mm layer of silica gel. The solvent system was chloroform: acetone (95:5). The plates were activated before use by heating at 110°C for 30 min. The alkyl resorcinol fraction was

eluted from the gel with acetone and its purity was checked by analytical thin-layer chromatography. When impurities were found, the fraction was rechromatographed increasing the polarity of the solvent system.

Grains, 0.5 g in weight, were extracted twice at room temperature with 5 cm^3 acetone for 24 hours. The extracts were combined and the acetone evaporated. The dry residue was dissolved in 0.5 cm^3 propanol.

Due to impurities which interfered in quantitative determination of the alkyl resorcinols, the propanol solutions were chromatographed on silica gel plates. The alkyl resorcinol fraction was eluted with acetone and colorimetrically assayed by the reaction with Fast Blue B (Tłuścik et al. 1981). It was determined that losses of material during thin-layer chromatography are minimal — 98% of the standard solution was recovered after chromatography.

Gas chromatography was used to analyse trimethylsilylate derivatives of 5-n-alkylresorcinols. To about 1 mg of alkyl resorcinols 0.3 cm^3 of the silanizing agent was added and the mixture was left at 70°C for 30 min. Gas chromatography was carried out under the following conditions: a column 3-m long with an internal diameter of 3 mm filled with 3% SE-30 on Gas-Chrom Q was used. The carrier gas was N_2 20 cm^3/min, H_2 20 cm^3/min, air 200 cm^3/min, the temperature of the injection chamber and of the detector was 320°C, a FID (flame-ionization) detector was used. The temperature program was: increase from 200°C to 300°C at T=1.5°C/min, 300°C for 60 min.

Chemicals. Fast Blue B.BF_4 — Chemapol (Czechoslovakia), BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) — Serva (FRG), 3% SE-30 on 125-150 μm Gas Chrom Q — Serva (FRG), chromatographic plates coated with silica gel (Merck # 5748) and silica gel G for thin-layer chromatography — Merck (FRG). The remaining reagents were from POCh, Gliwice.

Apparatus. Specord UV-Vis (Carl Zeiss, Jena, GDR), gas chromatograph GCHF (Chromatron, GDR).

RESULTS AND DISCUSSION

The presence of alkyl resorcinols was shown by thin-layer chromatography (stained with Fast Blue B and vanillin in orthophosphoric acid) in six studied species of the genus *Agropyron* and in two species of *Elymus* (positions 1-8, Table 1). Alkyl resorcinols were found in eight species belonging to the genus *Bromus* and in six representatives of other genera (positions 9-22, Table 1) when the chromatograms were stained with Fast Blue B (Mejbaum-Katzenellenbogen et al. 1978) which is much more sensitive. In five species of *Lolium* alkyl resorcinals were not found.

Table 1

The results of semiquantitative determination of alkyl resolcinols by thin-layer chromatography of acetone extracts* from grass grains

No.	Species	Mass of grains, g	Volume applied, mm^3	Staining	
				Fast Blue B**	vanillin
1	*Agropyron repens*	0.5	30	+++	+
2	*Agropyron canium*	0.5	30	+++	+
3	*Agropyron litorale*	0.5	30	+++	+
4	*Agropyron intermedium*	0.5	30	+++	+
5	*Agropyron trichophorum*	0.5	30	+++	+
6	*Agropyron trachycaulon*	0.5	30	+++	+
7	*Elymus arenarius*	0.5	40	++	+
8	*Elymus glaucus*	0.5	40	++	+
9	*Bromus macrostachys*	1.0	50	+	—
10	*Bromus mollis*	2.0	50	+	—
11	*Bromus intermis*	1.0	50	+	—
12	*Bromus ramosus*	1.0	50	+	—
13	*Bromus sterilis*	1.0	50	+	—
14	*Bromus racemosus*	1.0	50	+	—
15	*Bromus commutatus*	1.0	50	+	—
16	*Bromus tectorum*	1.0	50	+	—
17	*Hordeum murinum*	1.0	50	+	—
18	*Avenastrium* sp.	2,0	50	+	—
19	*Dactylis glomerata*	2,0	50	+	—
20	*Festuca rubra*	2.0	50	+	—
21	*Arrhenatherum elatius*	2.0	50	+	—
22	*Alopecurus pratensis*	2.0	50	+	—
23	*Lolium rigidum*	0.2	50	—	—
24	*Lolium perenne*	0.2	50	—	—
25	*Lolium multiflorum*	0.2	50	—	—
26	*Lolium remotum*	0.2	50	—	—
27	*Lolium temulentum*	0.2	50	—	—

* — acetone extracts were concentrated to a volume of 0.5 cm^3.

** — approximate amounts of alkyl resorcinols as determined by Fast Blue B staining: +++ — over 5 μg, ++ — about 5 μg, +— less than 5 μg.

A few typical chromatographic patterns of acetone extracts from the studied species are shown on Fig. 1. A fraction, F_2, (Fig. 1) is found in grains from the genera *Elymus*, *Agropyron*, *Hordeum* and *Bromus*. This fraction differs from the alkyl resorcinol fraction by its Rf value, nevertheless, it stains reddish-violet in a way similar to the alkyl resorcinols. This fraction is also found in trace amounts in rye and wheat. We did not study this fraction further — it is probable that it is a derivative of resorcinol.

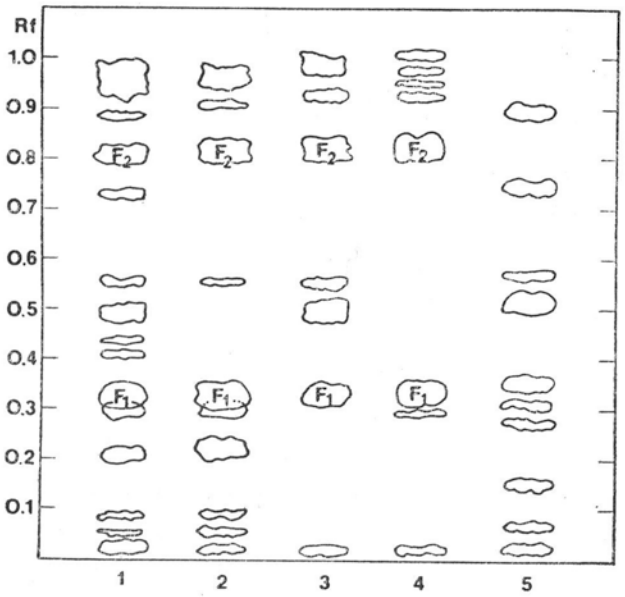

Fig. 1. Thin-layer chromatography on silica gel of acetone extracts from grass grains. The solvent system was chloroform:acetone (90:10). The chromatograms were stained with a 1% solution of Fast Blue B. 1 — *Elymus arenarius*, 2 — *Agropyron trachycaulon*, 3 — *Hordeum murinum*, 4 — *Bromus mollis*, 5 — *Lolium multiflorum*

Alkyl resorcinol fractions from 22 species (positions 1-22, Table 1) were obtained by preparative thin-layer chromatography, rechromatographed on analytical plates, eluted with acetone and dissolved in methanol after the acetone had been evaporated. The UV spectra of these methanol solutions had two absorption peaks (276 and 282 nm) which are characteristic for 5-n-alkyl derivatives of resorcinol (Fig. 2). The spectra of these fractions after reacting with Fiast Blue B were additionally studied in the visible range (Fig. 3). They were also identical with the spectrum of alkyl resorcinols.

In this way, it has been shown that in the studied species of grasses there is a phenolic fraction which has properties typical for 5-n-alkylresorcinols such as: the Rf value in thin-layer chromatography, UV spectrum and visible range spectrum after reacting with Fast Blue B, and, the presence of homologues.

The content of alkyl resorcinols as well as the composition of the homologue fraction was determined in: *Agropyron repens*, *A. caninum*, *Elymus arenarius*, *Bromus macrostachys* and *B. mollis*. A high content of alkyl resorcinols was found in *Agropyron repens* (700 μg/g grains) and in *A. caninum* (732 μg/g). These amounts are similar to those found in rye grains (Table 2). It should be noted that in the studied species there is a clearly higher amount of long-chain alkyl resorcinols than in rye

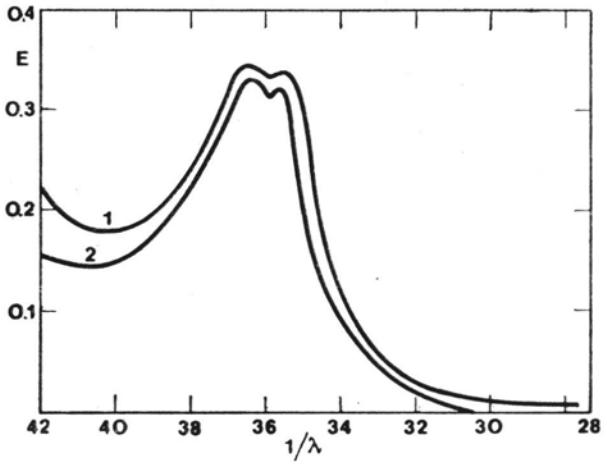

Fig. 2. UV absorption spectra of methanol solutions of alkyl resorcinols. 1 — spectrum of 5-n-pentadecylresorcinol (125 $\mu g \cdot cm^{-3}$), 2 — typical absorption spectrum of the alkyl resorcinol fractions from grass grains

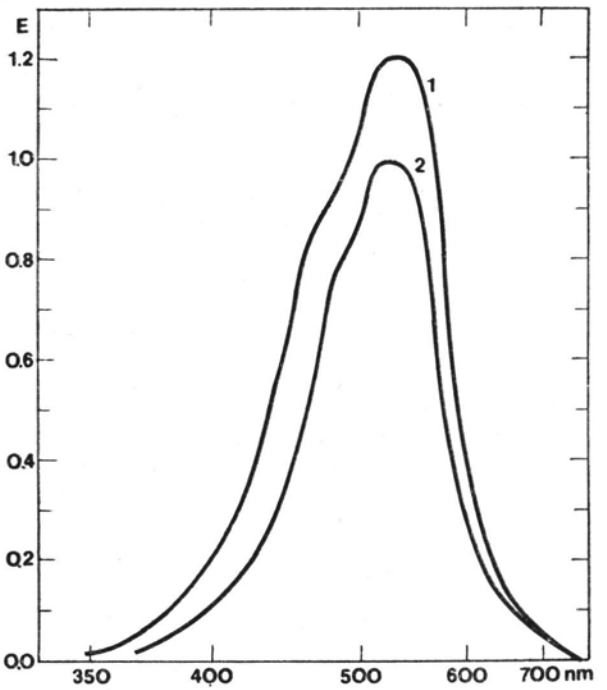

Fig. 3. Visible range absorption spectra of alkyl resorcinols after reacting with Fast Blue B. 1 — spectrum of 5-n-pentadecylresorcinol (10 $\mu g \cdot 2\ cm^{-3}$), 2 — a typical absorption spectrum of the alkyl resorcinol fractions from grass grains

Table 2

Quantitative determination of 5-n-alkylresorcinols* in grass grains

Species	Mass of grains, g	Number of grains	Mass of 1 grain, mg	Alkyl resorcinol content	
				$\mu g \cdot g^{-1}$	µg/grain
Secale cereale L. (cv. Dańkowskie Złote)	1.136	30	37.8	1050	35.00
Agropyron repens	0.5	123	4.06	700	3.09
Agropyron caninum	0.5	117	4.27	732	3.13
Elymus arenarius	0,5	36	13.90	272	3.75
Bromus macrostachys	0.5	108	4.63	71	0.33
Bromus mollis	0.5	124	4.03	50	0.20

* — Determination of alkyl resorcionols was done after eluting the fraction from a thin-layer chromatography plate

(Table 3). This is especially evident in the case of the homologue C_{27} which is found in trace amounts in rye and wheat but constitutes 24% of the total alkyl resorcinol fraction in *Bromus mollis*, 10% in *Agropyron repens* and 4% in *Elymus arenarius*.

Table 3

The results of determining the percentage of alkyl resorcinol homologues by gas chromatography

Species	Number of carbon atoms in the side-chain							
	C_{15}	C_{17}	C_{19}	C_{21}	C_{23}	C_{25}	C_{27}	C_{29}
Secale cereale L. (cv. Dańkowskie Złote)	1.6	19.4	23.5	15.8	7.9	7.4	1.3	—
Agropyron repens	2.8	2.6	8.4	19.1	18.6	38.4	10.1	trace
Bromus mollis	trace	3.1	9.7	19.8	22.8	20.8	23.8	trace
Elymus arenarius	0.5	12.3	24.3	29.8	15.7	13.5	3.9	trace

* — The surface of the peaks was computed using the triangulation method.

Unpublished studies on several scores of varieties of rye and wheat have shown that the homologue composition of alkyl resorcinols for a given species is constant. This feature may be of value in biochemical taxonomy. A n i o ł (1974) only showed the presence of alkyl resorcinols by thin-layer chromatography in *Agropyron* and *Elymus*. We have confirmed this observation and have also determined the amount of alkyl resorcinols in these species and their homologue composition. We have also shown that alkyl resorcinols are present in the genera *Bromus*, *Dactylis*, *Arrhenatherum*, *Alopecurus* and *Festuca*.

Acknowledgment

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Alkilorezorcynole w ziarniakach rodziny Gramineae

Streszczenie

Spośród przebadanych 27 gatunków traw wykryto 5-n-alkilorezorcynole w 22 gatunkach. U *Agropyron caninum* i *Bromus macrostachys* oznaczono tylko zawartość alkilorezorcynoli, natomiast u *Agropyron repens, Bromus mollis* oraz *Elymus arenarius* oznaczono również skład homologów alkilorezorcynoli. W przeliczeniu na gram powietrznie suchej masy ziarniaków zawartość alkilorezorcynoli wynosi: w rodzaju *Agropyron* około 715 μg, w rodzaju *Bromus* około 60 μg, a w *Elymus arenarius* 272 μg. Homologi alkilorezorcynoli w oznaczonych rodzajach traw różnią się od homologów pszenicy czy też żyta większą zawartością frakcji długołańcuchowych.