5-*n*-Alkylresorcinols from Grains of Winter Barley (*Hordeum vulgare* L.)

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The resorcinolic lipid content and homologue composition of winter barley grains harvested at two field locations were evaluated. Depending on the crop location, the predominant alkylresorcinols identified were 1,3-dihydroxy-5-*n*-heneicosylbenzene or 1,3-dihydroxy-5-*n*-pentacosylbenzene. Both resorcinol concentration and their homologue profiles were diverse in samples harvested at different fields indicating a prevailing role of the environment upon the alkylresorcinol biosynthesis in cereals.

Key words: Resorcinolic Lipids, Phenols, Cereals

Introduction

Cereals are not only a great source of essential nutritious proteins, carbohydrates, fibers, vitamins and minerals, but also contain many other substances often considered undesirable from a nutritional point of view. For example, 5-*n*-alkylresorcinols (ARs) are one group of those chemicals that were perceived to be antinutritional for a long time (Kozubek and Tyman, 1999). These polyketide-derived non-isoprenoid long-chain phenolic lipids (Fig. 1) occur in the majority of utilitarian cereal species as mixtures of saturated and unsaturated homologues. In general, the presence of ARs in high amounts has been reported in rye, wheat and triticale, and in low amounts in barley, oat, millet, and maize (Ross *et al.*, 2003). The significance of ARs in human and animal diet has been already demonstrated in several reports (Pawlik *et al.*, 1976; Pawlik, 1979; Ross *et al.*, 2001b; Ross, 2003) and, therefore, a lot of efforts have been put into breeding of new improved cereal cultivars. This improvement, however, can be quite variable and depends on geographical area, climate, weather and agronomy practices, where the crop was raised, as well as on genetic features of particular cultivars (Williams *et al.*, 1994, 1995; Magnucka *et al.*, 2001). These unique and complex conditions may improve, impair or cause no effects on the biosynthesis process of resorcinolic lipids. Because these factors can interact, the precise description of effects caused just by only one of them is almost impossible. In this paper we demonstrate the effect of crop localization on the AR synthesis in barley (*Hordeum vulgare* L.) grains harvested within the same year at two different locations in Poland. In addition, this is the first report demonstrating the presence of ARs in winter barley.

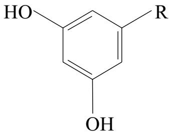

Fig. 1. General formula of resorcinolic lipids; R = alkyl or alkenyl carbon side chain.

Results and Discussion

Grains of winter barley cv. *Gil* harvested at two different distant field locations were analysed for content and composition of resorcinolic lipids (Table I). The use of GC/EIMS enabled us to identify and determine the length and unsaturation degree of alkyl chains in AR molecules. The extracts obtained after conversion into TMS-derivatives showed the base ion peaks characteristic for alkylresorcinols at *m/z* 267 and 268 with ratios varying from 1:5 to 1:4 (Vincieri *et al.*, 1981). Six parental molecular ions with *m/z* masses from 464 to 604 confirmed the presence of saturated homologues with C_{15} to C_{25} side-chains. There were much variations observed in homologue compositions between the two different barley crop field locations,

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Table I. 5-*n*-Alkyl- and 5-*n*-alkenylresorcinols in winter barley grains[a].

Localization	Content [μg/g]	Homologue composition (%)																	UI[c]
		$C_{15:0}$	$C_{17:1}$		$C_{17:0}$	$C_{19:1}$		$C_{19:0}$	$C_{21:1}$		$C_{21:0}$	$C_{23:1}$		$C_{23:0}$	$C_{25:1}$		$C_{25:0}$		
			I[b]	II		I	II		I	II		I	II		I	II			
Pawlowice	48.3	3.9	*t*	*t*	1.1	*t*	*t*	14.6	0.9	*n.d.*	47.9	*t*	*t*	28.2	*n.d.*	*n.d.*	0.9		0.034
Bakow	59.7	*t*	*t*	*n.d.*	3.9	0.6	*t*	11.7	*t*	*t*	27.7	*t*	*t*	19.4	*t*	*t*	35.5		0.018

[a] Means of three replications from three independent samples. Standard error did not exceed 3%.
[b] Congeners sharing the same molecular mass ions (MS), but having different retention times (GC).
[c] Unsaturation index represents the ratio of the sum of unsaturated homologues to total resorcinols.
t = Trace (less than 0.05%); *n.d.* = not detected.

however, their general mass fractions were within the range of those reported previously and amounted about 0.5% (Zarnowski *et al.*, 2002; Ross *et al.*, 2003). Depending on the field location, the predominant compounds found were 1,3-dihydroxy-5-*n*-heneicosylbenzene ($C_{21:0}$) in Pawlowice or 1,3-dihydroxy-5-*n*-pentacosylbenzene ($C_{25:0}$) in Bakow, respectively. The contents of 1,3-dihydroxy-5-*n*-nonadecylbenzene ($C_{19:0}$) and of 1,3-dihydroxy-5-*n*-tricosylbenzene ($C_{23:0}$) were also high, but diversed in two examined fields, whereas 1,3-dihydroxy-5-*n*-heptadecylbenzene ($C_{17:0}$) and 1,3-dihydroxy-5-*n*-pentadecylbenzene ($C_{15:0}$) were found in small quantities only.

According to our previous reports (Suzuki *et al.*, 1997; Zarnowski and Suzuki, 2004), rye and wheat grains contain diverse congeners of the same monounsaturated ARs that occur as mixtures with saturated homologues. In this study, unsaturated AR homologues were also detected (3.4 and 1.8%, respectively), however, the methodology applied enabled us to demonstrate the presence of mixtures of different AR congeners. Regardless of the field location, ARs of winter barley grains with 17, 19, 21, and 23 carbon atoms in the side chain consisted of two congeners, whereas seeds gathered at Bakow also contained two additional congeners of 1,3-dihydroxy-5-*n*-pentacosenylbenzene ($C_{25:1}$) (Table I). It is not out of the question that barley may contain more congeners like wheat and rye (Suzuki *et al.*, 1997; Zarnowski and Suzuki, 2004), however, the detection was limited due to their very low concentration in analysed samples. This is the first study demonstrating the occurrence of different congeners of unsaturated ARs in other than rye and wheat cereal species.

It is very important to have a better understanding of AR metabolism; all the more there are many recommendations for an increased consumption of disease-preventing whole grain cereals (Slavin *et al.*, 2001), which would also lead to an increased AR intake (Ross *et al.*, 2003). AR content and homologue composition in cereal grains have been demonstrated to be highly variable and dependent on cultivar and environmental conditions. However, it is still impossible to state unambiguously whether these variations were due to climate or genetic factors. Previous studies of wheat growing at different locations revealed no link between AR content and cultivar and/or location (Ross *et al.*, 2003). On the other hand, our recent experiments on spring barley proved the influence of environmental conditions upon AR content and composition (Zarnowski *et al.*, 2002). This variability can be even observed between samples harvested from different sites of the same field (Ross *et al.*, 2001b). Unfortunately this approach does it make impossible to define precisely factors that affect AR metabolism. In order to avoid such an undesirable situation in this study, we examined average samples prepared by mixing of several tentative samples collected from different sites of the same field. The results obtained clearly indicate a prevailing role of the environment (crop field location along with climate and soil parameters) upon the AR biosynthesis in winter barley.

Experimental

Grains of the qualified winter barley cv. *Gil* were studied. Plants were cultivated on field plots either at Wroclaw Agricultural University Plant Cultivation Experimental Station in Pawlowice, Poland or at “Hodowla Roslin Smolice” Plant Production Station at Bakow, Poland. A complete cultivar voucher is available from the Central Laboratory for Studies of Cultivable Plants (COBORU), Slupia Wielka, Poland. The plant material was harvested in 2001. Grains were extracted with acetone and fractions of ARs were isolated as described previously (Zarnowski *et al.*, 2002). The microcolorimetric method (Tluscik *et al.*, 1981) was used for quantitative determination of ARs. All determinations were made in triplicate. AR and homologue composition were itentified using GC and EIMS techniques (Zarnowski *et al.*, 2000). The standard of pure 5-*n*-pentadecylresorcinol was provided by Aldrich Chemical Co. (Milwaukee, WI, USA).

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