ELSEVIER

Chemistry and Physics of Lipids
78 (1995) 29–35

Chemistry and Physics of LIPIDS

Cereal grain resorcinolic lipids: mono and dienoic homologues are present in rye grains

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Received 2 May 1995; revision received 30 June 1995; accepted 5 July 1995

Abstract

Analyses (UV, IR, ^{1}H-NMR, MS) of the main phenolic fractions isolated by sequential separation on normal-phase and by argentation chromatography on silica gel confirmed the presence of monoenoic and dienoic homologues of 1,3-dihydroxy-5-*n*-alkylbenzene in acetone extracts from rye grains. Conversion of mono and dienoic homologue dimethyl ethers to the *cis*-diols with osmium tetroxide, transformation of the diol to the acetonide with acetone and subsequent MS analysis of resulting derivatives showed that the breakdown pattern for the monoenoic homologues was consistent with a double bond in all the homologous chain length at the 8-position. For dienes, the results were not so conclusive, although the 8- and 11-positions appear to be the favoured ones. It has been also shown that rye 5-*n*-ketoalkylresorcinols contain a previously unobserved C17 homologue. All identifications were confirmed by comparison with synthetically obtained C19:0 and C21:0 5-*n*-alkylresorcinols and a 5-*n*-(2-keto-heptadecyl)resorcinol. Other minor phenolic components present in the acetone extract were identified as homologous 5-*n*-(2-hydroxyalkyl)resorcinols.

Keywords: Phenolic lipids; Resorcinolic lipids; Alkylresorcinols; Cardol homologues; Cereal; Rye

1. Introduction

Since the pioneering papers of Wenkert et al. [1] and Wieringa [2], it is known that wheat and rye grains or the bran fraction obtained during their milling contain as a major fraction phenolic compounds that had been identified as long-chain alkyl derivatives of 1,3-dihydroxybenzene [1], a C_{19} and C_{21} homologous analogues of the C_{15} cardol component of the cashew phenols from *Anacardium occidentale* L. By contrast with the latter group, the long-chain resorcinols of cereal grains comprise a wide range of homologues. Further work has concerned quantitative and compositional analysis of these compounds and demonstrated their presence in other gramineaceous materials [3–8]. The level of alk(en)ylresorcinols is higher in rye (up to 5000 μg/g with enoic components equivalent to 15–

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SSDI 0009-3084(95)02480-7

50%) than in wheat (up to 900 μg/g and 6–20% of enoic components) and barley (up to 150 μg/g with traces of enocic components). An important feature of the rye and wheat compounds of interest is their localisation in the bran milling fraction (up to 1500 μg/g of wheat bran). Populations, particularly in Western countries, are concerned to have an adequate amount of fibre in their diet and large amounts of high-fibre products are consumed, prepared mostly with cereal grains. A detailed knowledge of the chemical and biological activity of the resorcinolic lipids is important. Results already obtained indicate that the resorcinolic lipids due to their amphiphilic structure are active biomembrane-altering agents with antioxidant properties, e.g. [9–19]. Recent work of Tsuge et al. [20] reports two resorcinolic lipids isolated from the antibiotic source *Streptomyces cyaneus*. These compounds have been found to be inhibitory to glycerol-3-phosphate dehydrogenase, a key enzyme of triglyceride synthesis, therefore suggesting a weight control function of resorcinolic lipids.

During our work on rye grain acetone extracts, it was found that besides the strong 5-*n*-alk(en)ylresorcinolic band, the extracts from rye and wheat reveal, on silica gel TLC, the presence of several additional minor phenolic fractions that specifically react with Fast Blue B. One of these bands was recently identified as containing alkyl- and alkenyl-resorcinol derivatives having a keto group attached to the carbon C2 of the aliphatic chain [21], which consist of approximately 10% of total resorcinolic lipids. Seitz also suggested [21] that these derivatives were previously identified by us as dienoic alkylresorcinol homologues [22, 23]. However, despite extensive analysis of resorcinolic lipid homologues according to the chain length, the position of unsaturation in cereal grain alkenylresorcinols had not yet been determined.

In this paper, we will present further analysis of cereal grain enoic homologues of resorcinolic lipids, together with synthetic work confirming the presence of ketoalkylresorcinols and not previously identified hydroxyalkylresorcinols, in grain extracts.

2. Materials and methods

Samples of rye and wheat grains or bran fractions, obtained in local markets and health food centres, represent the most popular varieties of Dankowskie Zlote (rye) and Grana (wheat).

2.1. Extraction of material

The whole kernels or bran (200 g) were extracted overnight at room temperature with 350 ml of acetone. The extracts were filtered through double paper filter to remove fine particulates and the solvent was removed in a rotary evaporator at 35°C. The residual, acetone oil, for further processing, was dissolved in chloroform.

2.2. Chromatography

The analyses were performed using HPTLC silica gel Si 60 analytical plates (Merck 5641) and PLC silica gel 60 preparative plates (Merck 5745). For development of chromatograms, the following solvent systems were used: chloroform/ethyl acetate (90:10 and 70:30 v/v), benzene/ethyl acetate (85:15 v/v) and petroleum ether/diethyl ether/formic acid (70:30:1 v/v). Bands of separated components were visualised by (i) iodine staining (ii) 50% aqueous H_2SO_4 spraying and charring at 140°C and (iii) spraying with 0.05% aqueous Fast Blue B (Serva) [22]. Individual bands of separated components were isolated either by preparative thin-layer chromatography in chloroform/ethyl acetate (90:10 v/v) or by column chromatography on a glass column (20 × 270 mm) packed with Sorbsil 40-60H (Rhone-Poulenc) silica gel suspended in chloroform/ethyl acetate (90:10 v/v) and eluted with the same solvent system at a flow rate of 3–4 ml/min. Fractions of 12 ml were collected, the solvent evaporated in vacuo and the residue dissolved in small volumes of chloroform, analysed by TLC. Argentation thin-layer chromatography was performed on HPTLC plates impregnated by dipping for 10 min into 20% silver nitrate solution in 50% methanol and drying for 30 min at 100°C. Plates were developed in chloroform/ethyl acetate (70:30 v/v). Column argentation chromatography was performed on a 20 × 400 mm column filled with silica gel Si 60 (J.T. Baker) impregnated with 5%

Table 1
Reaction of some phenolic lipids with aqueous 0.01% (w/v) Fast Blue B

Compound	Colour developed
5-alk(en)ylresorcinols from cereal grains	Violet
5-pentedec(en)ylresorcinols (cardol)	Violet
Cardol (one hydroxylic group methylated or acethylated)	Red-violet
Cardol (both hydroxylic groups methylated or acethylated)	Weak reddish-pink, long colour development
5-(2-Ketoalk(en)yl)resorcinols	Violet
5-(2-Hydroxyalk(en)yl)resorcinols	Deep violet
5-Pentadec(en)yl-4-methylresorcinol (methyl cardol)	Brown-reddish violet
3-Pentadec(en)ylphenol (cardanol)	Yellow-orange
2-Hydroxy-6-pentadec(en)ylbenzoic acid (anacardic acid)	Pale yellow, long colour development

of $AgNO_3$ and eluted with chloroform/ethyl acetate (85:15 v/v) in similar manner to that used for normal-phase column chromatography.

2.3. Analytical methods

Spectral analyses were carried out: UV spectra in a CECIL 5000 double beam spectrophotometer, IR spectra in a Perkin Elmer 1420 Ratio Recording IR spectrophotometer (thin films), ^{1}H-NMR analysis in a JEOL JNM-FX200 spectrometer, low resolution MS spectra in an AEI MS902 instrument. Accurate masses were determined at the SERC Mass Spectrometry Service Centre at University College of Swansea. The position of double bonds in enoic alkylresorcinols was established by mass spectral analysis of the breakdown pattern of derivatives obtained by osmium tetroxide oxidation of dimethyl ethers to glycols and conversion to acetonides as described by Jefferson and Wangcharentrakul [24].

2.4. Reference compounds

Hydrogenated cardol (5-*n*-pentadecylresorcinol) from cashew nut-shell liquid and synthetic 5-*n*-nonadecylresorcinol were used as reference compounds.

3. Results and discussion

Acetone extracts of whole cereal grains or bran fraction reveal upon separation on normal-phase silica gel plates and non-specific staining, the presence of several fractions, five of which are Fast Blue B positive indicating their phenolic nature. Experiments on cashew nut-shell liquid (technical industrial material), a source of several various phenolic lipids, was used as a control. The colours found with Fast Blue B were found to be diagnostic for various phenolic lipid constituents as depicted in Table 1. Extraction of whole cereal kernels or bran with acetone was chosen as this procedure avoided extensive extraction of other unnecessary lipids extracted with, for example chloroform/methanol from finely grounded material [25–27]. From a number of solvent systems examined, chloroform/ethyl acetate (85:15 and 90:10 v/v) and diethyl ether/petroleum ether/formic acid (30:70:1 v/v) were found to be most suitable in TLC analyses. In rye grain extract, two of the Fast Blue B positive bands (violet) are predominant, namely, one of alk(en)ylresorcinols (80% of total phenolics) and the other of ketoalk(en)ylresorcinols (R_f 0.27 and 0.16, respectively, in chloroform/ethyl acetate, 85:15 v/v, 10% of total phenolics). These bands were recovered and extracted with diethyl ether. Presence of unsaturated components in each band was shown by argentation thin-layer chromatography which was used in their subsequent separation. Both alk(en)ylresorcinols and ketoalk(en)ylresorcinols showed the presence of enoic homologues and TLC comparison on silver nitrate-impregnated silica gel (chloroform/ethyl acetate solvent systems) of cereal resorcinolic lipids with cardol homologues from CNSL clearly demonstrated the presence of saturated, monoenoic and dienoic members and the absence of trienoic homologues in grain extracts. Further analyses of enoic alkyl-

Table 2
IR and ^{1}H-NMR data for rye monoenoic and dienoic homologues of 5-*n*-alkylresorcinols

Compound	IR data (thin film)	^{1}H-NMR data
Rye monoenes		
	830, 980, 1160, 1310 w, 1470, 1600, 2860, 2930 3000 vw, 3330	0.88 (t), 1.25, 1.57, 1.96–2.02 (t), 2.48 (t), 5.35 (t) (J_{cis} 5 Hz), 5.20–5.40 (b, D_2O exc.) 6.17, 6.23
Rye dienes		
	800, 1000 w, 1100 w, 1160, 1260, 1300 w, 1350 w 1470, 1610, 2860, 2930, 3400	0.88 (t), 1.25, 1.57, 2.03 (t), 2.31 (t), 2.45 (t), 2.78 (m) 5.34 (t) (J_{cis} 5 Hz), 5.17–5.40 (b, D_2O exc.), 6.15, 6.23

resorcinol members were performed after their preparative isolation. The isolated material showed UV spectra identical to those given by natural and hydrogenated cardol (5-*n*-pentadecylresorcinol) with a double peak at 276 and 282 nm in agreement with literature data [28]. After hydrogenation these constituents showed, by argentation TLC only one spot, identical to hydrogenated cardol and synthetic 5-*n*-nonadecylresorcinol. Infrared spectra of thin films of the monoene and diene constituents were also comparable with those of corresponding cardol constituents and are listed in Table 2. The ^{1}H-NMR spectra in $CDCl_3$ with tetramethylsilane as standard again exhibited the expected chemical shifts, coupling constants confirming the presence of monoenoic and dienoic constituents with double bonds in the *cis* and which in the diene were methylene-interrupted. All these results, shown in Table 2, indicate that with respect to double bond configuration and the absence of conjugated bonds cereal grain resorcinolic lipids do not differ from compounds isolated from other sources [29]. Low resolution mass spectrometric analysis of mono and dienoic constituents showed the presence of peaks 124 and 123 with an m/z ratio (4:1), 137 and 166 characteristic of alkylresorcinols with m-substitution [4]. MS data for homologues found in analysed preparations (Table 3) indicate the presence of homologues C15–C29, in agreement with previously published data [4–7, 26]. The accurate mass of homologues in rye monoenes and dienes (Table 4) also confirmed the presence of these, as previously identified.

In the determination of the double bond position for the unsaturated constituents, the mass spectral analysis of their derivatives was employed. For this analysis, preparations containing mixed homologues were used in a preliminary approach on the probable assumption of constant double bond position regardless of homologue chain length. The material prior to MS analysis was methylated with dimethyl sulphate and subsequently converted to the *cis*-diol with osmium tetroxide. The resulting diols were condensed with acetone to form acetonides, derivatives which show characteristic breakdown patterns leading to enhanced peaks due to fission at the bond adjoining the original double bond [24, 29].

It was shown that the breakdown pattern for the monoenes was consistent with a double bond in all homologous chain length at the 8-position. With the diene, the results were not so conclusive, although the 8-position appeared to be the favoured one. The second double bond would be in consequence at the 11-position on account of the presence of methylene-interrupted conjugation. The presence of dienoic homologues can also be concluded from argentation HPTLC analysis which indicates the presence of other fractions of lower R_f value besides the main ones of the monoene and saturated constituents.

The data presented show that the occurrence of enoic constituents in cereal grain 5-*n*-alkylresorcinols is established without question despite stated doubts [21]. It is worth noting that the analysis of crude extracts or preparations enriched in phenolic fractions, i.e. without isolation of

Table 3
Mass spectrometric data for rye mono and dienoic alkylresorcinols

Compound	Characteristic ions (m/z)	Homologues found	Relative abundance (%)
Monoenes	123, 124 peak height	C15	1
	124/123 (4:1), 137, 166	C17	2
		C19	1
		C21	8
		C23	36
		C25	44
		C27	8
		C29	1
Dienes	123, 124 peak height	C15	1
	124/123 (4:1), 137, 166	C17	21
		C19	30
		C21	40
		C23	5
		C25	2
		C27	0.5
		C29	0.5

particular bands may indeed lead to misleading conclusions due do similar TLC mobilities on normal and Ag-impregnated silica gel of monoenoic alkylresorcinols and 2-ketoalkylresorcinols.

The isolated fraction of ketoalk(en)ylresorcinols gave UV and IR spectrometric patterns that were in agreement to data given by Seitz [21]. These components showed characteristic MS fragmentation patterns for m-substituted compounds with the intensity of m/z ion 123 stronger than that of 124. Table 5 lists the MS data and accurate masses of the most abundant homologues in the preparation of saturated 2-ketoalkylresorcinols. It is noteworthy that the presence of 5-*n*-(2-ketoheptadecyl)resorcinol indicated in our preparation which had not previously been noted and that, in comparison to 5-*n*-alk(en)ylresorcinols, the profile peak of the chain lengths present is shifted towards the shorter-chain members. The differences in the chain length composition of 2-ketoalkylresorcinols between our work and data presented by Seitz [21] can be related to cereal grain variety specificity of composition. On the other hand, in attempts at complete separation and isolation without cross-contamination of such heterogeneous mixtures that differ both in chain length and unsaturation, the usually rigorous, cut-and-shave chromatographic procedure for bands is employed. The cutting of leading and tailing parts of separated band may lead to the apparent alteration of homologue composition. Long chain homologues on hydrophilic silica gel migrate, due to their higher hydrophobicity, closer to the band leading edge, whereas short chain homologues are retained more strongly and migrate closer to the band tail. This observation has been confirmed when TLC mobility of individual homologues on silica gel was analysed [22].

To confirm spectroscopic identification of 5-*n*-(2-ketoalkyl)resorcinols in acetone extracts from cereal grains, one of their homologues, 5-*n*-(2-ketoheptadecyl)resorcinol was chemically synthe-

Table 4
Accurate MS data for main homologues of rye monoenes and dienes

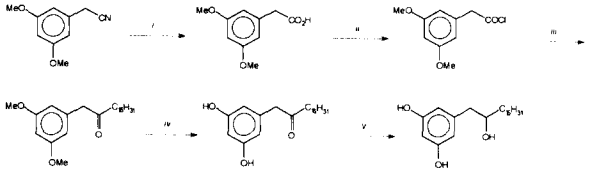
Compound	Measured masses	Calculated masses
Monoenes	402.3498	402.3495
	430.3811	430.3810
	458.4124	458.4121
	486.4440	486.4438
Dienes	345.2794	345.27916
	373.3107	373.31044
	401.3420	401.34172

Table 5
Mass spectrometric data for rye 5-(2-ketoalkyl)resorcinols

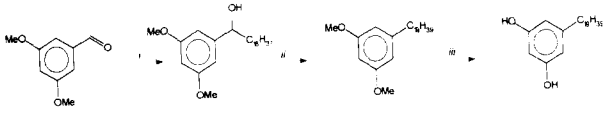
Compound	Identified homologs	Relative abundance (%)	Measured masses	Calculated masses
5-(2-Ketoalkyl)resorcinol	C17	9	362.2821	362.28189
	C19	41	390.3134	390.31317
	C21	23	418.3447	418.34445
	C23	19	446.376	446.37573
	C25	6		
	C27	2		

sised. The route presented in Scheme 1 was chosen. 3,5-Dimethoxybenzyl nitrile was converted to 3,5-dimethoxyphenylacetic acid and, thence, to the acid chloride. Reaction of the acid chloride with di(*n*-pentadecyl)cadmium prepared by way of the Grignard reagent, afforded 3,5-dimethoxybenzyl-*n*-pentadecyl ketone, which was demethylated by boron tribromide to 5-*n*-(2-keto-*n*-heptadecyl)resorcinol in an overall yield of 20%. The product showed all characteristic chromatographic and spectrometric features identical to those of natural isolated rye compounds. A comparison of natural 2-ketoalkylresorcinols methylated with dimethyl sulphate was identical in properties and TLC behaviour to synthetic 2-ketoheptadecylresorcinol dimethyl ether. The homologous 5-*n*-alkylresorcinols were also synthesised from 3,5-dimethoxybenzaldehyde and the required *n*-alkyllithium by the procedure shown in Scheme 2.

Comparison of the sodium borohydride-reduced synthetically obtained 2-ketoalkylresorcinol with the isolated natural mixture of these derivatives treated the same way gave support to the likely presence of 2-hydroxyalkylresorcinols as a third component, in order of colour intensity with a Fast Blue B reaction in crude acetone extracts from cereal grains or bran (R_f 0.05 on silica gel in chloroform/ethyl acetate 85:15 v/v, ~ 10% of total resorcinolic lipids). These derivatives can be readily converted back to the original keto state by mild oxidation with pyridinium chlorochromate.



Scheme 1. The route of synthesis of 5-*n*-(2-ketoheptadecyl)resorcinol. (i) NaOH H_3O^+; (ii) $SOCl_2$; (iii) $(C_{15}H_{31}Br)_2Cd$; (iv) BBr_3, −77°C, 72 h; (v) $NaBH_4$.



Scheme 2. The route of synthesis of 5-*n*-nonadecylresorcinol (i) $C_{18}Li$; (ii) Pd/C, H_2 H_3O^+; (iii) BBr_3.

Acknowledgement

This work was supported by the Commission to the European Communities COST Programme Grant No. 10625 and KBN Grant No 4 1294 91 01.

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