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Absorption of dietary alkylresorcinols in ileal-cannulated pigs and rats

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Alkylresorcinols (AR) are amphiphilic 1,3-dihydroxy-5-alkyl phenolic lipids. AR in food are only found in the outer layers of wheat and rye grains, and in whole grains are present at concentrations of 500–1000 μg/g. In wheat and rye, there are five main homologues, differing in the length of the odd-numbered alkyl chain (from seventeen to twenty-five C atoms long). Because AR may be bioactive, and might serve as biomarkers for these cereals, their absorption was investigated in model experiments with pigs and rats. Pigs with a cannula in the terminal ileum were fed four diets containing rye fractions with different levels of AR and the ileal effluents were analysed. The ileal recovery of AR was found to vary between 21 and 40 %, with no major difference between different chain-length homologues. The absorption of AR by rats was investigated by feeding ^{14}C-labelled heneicosylresorcinol ($C_{21:0}$). Of the total activity, about 34 % was recovered in the urine, showing that the labelled AR was absorbed and metabolised by rats. AR were mostly cleared from rats by 60 h. It is concluded that AR are absorbed in the small intestine of single-stomached animals and excreted in metabolised form in the urine, and might contribute to the nutritional qualities of wholegrain wheat and rye diets.

Alkylresorcinols: Ileal absorption: Wholegrain rye

Cereals contain a large number of phenolic compounds that may play a role in the observed health effects of plant-based foods (Clifford, 2000). Alkylresorcinols (1,3-dihydroxy-5-alkylbenzenes) (AR) are phenolic lipids with a hydrocarbon side chain (Fig. 1). The side chain in cereal AR consists of an odd-numbered straight hydrocarbon chain (fifteen to twenty-seven C atoms), most commonly saturated, but small amounts of analogues with unsaturated and keto modifications of the side chains also exist (Kozubek & Tyman, 1999). Among cereals, AR are found mainly in wheat, rye and triticale, with small amounts reported in barley (Ross *et al.* 2003). AR are present in the outer part of the kernel (Tłuścik, 1978; Ross *et al.* 2001) and are therefore only found in high amounts in wholegrain wheat and rye products. Almost no AR are found in white sifted flour (starchy endosperm fraction) of rye and wheat grains (Ross *et al.* 2003).

The consumption of foods rich in fibre, such as wholegrain cereals, is believed to be associated with a reduced risk of major chronic diseases including various cancers, and cardiovascular and other age-related diseases (Truswell, 2002). The dietary-fibre complex, which includes a wide range of vitamins, phytohormones and other bioactive compounds, is essential for good health (Åman *et al.* 1997). AR are specific to the outer parts of kernels of wheat and rye, and are stable during cooking and baking and during sample storage and analysis (Ross *et al.* 2003). This suggests that if AR are absorbed and traced in blood and/or urine, they might serve as biomarkers for diets rich in whole grains and/or brans of wheat and rye. Knowledge

Abbreviation: AR, alkylresorcinols.

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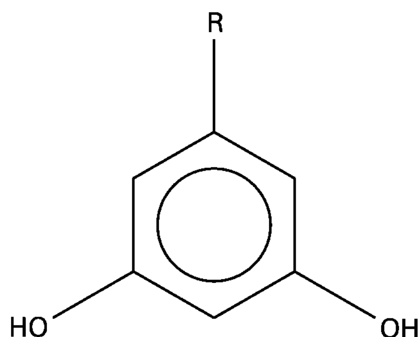

Fig. 1. General structure of alkylresorcinols. In cereals, the length of the odd-numbered hydrocarbon chain at 'R' can vary in length from fifteen to twenty-seven C atoms.

about the absorption of AR is also important to understand their bioavailability and any possible biological effects *in vivo* (for a comprehensive review of the bioactivities of AR, see Kozubek & Tyman, 1999).

There is only one published paper on the digestion of AR, showing that 36–48 % of dietary AR are excreted in their intact form in the faeces in rats (Tłuścik *et al.* 1990). Since some intestinal bacteria might metabolise AR (Kozubek & Tyman, 1999), incomplete faecal excretion of AR cannot be taken as unequivocal evidence for their absorption. In the present study, two animal models were used to demonstrate the absorption of AR. A pig model was used to determine the ileal recovery of AR from four different rye-grain fractions containing different amounts of AR, and a rat model was used to determine the absorption of a pure ^{14}C-labelled AR and measure its presence in blood, and excretion in faeces and urine. The two models complement each other in providing information about the possible site of absorption and fate of absorbed AR.

Materials and methods

Chemicals and reagents

β-Glucuronidase–sulfatase from *Helix pomatia* was purchased from Sigma (Poole, Dorset, UK) and [2-^{14}C]diethyl malonate was purchased from Amersham International (Little Chalfont, Bucks, UK). Standards for GC calibration were olivetol (pentylresorcinol, $C_{5:0}$; Sigma Chemicals, St Louis, MO, USA), pentadecylresorcinol ($C_{15:0}$) (purified from Aldrich pentadecylresorcinol; Kamal-Eldin *et al.* 2000) and heneicosylresorcinol ($C_{21:0}$) (for details of synthesis, see later). Dimethyl acetylmethylphosphonate and diethyl malonate (unlabelled) used for AR synthesis were obtained from Aldrich (Poole, Dorset, UK). The internal standard used for GC analysis was methyl behenate ($C_{22:0}$; Larodan Fine Chemicals AB, Malmö, Sweden). All solvents were of analytical grade (E Merck GmbH, Darmstadt Germany) and were used without further purification.

Ileal digestibility of alkylresorcinols in pigs

Rye-bread diets fed to pigs. Rye (*Secale cereale*, cv Marder) was separated by dry milling into fractions rich in the pericarp and testa, the aleurone layer, and starchy endosperm (Glitsø & Bach Knudsen, 1999). Although the milling fractions were not pure, they are referred to by their botanical names. Rye breads used for pig feeding were based on the whole rye and rye-milling fractions, enriched with wheat starch, casein, gluten, lard, vegetable oil, vitamins, minerals, yeast and water so that the breads would make up fully adequate diets for the pigs (Table 1). Breads were baked in an industrial bakery (Pandrup Brød, Schulstad A/S, Pandrup, Denmark). The energy contribution from fat, protein and carbohydrates was approximately 25, 15 and 60 % respectively. AR in rye fractions added to the diets were analysed as explained later, and other dietary components analysed using the methods of Glitsø *et al.* (1998).

Ileal recovery of rye alkylresorcinols in pigs. Twenty growing castrated male pigs (five pigs/diet) were fed the experimental diets and two sets of pooled ileostomy sample were taken over 4 d (two samples/pig, ten samples/diet). The pigs weighed approximately 30 kg at surgery (Danish Institute of Agricultural Sciences swine herd, Foulum, Denmark) and were fitted with simple T-cannulas about 150 mm anterior to the ileo-caecal junction and allowed to recover for 8–10 d. After an adaptation period of 8–9 d on the experimental diets, the pigs were placed in metabolism cages for recovery measurements. Before feeding, the breads were cut into small pieces (Robot Coupe SA, Rosney, France) and mixed 9:1 with an unheated raw diet based on the cereal fractions with approximately the same dietary composition as the breads, and 10 g Cr_2O_3/kg diet as an indigestible marker of recovery (Saha & Gilbreath, 1991). The breads and raw diet were thoroughly mixed with water in the proportion of 1:2:5, and fed three times daily (07.00, 15.00 and 23.00 hours) with the amount of diet (based on DM) adjusted to 3 % of the body weight of the individual pigs. The pigs ate all the experimental diet given to them. Ileal contents were collected from 07.00 hours (morning feeding) to 15.00 hours (afternoon feeding), and repeated 2 d later. The ileal collections were performed by attaching small plastic tubes to the cannula, and were replaced when full or after 90 min. The ileal samples were frozen and later thawed by careful heating and stirring for a minimum time to restrict any bacterial degradation of AR, and finally pooled (per pig per balance period per time of day). The pigs rested for 1 week, still consuming the experimental diets, and were then subjected to a similar second balance period in the metabolism cages. Freeze-dried ileal samples were analysed for AR. All aspects of this protocol were approved by the Danish Animal Experiments Inspectorate, Copenhagen, Denmark. For more details regarding the experimental design, see Glitsø *et al.* (1998).

Analysis of alkylresorcinols in pig diets and ileal effluent. The AR content of rye fractions and ileostomy samples was analysed in triplicate (CV < 5 %). Analysis was performed without derivatisation using a GC–flame ionisation detector method reported previously (Ross *et al.* 2001). Freeze-dried ileal samples were ground to a particle size less than 0·5 mm before analysis. The DM content was determined by drying the samples at 105°C for 6 h.

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Table 1. Ingredients (g/kg), chemical composition (g/kg dry matter) and alkylresorcinol (AR) content (mg/kg diet dry matter) of rye-bread-based diets fed to pigs

Diet…	Whole rye	Pericarp–testa	Aleurone	Starchy endosperm
Ingredients (g/kg)				
Rye raw material	866	213	591	762
Wheat starch	–	546	277	–
Casein	26	112	28	104
Gluten	9·5	9·6	9·5	9·5
Soyabean oil	35	46	33	49
Lard	29	38	27	41
Baker's yeast	7·1	7·2	7·1	7·1
Vitamin–mineral mixture	26	27	26	26
Cr_2O_3	0·77	0·87	0·80	0·77
Chemical composition (g/kg DM)				
Protein (N × 6·25)	133	144	148	174
Fat	94	106	99	103
Starch	519	503	471	559
Dietary fibre	156	177	180	94
Water content (%)	8·5	5·7	8·6	10·3
Total AR* (mg/kg diet DM)	484	204	1290	27
AR (% of total)				
17:0	21	18	21	16
19:0	30	26	29	29
21:0	26	24	26	33
23:0	11	15	11	12
25:0	12	17	13	11

* AR were analysed in triplicate, CV < 5 %.

GC–MS was used to confirm the presence of AR in the ileal excreta by selective ion recording at m/z 124 and at the molecular ion of individual saturated AR (Fig. 2). Selective ion recording was also run at m/z 311 and 354 to detect the methyl behenate internal standard. Standard $C_{15:0}$ AR was used to confirm the fingerprint of saturated AR. The GC–MS method is described elsewhere (Ross *et al.* 2001).

Calculations and statistical calculations of pig ileostomy study. Recovery of the dietary AR in ileal effluents was calculated on the basis of the concentrations of AR and marker (Cr_2O_3) in the diet and ileal digesta using the following formula:

$$\text{Recovery of AR} = \frac{AR_{ileum} \times Cr_2O_{3\,diet} \times 100}{AR_{diet} \times Cr_2O_{3\,ileum}},$$

where AR_{ileum} is the concentration of AR in ileal effluents, AR_{diet} is the concentration of AR determined in the diet and $Cr_2O_{3\,diet}$ and $Cr_2O_{3\,ileum}$ are the concentrations of Cr_2O_3 in the diet and ileum respectively.

Statistics of pig ileostomy study. Data from the pig ileostomy study were compared using Student's *t* test with the Statistical Analysis Systems statistical software package version 8.0 for Windows® (SAS Institute, Inc., Cary, NC, USA).

Absorption of alkylresorcinols by rats

Synthesis of heneicosylresorcinol ($C_{21:0}$). 5-*n*-Heneicosylresorcinol and [4-^{14}C]5-*n*-heneicosylresorcinol were synthesised according to Fig. 3. The synthesis of 5-*n*-heneicosylresorcinol, starting from 3,5-dimethoxybenzaldehyde, was described by Wenkert *et al.* (1964). However, since the present study called for the incorporation of labelled C into the aromatic ring, it was decided to build the aromatic ring at a late stage of the synthesis, using diethyl malonate or [2-^{14}C]diethyl malonate at that point. This apparently straightforward route was used successfully for the synthesis of olivetol ($C_{5:0}$) by Focella *et al.* (1977). However, with the twenty-one-C chain needed for our work, yields were found to be unreliable in the last two steps. Nevertheless, sufficient material was obtained for the requirements of the present study. The procedure was detailed as follows.

Oxidation of 1-docosanol was carried out with pyridinium chlorochromate in dichloromethane at room temperature to give the aldehyde (yield of 91 %). The Emmons-Wittig reaction of the aldehyde with dimethyl acetylmethylphosphonate in refluxing diethyl ether using NaH as a base was slow (required 2–3 d to go to completion) but efficient, giving the enone as a mixture of *cis*, *trans* isomers (yield of 95 %). The Michael addition-Claisen condensation of the enone using diethyl malonate with sodium methoxide at room temperature was also slow, but variable yields of up to 75 % 5-alkyl-methoxycarbonyl-1,3-cyclohexanedione were obtained after 2–3 d. The most difficult step in the synthesis was the aromatisation-decarbomethoxylation sequence; 5-alkyl-methoxycarbonyl-1,3-cyclohexanedione to 5-*n*-heneicosylresorcinol. Treatment of 5-alkyl-methoxycarbonyl-1,3-cyclohexanedione or ^{14}C-labelled 5-alkyl-methoxycarbonyl-1,3-cyclohexanedione with bromine in dimethyl formamide proceeded smoothly at room temperature, but conversion to 5-*n*-heneicosylresorcinol and [4-^{14}C]5-*n*-heneicosylresorcinol required heating the mixtures under reflux for 10 d. After isolation, each

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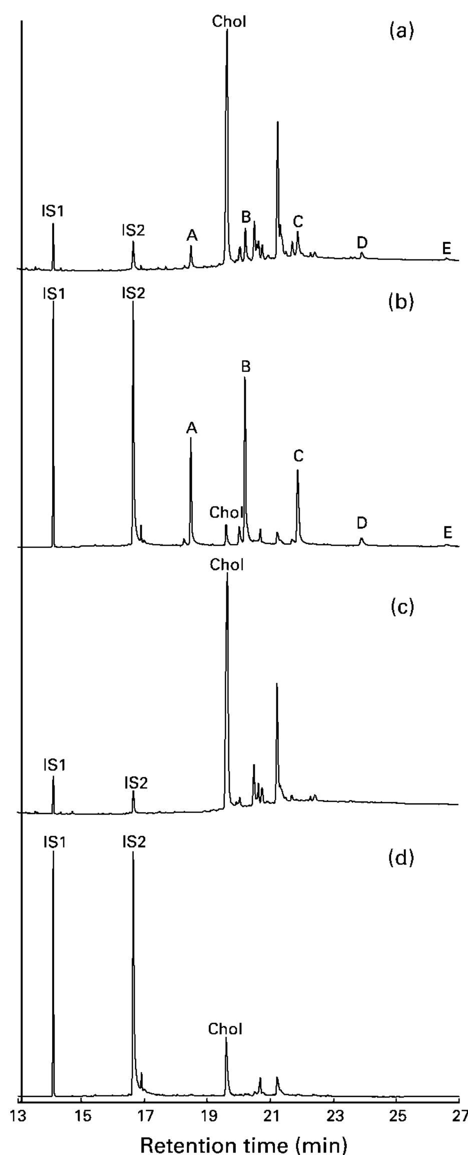

Fig. 2. GC–MS chromatograms of pig ileostomy samples. Total ion chromatograms (m/z 100–700; (a) and (c)) and selective ion recordings (m/z 124; (b) and (d)) of an ileostomy sample from a pig fed a rye-aleurone-layer-enriched diet (a, b) or a rye-endosperm-enriched diet (c, d). IS1, internal standard 1 (methyl behenate); IS2, internal standard 2 (5-pentadecylresorcinol, $C_{15:0}$); A, 5-heptadecylresorcinol ($C_{17:0}$); B, 5-nonadecylresorcinol ($C_{19:0}$); C, 5-heneicosylresorcinol ($C_{21:0}$); D, 5-tricosylresorcinol ($C_{23:0}$); E, 5-pentacosylresorcinol ($C_{25:0}$); Chol, cholesterol. For details of chromatographic conditions, see p. 789.

product was purified by two chromatographic separations on silica gel (the first column being eluted with CH_2Cl_2 followed by 0·5 % (v/v) methanol in CH_2Cl_2 and the second column with diethyl ether–light petroleum; 9 : 1 (v/v)) and then by distillation (short path, 220°C, 0·03 mmHg) to > 99 % purity. By this means the unlabelled compound 5-*n*-heneicosylresorcinol was obtained in yields of up to 46 % based on the dione (5-alkyl-methoxycarbonyl-1,3-cyclohexanedione) and of 18–34 % based on diethyl malonate. In the case of the labelled compound ([4-^{14}C]5-*n*-heneicosylresorcinol), only 2·3 % was obtained, based on diethyl malonate. Melting points and appearances were: 5-*n*-heneicosylresorcinol, 103·5–104·5°C, colourless solid; [4-^{14}C]5-*n*-heneicosylresorcinol, 101·5–102·5°C, pale yellow solid. The specific activity of [4-^{14}C]5-*n*-heneicosylresorcinol was 44 MBq/mmol. The identity of 5-*n*-heneicosylresorcinol was confirmed by carbon–hydrogen analysis, electron impact MS, and u.v., i.r., and NMR (^{1}H and ^{13}C) spectroscopies. The identity and purity of [4-^{14}C]5-*n*-heneicosylresorcinol was confirmed by HPLC using 5-*n*-heneicosylresorcinol as the reference.

The composition of 5-*n*-heneicosylresorcinol as calculated for $C_{27}H_{48}O_2$ (404·68) was: C, 80·14 %; H, 11·96 %. The composition as found was: C, 80·24 %; H, 12·08 %. The relative abundance for the MS spectra was as follows: m/z 406, 1·3 % (M^++2); m/z 405, 7·3 % (M^++1); m/z 404, 23·8 % (M^+); m/z 124, 100 %. u.v. (Ethanol; λ_{max}) data were: 276 nm, (ε_{max} 1469); 282 nm, (ε_{max} 1432). Fully consistent i.r. and NMR spectral data were also obtained.

*Absorption of radiolabelled [4-^{14}C]5-*n*-heneicosylresorcinol by rats.* A study to determine the fate of ^{14}C-radiolabelled AR in rats consisted of a pilot study to determine if and where AR are excreted, a study to determine the presence of AR in blood over time, and a study to determine the proportion of AR in the faeces and urine of rats. Twelve 8-week-old male Fisher F344 rats (Harlan OLAC Ltd, Bicester, Oxon, UK) with body weights in the range of 200–230 g were used for these studies. Rats were housed individually in metabolism cages and given single oral doses of 4·6–5·0 mg [4-^{14}C]5-*n*-heneicosylresorcinol/kg body weight (0·67–0·73 MBq/kg) in maize oil, a level estimated to correspond to the daily intake of a human consumer with a high intake of wholegrain bread. Rats had free access to food (R&M no. 1 (E) SQC diet; Special Diet Services, Witham, Essex, UK) and tap water during the study. Ethical approval for the rat study was granted by a UK Home Office licence.

Three rats were used in a pilot study where radioactivity was measured in the urine, faeces and tissues (liver, gut, lungs, kidneys, peri-renal fat, heart, spleen, brain and carcass). Expired CO_2 was measured from 0 to 6 h, and a cage wash was also analysed for radioactivity. Kidneys, spleen, heart and samples of blood were oxidised directly; while fat, brain, lungs and carcass were digested using methanolic potassium hydroxide containing Triton X-100 (Sigma), and samples were oxidised as for the faeces and liver (see later). Another three rats had blood taken from the tail vein over a 144 h period, and from the dorsal aorta post mortem to monitor the presence of AR and/or AR metabolites in blood.

To determine the presence of AR and AR metabolites in the urine and faeces of rats, six rats were fed the same diet, and faeces and urine were collected for analysis. Radioactivity in the urine was determined directly by liquid scintillation counting in an Optiphase Safe cocktail (Perkin Elmer, Cambridge, UK). Faeces were homogenised in water and samples assayed by liquid scintillation counting after oxidation (Packard 307 Sample Oxidiser; Packard Bioscience Ltd, Reading, Berks, UK). Faeces and urine were extracted with ethyl acetate, and then hydrolysed with β-glucuronidase–sulfatase overnight at

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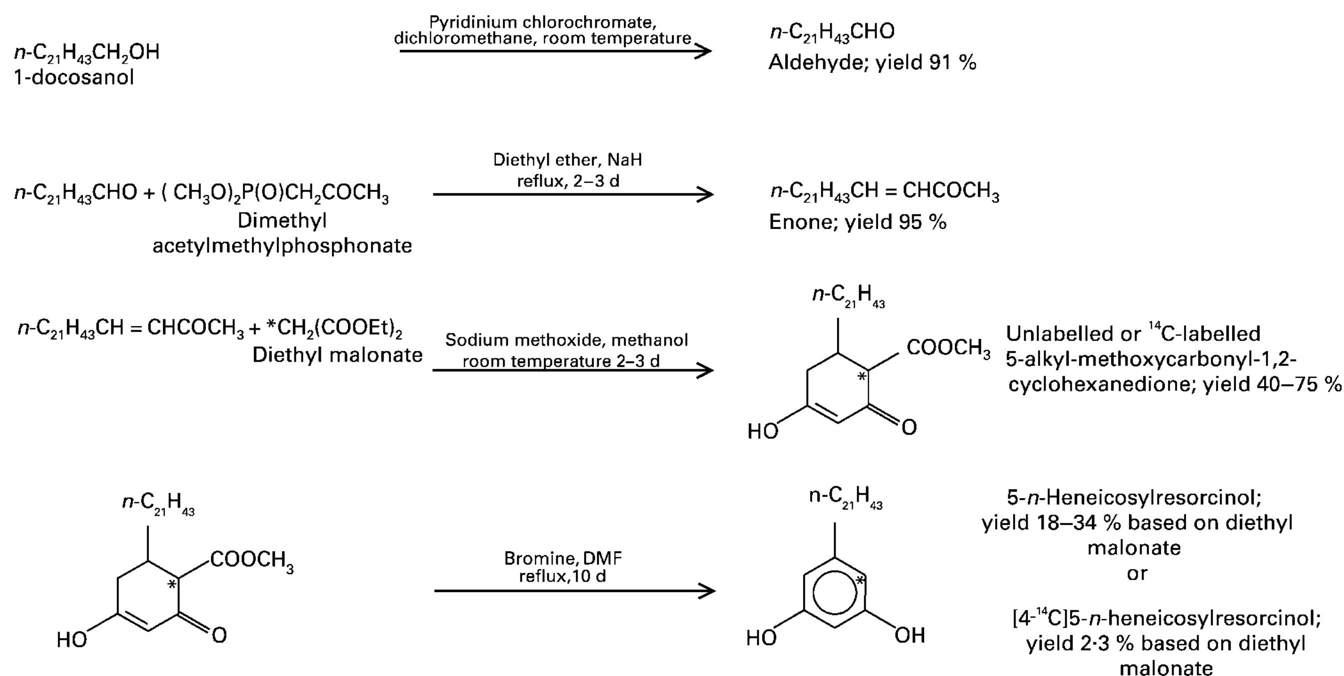

Fig. 3. Synthesis of 5-*n*-heneicosylresorcinol and [4-^{14}C]5-*n*-heneicosylresorcinol. *Position of ^{14}C. COOEt, ethyl ester; DMF, dimethyl formamide. For full details of the synthesis procedures, see p. 789.

37°C, and partitioned again with ethyl acetate to extract any AR that may have been conjugated.

HPLC was carried out on rat plasma, urine and faecal samples. Separation was performed on a Spherisorb ODS2 column (Hichrom, Reading, Berks, UK) (250 mm × 4·6 mm internal diameter) and detection was performed at 275 nm with a u.v. detector (Spectra-Physics, Darmstadt, Germany). The injection volume was 50 μl and the mobile-phase flow rate was 1 ml/min. A linear gradient of the solvents, methanol–water (90:10, v/v) and 100 % (v/v) methanol, from 0 % to 100 % methanol over 60 min, followed by an isocratic hold at 100 % methanol for 10 min was used. The peak from the faecal extract eluting with the same retention time as standard $C_{21:0}$ contained 90 % of the total faecal ^{14}C activity. This was collected and analysed by GC–MS to confirm its identity as $C_{21:0}$.

Results and discussion

In the present study, two models were used to determine if AR are absorbed, where they may be absorbed, and whether they are metabolised or not. As AR are phenolic lipids, it was assumed that their most probable point of absorption would be in the upper intestine; so a pig ileostomy model was used to estimate the amount of absorption. Rats were used for the radiolabelled AR study as they are easier to handle than pigs and require less radiolabelled AR. Using radiolabelled AR, it was also possible to follow the fate of AR in the tissues and urine of the rats without requiring specific analytical techniques. At the time of the study no method was available for the analysis of AR in plasma samples; however, a method for this analysis was later published by Linko *et al.* (2002).

Analysis of alkylresorcinols in diets and ileostomy samples from pigs

The AR levels in the rye pericarp and testa, aleurone, and starchy endosperm diets used in the pig experiment are shown in Table 1. The aleurone layer contained the highest amount of AR, consistent with reports that AR are located in the bran fraction of rye (Tłuścik, 1978; Ross *et al.* 2001). The starchy endosperm has a very small amount of AR present, most probably due to contamination by the aleurone layer because of the difficulty of completely separating the aleurone layer from the rest of the endosperm in rye (Slavin *et al.* 2001*a*). In the endosperm-based diets fed to pigs, AR were present, but only in minute quantities. The distribution of AR homologues is similar for the whole grain, pericarp and testa, and aleurone layer. Minor AR homologues, compounds similar to AR, but with a modified side chain, make up to 20 % total AR in rye grains. These compounds elute before the main AR peaks (Fig. 2), but their exact identity is not certain (Ross *et al.* 2001) so they were not included in the calculation of AR absorption. There was no clear evidence for any difference in uptake between the main, saturated AR, and minor AR homologues, however.

AR could be measured in the ileostomy effluent using the same method employed for analysing the diets. The presence of AR in the ileostomy effluent from pigs fed the enriched diets was confirmed by GC–MS (Fig. 2) and their levels in ileal digesta were quantified by the GC–flame ionisation detector method. A comparison of the selective ion recording chromatograms of the ileal effluent from pigs fed diets enriched with the rye-aleurone layer, or rye endosperm, clearly shows that no AR are present in the ileal effluent from endosperm diets. The response of

the long-chain AR (C_{21}, C_{23} and C_{25}) is low compared with the earlier eluting AR when using a mass selective detector. This chain-length discrimination is not evident when using the GC-flame ionisation detector method.

Ileal digestibility of alkylresorcinols in pigs

The recovery of AR in the ileal digesta varied depending on the diet. The whole-grain and aleurone-based diets had AR recoveries of 37 and 40 % respectively; while the pericarp–testa diet had a significantly lower ($P < 0.01$) recovery of 21 %. No AR were detected in the ileostomy samples taken from pigs on the starchy endosperm diet (Table 2). As the diet containing the lowest level of AR (pericarp–testa diet) had the lowest recovery of AR, ileal absorption might depend on the dose as well as the dietary source of AR. Tłuścik *et al.* (1990) found that 36 % of dietary AR were recovered in the faeces of rats fed a wheat-bran diet (718–1251 μg AR/g), and 48 % of AR were recovered when the rats were fed rye-based diets (1352–2734 μg AR/g). However, it is possible that the metabolism of AR by the intestinal microflora may be responsible for these differences, though this was not observed in the rat model (see later).

There was no clear relationship between homologue chain length and recovery of AR (Fig. 2) though $C_{17:0}$ tended to have a lower recovery compared with the longer-chain homologues ($C_{23:0}$ and $C_{25:0}$). However, in the pericarp–testa diet, $C_{23:0}$ and $C_{25:0}$ had the lowest recovery.

Recovery of radiolabelled alkylresorcinols from rat faeces, urine and blood

To test whether AR were present in the plasma, urine and body tissues, rats were fed radiolabelled AR. The tissue distribution of ^{14}C in the group of three rats given single oral doses of [4-^{14}C]5-*n*-heneicosylresorcinol at 4·6 mg/kg (0·67 MBq/kg) showed that < 1 % radioactivity remained after 100 h in the organs analysed (gut, lungs, kidneys, peri-renal fat, heart, spleen, brain and carcass). The overall recovery of ^{14}C from six rats fed a single dose of radiolabelled AR was 92 %, with 61 % of the radioactivity being found in the faeces, and 31 % in the urine. Collection of expired CO_2 was stopped after 6 h, as negligible radioactivity was detected, and it is possible that a later production of CO_2 might account for some additional activity. The maximum amount of activity in the faeces and urine was at 24 h (Fig. 4). In a separate experiment, the blood levels of ^{14}C were monitored from three rats given a single oral dose of [4-^{14}C]5-*n*-heneicosylresorcinol at 5·0 mg/kg (0·73 MBq/kg) (Fig. 5). The peak value of radioactivity in blood was between 7 and 12 h, and decreased rapidly after this. Almost no radioactivity was detected in blood at 144 h. The results suggest that most of the radioactivity is cleared from the rats within 60 h (Figs. 4 and 5). However, there is a possibility that AR reappear in the bloodstream due to enterohepatic circulation, as the level of radioactivity in the blood never reaches zero, even after 144 h.

The urine contained one-third of the ^{14}C dose. When urine was analysed by HPLC, no peak corresponding to the parent $C_{21:0}$ was found, but all the activity was present in an early eluting, and therefore more polar, peak that must have belonged to a metabolite of the AR. The log octanol–water partition coefficient for 5-*n*-nonadecylresorcinol ($C_{19:0}$) is about 11 (Kozubek, 1995), so AR would probably partition into body fat if not metabolised in some way to make them water soluble.

The radioactivity found in the faeces could be completely extracted (mean of 106·5 % recovery) into ethyl acetate regardless of enzyme treatment. Over 90 % of the radioactivity from the faeces was recovered as unchanged $C_{21:0}$. Very little activity (< 1 %) was found in the body tissues, despite the considerable bioavailability demonstrated by the radioactivity occurring in the urine. The radioactivity that partitioned into ethyl acetate from urine extracted without enzymic hydrolysis was 8 % of the total activity in urine, while 30–54 % was recovered in ethyl acetate when urine samples had been hydrolysed with β-glucuronidase–sulfatase before extraction. This suggests that AR are at least to some extent metabolised

Table 2. Intake of alkylresorcinols (AR; mg/d) and recovery (%) from ileal samples*
(Mean values and standard deviations)

	Diet tested										
	Whole-grain			Pericarp–testa			Aleurone			Starchy endosperm	
		Recovery			Recovery			Recovery			
AR homologue	Intake	Mean	SD	Intake	Mean	SD	Intake	Mean	SD	Intake	Recovery
17:0	154	29^a	5	54	21^a,b	6	406	29^a	5	6	nd
19:0	217	38^b	7	79	22^a,b	6	568	40^b	6	11	nd
21:0	188	40^b	8	72	28^a	13	492	45^b,c	6	13	nd
23:0	81	43^b	10	46	16^b,d	9	219	46^c	7	5	nd
25:0	86	40^b	11	53	12^c,d	3	242	43^b,c	8	4	nd
Total	726	37	7	306	21†	7	1927	40	5	40	0

nd, Not detected. The detection limit was 5 μg/g.
a,b,c,d Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).
* Values are means of three determinations, CV < 5 %.
† Recovery of AR from the pericarp–testa diet was significantly different from the whole-grain and aleurone diets ($P < 0.01$).

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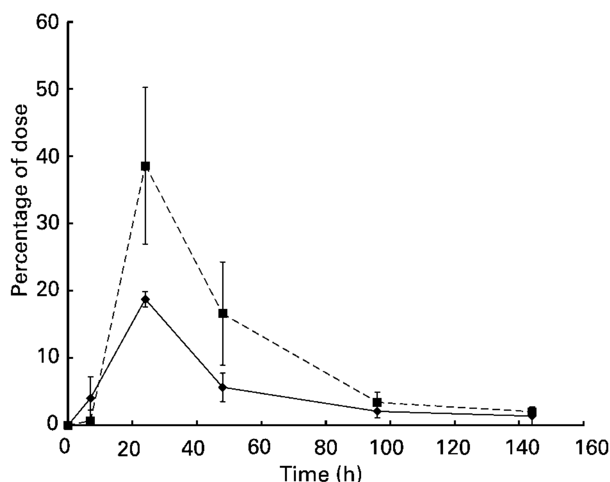

Fig. 4. Appearance of ^{14}C-labelled $C_{21:0}$ in rat faeces (–■–) and urine (-◆-) (*n* 6). Values are means, with standard deviations represented by vertical bars.

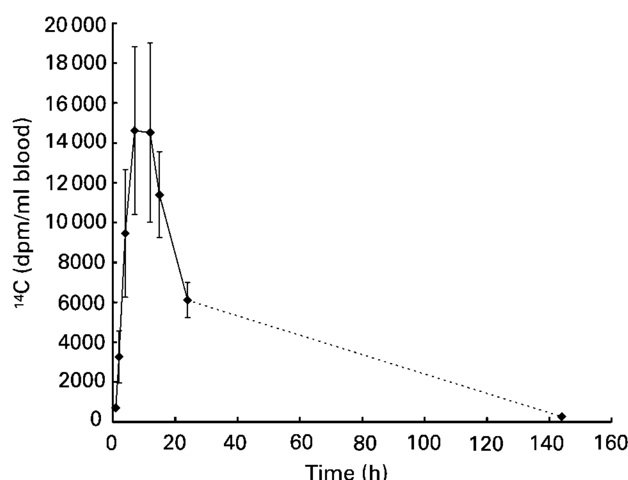

Fig. 5. Appearance of ^{14}C-labelled $C_{21:0}$ in rat blood (*n* 3). Values are means, with standard deviations represented by vertical bars. (– – –), Uncertainty due to lack of data points; dpm, disintegrations per min.

by phase II enzymes. No attempt was made to determine whether the metabolites were simple glucuronide or sulfate derivatives, or if AR had been more extensively metabolised.

Insights into alkylresorcinol absorption from the pig and rat models

The results for the absorption of radiolabelled AR provide direct evidence that AR are absorbed by rats. As ileostomy studies do not directly measure absorption *per se* but measure apparent disappearance (absorption) of selected compound(s) up to the end of the small intestine, the results from the rat study are important in giving an idea of the fate of AR in animals after digestion. The percentages of absorption reported in the present paper for the pig ileostomy study, and by Tłuścik *et al.* (1990), can only be assumed as a crude measure of absorption because of the possibility of enterohepatic circulation and/or degradation of AR by the bacteria in the upper part of the gastrointestinal tract. However, pigs have been found to be comparable with human subjects in the case of ileal digestibility for amino acids, and are considered a good model for human absorption (Rowan *et al.* 1994). In both pigs and rats, there were large individual variations in the apparent absorption of AR. Studies on the absorption of phenolic compounds from plants also show considerable variation, both within and between species (Clifford, 2000). For example, the absorption of vitamin E has been reported to vary from 21 to 86 % in human volunteers (Bramley *et al.* 2000).

The present paper shows that AR are absorbed to a significant extent in both pigs and rats. The pig study shows that AR are absorbed in the upper part of the intestine, suggesting lymphatic absorption. The rat study shows that absorbed AR are metabolised quickly and excreted in the form of metabolites in urine. Most probably AR are metabolised by shortening the alkyl tail (McClanahan & Robertson, 1984) in a similar fashion to vitamin E (Birringer *et al.* 2001). Recently AR have been found in unmetabolised form in human plasma (Linko *et al.* 2002) showing conclusively that AR are also absorbed in man.

Significance of alkylresorcinol absorption

There is sound epidemiological evidence suggesting a link between a diet rich in wholegrain cereals and a lower risk of many ‘Western’ diseases, especially CHD, and also some cancers, diabetes and obesity (Slavin *et al.* 2001*b*; Truswell, 2002; Hallmans *et al.* 2003). A diet rich in wholegrain wheat and rye is likely to contain a high amount of AR. A UK adult extreme consumer (97·5 % percentile) of wholegrain wheat bread may eat 250 g bread/d (S Church, Ministry of Agriculture, Fisheries and Food, UK, personal communication). This consumption corresponds to an intake of about 200 mg AR/d. AR are not destroyed by the baking process, so wholegrain wheat or rye bread may contain high amounts of AR (Ross *et al.* 2003).

That AR are present in abundant levels in wholegrain wheat and rye and are absorbed to a significant extent in pigs and rats, and can be measured in human plasma (Linko *et al.* 2002), suggests that they may be possible biomarkers for wholegrain wheat and rye intake by human consumers. A biomarker for wholegrain cereal intake would help to explore the proposed link between a diet rich in wholegrain cereals and the observed decrease in the risk of several ‘Western’ diseases. However, the time-course experiments with rats in the present study show that AR taken up from the gut are cleared quickly from the body pool. There was, however, a small amount of activity remaining in the blood at the end of the study. Further studies are needed to determine the kinetics of AR absorption, how long AR persist in the blood, if enterohepatic circulation occurs and what metabolite(s) are formed.

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