# Alkylresorcinol Homologs in *Pisum sativum* L. Varieties

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Alkylresorcinols, Cardol Homologs, Pea, *Pisum, Leguminaceae*

Acetone extracts from the seeds of *Pisum sativum L. sensu lato* (*Leguminoseae*) separated by thin layer chromatography revealed the occurrence of bands with chromatographic mobility and color reaction with Fast Blue B characteristic for 1,3-dihydroxy-5-alkylbenzenes. These polyketide metabolites have been isolated and identified by spectroscopic means. The occurrence of homologous series of saturated (approximately 70%) and enoic (mono and diunsaturated) homologs with chain length of C15 to C25 has been revealed with C17 as the main homolog.

## Introduction

The Leguminosae (Papilionaceae, Fabaceae) is a large family of utilitarian dicotyledoneous plant family that are characterized by large seeds. Most of leguminaceous species are annual plants cultivated for seeds and as a green feeding stuff for animals or as ornamentals, either as houseplants or glasshouse subjects. The seeds are utilized in human nutrition as the source of high quality of protein, in animal breeding as well as in food industry. Besides high protein content (up to 45% of a dry mass) leguminaceous seeds contain numerous antinutricious components together with tannins, alkaloids, phytin and phenolic compounds. Among phenolic compounds, a nonisoprenoic long-chain phenols and dihydroxy phenols (resorcinolic lipids), have been demonstrated and identified in some leguminaceous plants (*Ononis* sp.) by Barrero and co-workers (Barrero *et al.*, 1989; Barrero *et al.*, 1994a; Barrero *et al.*, 1994b; Barrero *et al.*, 1997; Barrero *et al.*, 1991; Barrero *et al.*, 1990). In this report the occurrence of homologous series of 5-*n*-alk(en)ylresorcinols (Fig. 1) in other, perhaps more significant from human nutrition point of view, group of leguminaceous plants, namely *Pisum sativum L. sensu lato* is demonstrated.

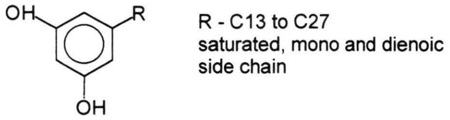

Fig. 1. General formula of alkylresorcinols (resorcinolic lipids).

## Materials and Methods

Seeds from respective varieties were obtained from authorized Plant Breeding stations distributing qualified material stated in the legend of Table I. Standard mixture of 5-*n*-alkylresorcinol homologs was isolated chromatographically from rye (Dankowskie Zlote variety) according to the procedure described elsewhere (Kozubek, 1985).

### *Isolation of alkylresorcinolic fraction*

The fraction of the material containing alkylresorcinols was isolated from whole dry seeds (1 kg) by 24 hr extraction with acetone using the volume of the solvent sufficient to soak the material completely. The extracts were filtered through filter paper to remove any solid particulates and the solvent was removed by vacuum evaporation on rotavapor. The oily residue was dissolved in 0.2 ml of chloroform and applied on 1.5 x 15 cm glass open-air column filled with silica gel 60 (J. T. Baker B. V., Deventer, Holland) equilibrated with chloroform. Separation was carried out first with 2 vol. of chloroform than with chloroform:ethyl acetate (85:15, v/v) until alkylresorcinols were eluted. The eluate has been collected in 2 ml frac-

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tions and analyzed by thin-layer chromatography. Fractions containing alkylresorcinols have been combined, the solvents were removed, the residues were dissolved in 1 ml of chloroform and used for further experiments.

### *Thin-layer separations*

Normal-phase thin-layer chromatographic separations have been done on analytical and preparative layers on glass plates (20 x 20 cm and 10 x 10 cm) covered with silica gel Si 60 (Merck 5745). The analytical chromatograms were developed in chloroform:ethyl acetate (85:15, v/v) whereas preparative runs were done with n-hexane:ethyl ether:formic acid (70:30:1, v/v/v). After separation and evaporation of the solvents a 1 cm wide strips of the gel on both sides of the plate were sprayed with aqueous 0.05% Fast Blue B x $BF_4$ (Chemapol, Prague, Czech Republic). Alkylresorcinols have been identified by their characteristic red-violet color and Rf value (Kozubek and Tyman, 1995; Mejbaum-Katzenellenbogen *et al.*, 1978). Parts of the gel containing compounds of interest have been scrapped off the plates and the materials have been extracted with chloroform:ethyl acetate (85:15, v/v) for 30 min. After filtration and removal of the solvent the residue was dissolved in 0.3 ml of chloroform and used for further analyses. To check for the presence of non-alkylresorcinolic contaminating compounds, the material was chromatographed on silica gel and stained with iodine vapors.

### *Analyses of the homolog composition*

To determine composition of the homologs according to the length of the side-chain the reversed-phase thin-layer chromatography on Merck 5914 RP18 HPTLC plates (Kozubek, 1985) and normal-phase chromatography on aluminum oxide (Tluscik and Kozubek, 1984) (Merck 5581)were used. For analysis of the presence and composition of homologs according to the unsaturation of the side-chain the argentation chromatography on silica gel was used (Kaczmarek and Tluscik, 1984).

The microcolorimetric method (Tluscik *et al.*, 1981) was used for quantitative determination of alkylresorcinols in the material. Quantitative determinations were made at least in triplicate and the results were analyzed statistically.

### *Instrumental analyses*

UV spectra of ethanolic solutions were taken in Cary 1E spectrophotometer (Varian), IR spectra of the samples in KBr tablets were taken in Perkin-Elmer System 2000 FT-IR spectrophotometer (at the Faculty of Chemistry, University of Wroclaw) and the data were compared with those of 5-*n*-pentadecylresorcinol (The Aldrich Library of Infrared Spectra, Aldrich, Poznan, Poland) and alkylresorcinol homologs (Kozubek and Tyman, 1995; Tyman, 1979; Tyman, 1991; Tyman, 1996). Mass spectral analyses were performed at The Instrumental Analyses Laboratory at the Faculty of Chemistry, Adam Mickiewicz University, Poznan, Poland. Spectra were obtained by ESI MS (Finnigan Mat PSQ 700) and GC/MS on Hewlett Packard 5890 Series II gas chromatograph coupled with EI mass spectrometer (AMD Intectra) and compared with the spectra obtained for pure 5-*n*-pentadecylresorcinol and literature data (Kozubek and Tyman, 1995; Vincieri *et al.*, 1981).

Thin-layer chromatography plates were from Merck, Darmstadt, Germany, solvents and remaining reagents of the highest available purity were from POCh, Gliwice, Poland.

## Results and Discussion

Acetone extract from dry mature pea (*Pisum sativum* L.) seeds separated by TLC on silica gel showed presence of the band exhibiting characteristic to 1,3-dihydroxy-5-alk(en)ylbenzene derivatives (alkylresorcinols) color reaction (Kozubek and Tyman, 1995) with aqueous Fast Blue B. In the first approach the identification of the presence of alkylresorcinolic compounds was done by TLC analysis of the samples comigration with authentic alkylresorcinols biosynthetically labeled with $^{14}C$. Analyzed band showed identical chromatographic mobility in various systems tested to that of alkylresorcinols. Further, the band was isolated by preparative TLC on silica gel and compounds were eluted from scrapped off gel with chloroform-methanol and concentrated *in vacuo*. Isolated material showed UV spectrum characteristic to alkylresorcinols with two close peaks at 278 nm and 282 nm characteristic to authentic 1,3-dihydroxy-

5-alkylbenzenes isolated from *Anacardium occidentale* L. (Cashew). The IR spectrum showed the presence of characteristic for alkylresorcinolic molecules bands and was in agreement to the spectrum of reference 5-*n*-pentadecylresorcinol and IR-library data. Final identification of the composition of alkylresorcinols in studied material was done by GC-MS analysis. The EI spectra showed occurrence of characteristic for alkylresorcinols base ionic peaks. The peak at *m/z* 124 is due to the rearrangement occurred *via* four- or six-membered transition complex of a hydrogen atom of the side chain, while *m/z* at 123 is due to the dihydroxytropylium ion formed by direct β-fission. The 124/123 abundance ion ratio is about 5 to 1, in agreement to the meta position of alkylresorcinols (Tyman, 1991; Tyman, 1996; Vincieri *et al.*, 1981). The occurrence of several parent molecular ions with the *m/z* masses from 292 to 460 confirms presence of homologs with the side chain length from C15 to C25. The same homologs were identified by reversed-phase TLC analysis of the isolated from the plates alkylresorcinolc band. For example, the percentage composition of homologs in alkylresorcinols isolated from the Opal variety was as follows: C15−9±4; C17−15±3; C19−31±4; C21−21±2; C23−15±3 and C25−9±3. Thin-layer chromatographic analysis on silver ions-impregnated silica gel revealed occurrence mainly of homologs with saturated side chain and smaller amounts of monoenoic and dienoic congeners. The relative percentage of saturated, monoenoic and

Table I. Alkylresorcinols in seeds, pericarps and cotyledones of *Pisum sativum* L. varieties.
The source of varieties is shown as superscript number: 1 – Hodowla Roslin Szydlak Sp. z o.o., Szydlak; 2 – Stacja Hodowli Roslin Wiatrowo, Wagrowiec; 3 – Gospodarstwo Hodowli Roslin Kosieczyn, Zbaszynek; 4 – Zaklad Doswiadczalny Hodowli i Aklimatyzacji Roslin Przebedowo, Murowana Goslina; 5 – Stacja Hodowli Roslin Wierzenica, Kobylnica; 6 – Stacja Hodowli Roslin Marchwacz, Rajsko; 7 – Instytut Hodowli i Aklimatyzacji Roslin Radzikow, Blonie. The remaining material was obtained from Agricultural Academy Wroclaw.
* – According to the List of Cultivable Plant Varieties published yearly by Central Laboratory for Studies of Cultivable Plants (COBORU), Slupia Wlk., Poland.

| | Class | Variety name | COBORU registry code of cultivable plants* | Alkylresorcinols (μg/g ± SE) | | |
|---|---|---|---|---|---|---|
| | | | | whole seeds | pericarps | cotyledons |
| 1 | coloured, fodder | SZC 1938$^1$ | SZC 1938 | 0.33 ± 0.04 | 26.50 ± 1.86 | 0.72 ± 0.05 |
| 2 | | Turkan$^1$ | SZC 789 | 0.25 ± 0.03 | 21.90 ± 2.09 | 0.71 ± 0.06 |
| 3 | | Kormoran$^1$ | SZC 584 | 0.39 ± 0.03 | 22.10 ± 1.55 | 0.32 ± 0.03 |
| 4 | | Perkoz$^1$ | SZC 686 | 0.28 ± 0.05 | 13.98 ± 2.52 | 0.63 ± 0.11 |
| 5 | | SZC 1930$^1$ | SZC 1930 | 0.19 ± 0.03 | 16.04 ± 2.09 | 0.36 ± 0.05 |
| 6 | | Fidelia$^2$ | - | 0.13 ± 0.01 | 7.86 ± 0.86 | 0.45 ± 0.05 |
| 7 | | Bart$^3$ | KOB 491 | 0.19 ± 0.02 | 5.12 ± 0.61 | 0.46 ± 0.06 |
| 8 | | Idol$^2$ | WTD 2493 | 0.15 ± 0.02 | 4.15 ± 0.58 | 0.43 ± 0.06 |
| 9 | | SZC 1495$^1$ | SZC 1495 | 0.10 ± 0.02 | 6.04 ± 0.54 | 0.16 ± 0.02 |
| 10 | | Grapis$^2$ | - | 0.09 ± 0.01 | 3.40 ± 1.73 | 0.20 ± 0.03 |
| 11 | | Helia$^6$ | RWT 2814 | 0.13 ± 0.02 | 2.35 ± 0.21 | 0.34 ± 0.03 |
| 12 | | Pegro$^5$ | WTD 583 | 0.09 ± 0.01 | 2.08 ± 0.37 | 0.24 ± 0.04 |
| 13 | | Vatra$^2$ | WTD 288 | 0.07 ± 0.01 | 1.67 ± 0.23 | 0.24 ± 0.03 |
| 14 | | Davo$^2$ | WTD 2293 | 0.08 ± 0.01 | 1.41 ± 0.16 | 0.20 ± 0.02 |
| 15 | | SZC 1394 | SZC 1394 | 0.09 ± 0.01 | 2.65 ± 0.32 | 0.17 ± 0.02 |
| 16 | | KOB 692$^3$ | KOB 692 | 0.06 ± 0.01 | 2.24 ± 0.38 | 0.10 ± 0.02 |
| 17 | white, fodder | KOB 794$^3$ | KOB 794 | 0.04 ± 0.01 | 1.06 ± 0.11 | 0.12 ± 0.01 |
| 18 | | Mige$^4$ | PRH 179 | 0.06 ± 0.01 | 1.01 ± 0.12 | 0.12 ± 0.01 |
| 19 | | Kama$^3$ | KOB 385 | 0.04 ± 0.01 | 1.02 ± 0.13 | 0.15 ± 0.02 |
| 20 | | Albatros$^1$ | SZC 990 | 0.02 ± 0.01 | 0.45 ± 0.03 | 0.06 ± 0.01 |
| 21 | white edible | Opal | RWT 4025 | 0.15 ± 0.02 | 2.37 ± 0.28 | 0.29 ± 0.03 |
| 22 | | Mazurek$^1$ | SZC 1293 | 0.12 ± 0.01 | 3.18 ± 0.48 | 0.10 ± 0.01 |
| 23 | | Miko$^7$ | PRH 583 | 0.07 ± 0.01 | 2.18 ± 0.28 | 0.14 ± 0.02 |
| 24 | | Rubin | SOD 986 | 0.05 ± 0.01 | 3.12 ± 0.27 | 0.27 ± 0.02 |

dienoic homologs in Opal variety was 79±5, 11±6 and 10±2, respectively. The data are in agreement with reports of Barrero *et al.* (Barrero *et al.*, 1989; Barrero *et al.*, 1994a; Barrero *et al.*, 1994b; Barrero *et al.*, 1997; Barrero *et al.*, 1991) who demonstrated occurrence of alkylresorcinol derivatives in the genus *Ononis.*

Further, the content and the composition of alkylresorcinols in 24 varieties of *Pisum sativum* L. present in Poland, have been analyzed. Data presented in Table I indicate practical absence of alkylresorcinols in cotyledons and that their content is up to several folds higher in pericarps of colored fodder varieties. These results are in good agreement to the data of Tluscik, who demonstrated that in rye grains alkylresorcinols are exclusively localized in the pericarp (Tluscik, 1978). This finding supports the thesis of the protective role of alkylresorcinol-type phenols in grain biology (Suzuki *et al.*, 1996). In most of studied plants, contrary to most of the known sources, the occurrence of a wide range of homologs was demonstrated. The occurrence of a particular homolog is dependent on the genetic variety of studied plant material. The relative abundance of alkylresorcinol homologs estimated by reversed-phase HPTLC analyses is presented in Table II. After analysis of the composition of the homologs in all of the studied material the statistic abundance of alkylresorcinol homologs has been calculated. The most prominent homologs according to their occurrence and relative amounts were C19 and C21, 24% and 26%, respectively. Other homologs (C13, C15, C17, C23, C25 and C27) were less frequently present, and their statistical abundance ranged from 1–2%, for short- and very long-chain ones to 12–17% for intermediate length homologs.

The results indicate that resorcinolic lipids (alkylresorcinols and their derivatives) that initially were shown in only a few plant families (see e.g. (Kozubek and Tyman, 1998; Tyman, 1991)) might be, in fact, more abundant among plant kingdom. Their presence in seeds used in both human and animal nutrition together with already described biological activities support the necessity of further studies that may explain in more details their possible biological and biomedical role.

Table II. Alkylresorcinol homologs in varieties of *Pisum sativum* L.
Number of crosses indicates relative amount of particular homolog.

| | Variety | Homolog | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | C 13 | C 15 | C 17 | C 19 | C 21 | C 23 | C 25 | C 27 |
| 1 | SZC 1938 | + | + | ++ | ++ | +++ | | | |
| 2 | Turkan | + | + | ++ | | | | | |
| 3 | Kormoran | | | + | ++ | +++ | ++ | + | |
| 4 | Perkoz | + | + | + | ++ | +++ | | | |
| 5 | SZC 1930 | | | + | + | ++ | | | |
| 6 | Fidelia | | + | ++ | +++ | ++ | + | + | |
| 7 | Bart | | | + | + | +++ | ++ | + | + |
| 8 | Idol | | ++ | ++ | +++ | +++ | ++ | + | + |
| 9 | SZC 1495 | | + | ++ | +++ | ++ | + | + | |
| 10 | Grapis | | + | ++ | +++ | ++ | + | + | + |
| 11 | Helia | | + | + | ++ | +++ | ++ | + | |
| 12 | Pegro | | ++ | ++ | ++ | +++ | + | + | |
| 13 | Vatra | | | + | + | +++ | ++ | | |
| 14 | Dawo | | +++ | ++ | + | | | | |
| 15 | SZC 1394 | | | ++ | +++ | + | + | | |
| 16 | KOB 692 | | ++ | + | + | | | | |
| 17 | KOB 794 | | ++ | + | + | | | | |
| 18 | Mige | | + | + | + | +++ | ++ | ++ | |
| 19 | Kama | | | + | ++ | +++ | ++ | + | + |
| 20 | Albatros | | +++ | + | + | + | | | |
| 21 | Opal | | + | ++ | +++ | ++ | ++ | + | |
| 22 | Mazurek | | +++ | ++ | ++ | ++ | + | + | |
| 23 | Miko | | + | ++ | +++ | ++ | + | + | |
| 24 | Rubin | | | + | +++ | +++ | ++ | + | |

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