ALKYLRESORCINOLS IN THE FAMILY FABACEAE

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ABSTRACT

The Fabaceae are a large family, members occurence of which is very common at various latitudes. The characteristic feature of this plant family is the symbiosis with the bacteria genus *Rhizobium* and *Bradyrhizobium*.

The analysis of acetone extracts from dried seeds of the subfamily Papilinioideae revealed the occurrence of alkylresorcinols in about 85% of the examined samples. Unfortunately, it was not possible to verify this observation in the second subfamily of Fabaceae-Caesalpinioideae. It was partly because there is merely one species belonging to this subfamily, in Poland.

In reference to the common occurence of alkylresorcinols in Papilinioideae some interspecific differences were indicated. This survey is a basis for further studies on the Fabaceae family.

KEY WORDS: Fabaceae, chemotaxonomy, alkylresorcinols, Papilinioideae, Caesalpinioideae.

INTRODUCTION

The Fabaceae are a large plant family in the dicotyledons of about 16 400 species in 657 genera. They are of cosmopolitan distribution, living in every soil and climate. The family is divided into Mimosoideae and Caesalpinioideae which are mostly tropical, and Lotoideae (Papilinioideae) which are both tropical and temperate (Harborne et al. 1971). They are all perennial utilitarian plants, characterized by large seeds. Particular pea species are widely used in cultivation not only for seeds and nutritious green fodder for animals but also as ornamentals, either as house- or glasshouse plants. The seeds have the application in human nutrition and animal breeding for the sake of their high nourishing content, as well as in food processing industry. The legumes, in comparison with cereals are a richer source of high quality proteins, predominantly of lysine-rich proteins. Besides, the green crops are also used both in dry form and silage. The legumenoceous plants possess the favourable property to make additions to physical and chemical properties of the soil and its fertility. The roots of most species bear tubercles containing bacteria of the genus *Rhizobium* and *Bradyrhizobium*, and plants provided with these tubercles are able to take up more atmospheric nitrogen. The plants appear to consume the nitrogen-rich bacteria, which live in their cells (Lynch and Poole 1979).

The major factors, which determine the cultivation of the particular legume species, are climatic and soil conditions. It results from the fact that the response of these plants is specific in relation to soil conditions and water relationship. In consequence the plant development may be disturbed and it may manifest itself as changes in chemical constituents composition.

The chemical composition of seed of certain legume species is very diversified, both qualitative and quantitative. This is caused by genetic differences and environmental conditions. Proteins are the main component of legume plant seeds. The content of proteins in seeds varies from 20 to 45% of dry weight. Besides proteins, legume seeds contain carbohydrates, lipids and a considerable amount and number of substances that are definied as antinutritious. The latter compounds may have a disadvantageous effect on the use of nutrient substances. Tannins, alkaloids, inhibitors of proteases, heamaglutinins, phytin and phenolic compounds are the most frequent representatives of this group (Jasińska and Kotecki 1993). The mode of action of some of them on animal organisms is quite well know, but not much is known about the influence of environmental conditions on the biosynthesis of e.g. phenolics in seeds. It is a well recognized phenomenon that plant varieties with decreased concentration of these compounds, are more sensitive to different plant-disease agents. Therefore these antinutritious compounds are accepted as phytoalexins. The mentioned above phenolic lipids belong to the large group of organic substances, which are aromatic compounds with hydroxyl substituent(s). Among the plant phenols, of which over 8000 are known, the non-isoprenoic lipids, which origin from polyketide pathway, form the important group of secondary metabolites. It is a relatively less known group, which includes derivatives of phenol (alkylphenols, e.g. cardanols and alkylphenolic acids, e.g. anacardic acids), derivatives of catechol (e.g. urushiols, laccols) and

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derivatives of resorcinol (alkylresorcinols, e.g. cardols, bilobol, grevillol) and alkylresorcinolic acids (e.g. merulinic acid) (Kozubek and Tyman 1999).

Characteristic features of these non-isoprenoic phenolic lipids are the odd numbers of carbon atoms in the aliphatic chain and the *cis* configuration of a double bond present in the enoic members of the group. Aliphatic chains are saturated or may contain one, two or three double bonds. Usually these phenolic lipids occur as a mixture of several homologs with varied degree of saturation and/or chain length.

The essential reason for particular interest in this group of compounds, is the fact of the economic significance of phenolics. They are important components in plants, because they make the major contribution to the taste, flavour and colour of food and beverages, e.g. the bitterness of beer is due to the content of the 1,3,5-trihydroxybenzene derivative, humulone (Fung et al. 1997). On the other hand, phenolic lipids have been applied in the synthesis of resins, varnishes, dyes, antioxidants, detergents, printing paints, plastics, drugs and additives improving the oil and grease properties (Tyman 1991).

Alkylresorcinols (1,3-dihydroxy-5-*n*-alk(en)ylbenzenes), which are alternatively called resorcinolic lipids, are a widespread group of natural substances. Although they are found throughout the plant kingdom (e.g. Dring 1995), they are also present in bacteria and fungi. Alkylresorcinols play a significant role in protecting plant tissue from herbivores or infection by plant pathogens. They may also act as chemical signals in the host-guest interaction between plant and microorganisms, and play an important role in the complex plant resistance system. These compounds display also some other useful biological properties. It has been demonstrated that they exhibit antibacterial, antifungal, antiparasitic and anticancer activities (e.g. Kozubek and Tyman 1999). Additionally, monoterpene-substituted resorcinol derivatives (e.g., δ^1-tetrahydro-cannabinol) are the active hallucinogenic component of marihuana smoke.

The occurrence of resorcinolic lipids have been demonstrated in some legume plants of the genera *Ononis* and *Pisum*. In this study, the distribution of these compounds in the most prominent in Poland legume plant species (approximately 50% of reported species), is described.

MATERIAL AND METHODS

Plant material

Seeds from members of the Fabaceae family were taken from the collection at the Botanic Garden of the University of Wrocław, where voucher specimens are kept. The identification of plant material by scientific staff of this garden is gratefully acknowledged.

Isolation of alkylresorcinols

Resorcinolic lipids were isolated from whole seeds, which had been dried before extraction for two months in room temperature. Dry seeds were extracted for 24 hours with a volume of acetone sufficient to soak the material completely. The extracts were filtered through filter paper to remove solid particulates. The solvent was than removed by vacuum evaporation on rotavapor, the residue was dissolved in chloroform and applied on 1.5 × 15 cm glass column filled with silica gel Si 60 (Merck, Darmstadt, Germany) equlibrated with chloroform. The column was eluted with chloroform and then with chloroform: ethyl acetate (85: 15, v/v) till the fraction containing alkylresorcinols was obtained. The eluate was collected in 2 ml fractions and analyzed by thin-layer chromatography. Fractions containing alkylresorcinols were combined, concentrated in vacuo and dissolved in chloroform for the use of further experiments. To check for the presence of non-alkylresorcinolic contaminations comopunds, the iodine vapors staining of the chromatograms was used.

Qualitative determination of resorcinolic lipids

Alkylresorcinolic compounds have been characterized and identified by means of their chromatographic behaviour and spectral properties. The Fast Blue B × BF_4 salt was a kind gift of Lachema, Prague, Czech Republic. Standard alkylresorcinol homologues were isolated at our laboratory from natural sources using the procedure described elsewhere (Kozubek 1985). Solvents and remaining reagents of the highest available purity were from POCh (Gliwice, Poland).

Thin-layer chromatography: Fractions containing alkylresorcinols were analyzed on glass plates covered with silica gel Si 60. The chromatograms were developed in chloroform: ethyl acetate (85: 15, v/v). After separation and evaporation of solvents, the plates were sprayed with aqueous 0.05% Fast Blue B × BF_4. Alkylresorcinols have been identified by their characteristic reddish violet colour and R_f value, which were compared to the standard mixture of 5-n-alkylresorcinol homologues isolated from rye bran (Mejbaum-Katzenellenbogen et al. 1978).

Spectral analysis. UV: Varian Cary 1E; IR: Perkin Elmer System 2000 FT-IR; GC: Hewlett Packard 5890; MS: Finningan Mat PSQ 700; NMR: AMX Bruker 300. Spectra were recorded for some randomly freely selected samples. Obtained data were compared with those obtained for pure 5-*n*-pentadecylresorcinol as well as those taken from the literature data.

Quantitative determination of resorcinolic lipids

For quantitative determination of alkylresorcinols the microcolorimetric method of Fast Blue B was used (Tłuścik et al. 1981). Briefly, the sample containing alkylresorcinols, dissolved in chloroform was put into a dry glass tube and the solvent was evaporated with the stream of nitrogen gas. To the dry residue 4 ml of the reagent prepared by 5-fold dilution with n-propyl alcohol of 0.05% (w/v) Fast Blue B × BF_4 in 5% acetic acid were added. The content was thoroghly mixed using a Vortex mixer and left in a dark place for an hour. The absorbance of the developed colour was read at 520 nm against the reagent blank. The content of alkylresorcinols was estimated using a calibration curve (1-10 µg) prepared for 5-n-pentadecylresorcinol as a reference compound. The latter compound was isolated chromatographically from the extracts of *Azotobacter* strain that was producing it (Kozubek et al. 1996). All determinations were made at least in triplicate and the results were analyzed statistically. The standard error did not exceed 5% in all determinations.

RESULTS

In this work the presence and contents of alkylresorcinols have been analyzed in 66 samples of seeds of 45 species attributed to 23 genera of the Fabaceae family. The used isolation procedure allowed to obtain pure 5-*n*-alkylresorcinol fractions from the studied legume seeds. Extracts separated by thin-layer chromatography on silica gel showed the presence of characteristic of resorcinolic lipids bands, as revealed by their specific

TABLE 1. The occurrence and contents of alkylresorcinols in Fabaceae seeds, classified according to presence and absence of resorcinolic lipids (+ indicates presence, – indicates absence of resorcinolic lipids).

Plant source	No. of tested samples	Occurence of alkylresorcinols	Contents of alkylresorcinols (mg/g)	No. of total species in genus	Percentage tested to total no. of Polish species
Subfamily Caesalpiniodeae					
Genus *Gleditschia* L.	6			1	100
G. tricanthos L.	6	–	0.0		
Subfamily Papilionoideae					
Genus *Ulex* L.	1			1	100
U. europaeus L.	1	+	3.0		
Genus *Genista* L.	5			5	80
G. anglica L.	1	–	0.0		
G. pilosa L.	1	+	4.3		
G. sagittalis L.	1	+	33.8		
G. tinctoria L.	2	±	10.7		
Genus *Cytisus* L.	5			9	33
C. hirsutus L.	2	+	53.7		
C. albus (Lam.) Link, non Hacq.	1	+	15.1		
C. podolicus Błocki	2	+	103.4		
Genus *Laburnum* Fabr.	2			1	100
L. anagyroides Medicus	2	±	3.2		
Genus *Lupinus* L.	1			3	33
L. polyphyllus Lindley	1	+	0.2		
Genus *Ononis* L.	2			3	67
O. repens L.	1	+	33.4		
O. spinosa L.	1	+	18.2		
Genus *Trigonella* L.	2			1	100
T. caerulaea (L.) Ser.	2	±	2.6		
Genus *Melilotus* Miller	1			4	25
M. officinalis auct., non (L.) Pallas	1	+	1.5		
Genus *Trifolium* L.	3			21	14
T. alpestre L.	1	+	0.4		
T. pannonicum Jacq.	1	–	0.0		
T. rubens L.	1	+	10.4		
Genus *Anthyllis* L.	2			2	100
A. alpestris Hegetschw., non Reichenb	1	+	36.8		
A. vulneraria L.	1	+	39.2		
Genus *Lotus* L.	1			4	25
L. corniculatus L.	1	+	1.0		
Genus *Galega* L.	1			1	100
G. officinalis L.	1	–	0.0		
Genus *Robinia* L.	3			1	100
R. pseudoacacia L.	3	+	2.8		
Genus *Colutea* L.	1			1	100
C. arborescens L.	1	–	0.0		
Genus *Caragana* Fabr.	1			2	50
C. frutex (L.) C. Koch	1	+	2.5		

TABLE 1. cont.

Plant source	No. of tested samples	Occurence of alkylresorcinols	Contents of alkylresorcinols (mg/g)	No. of total species in genus	Percentage tested to total no. of Polish species
Genus *Astragalus* L.	2			11	18
A. glycyphyllos L.	1	–	0.0		
A. oroboides Hornem.	1	–	0.0		
Genus *Oxytropis* DC.	2			4	25
O. campestris (L.) DC.	2	±	1.2		
Genus *Hedysarum* L.	1			1	100
H. obscurum L.	1	+	27.3		
Genus *Onobrychis* Miller	1			3	33
O. vicifolia Scop.	1	+	6.8		
Genus *Vicia* L.	5			18	17
V. angustifolia L.	2	±	0.6		
V. villosa Roth	1	+	1.2		
V. sativa L.	2	±	0.9		
Genus *Lathyrus* L.	13			14	78
L. hirsutus L.	1	+	1.4		
L. heterophyllus L.	1	–	0.0		
L. latifolius L.	2	+	1.6		
L. niger (L.) Bernh.	1	+	2.9		
L. palustris L.	1	+	35.1		
L. pratensis L.	1	+	3.0		
L. tuberosus L.	1	+	1.4		
L. silvestris L.	2	±	1.2		
L. maritimus Bigelow	1	–	0.0		
L. vernus (L.) Bernh.	2	±	11.8		
Genus *Phaseolus* L.	5			2	100
P. coccineus L.	4	+	12.8		
P. vulgaris L.	1	+	4.2		

colour in reaction with aqueous Fast Blue B solution, and identical values of retention factor (R_f) in various systems tested to that of standard alkylresorcinols. Isolated from the bands resorcinolic lipids showed the UV spectra characteristic of alkylresorcinols in which two close peaks appeared at 278 and 282 nm. The IR spectra showed the presence of characteristic of the reference alkylresorcinols bands (3332 (3600-3100), 2917, 2849, 1606, 1512, 1458, 1375, 1331, 1259, 1199, 1147, 842, 720 cm^{-1}), which was in agreement to the data from IR-spectra reference library. The ^{1}H-NMR spectra revealed also characteristic bands identical to those of reference compounds. The final confirmation of identity of the compounds was done by GC-MS analysis. The occurence of two characteristic base peaks at 124 and 123 (m/z) and their ratio approx. 1 : 5, was in agreement to the meta position of hydroxyl groups in the aromatic ring and to literature data (Vincieri et al. 1981; Tyman 1991; Tyman 1996)

Further, the content of resorcinolic lipids in the studied legume seeds was analyzed. Data were compiled in Table 1, where both the occurrence/absence and the contents of alkylresorcinols are presented.

DISCUSSION

In the literature reports the presence of alkylresorcinols have been demonstrated in the representatives of the genus *Ononis*, where they were found to occur in leaves and stems of several species (Barrero et al. 1989; Barrero et al. 1990; Barrero et al. 1991; Barrero et al. 1994). Our previous paper was focused on the presence of resorcinolic lipids in the genera of the Fabaceae family, more significant from the human nutrition point of view, namely *Pisum sativum* L. *sensu lato* (Żarnowski and Kozubek 1999). The present work extends our investigation to further 45 species, that constitutes almost 50% of species which can be found in Poland.

Seeds of two subfamilies of Fabaceae have been sampled, and alkylresorcinols have been found only in Papilionaideae. The presence of resorcinolic lipids have been verified in almost 85% of the analyzed species. This result indicates that alkylresorcinols are widespread in the Papilionoideae subfamily. The absence of studied compounds may result from relevant wide-comprehended environmental parameters to the biosynthesis of resorcinolic derivatives in plant organisms. The biosynthesis process in plants may be partially or completely inhibited by disadvantageous environmental conditions. Additionally, the occurrence of this kind of phenols may coincide in an individually fitted way, contrary to either of the organisms occupying the same ecological niches. This phenomenon may be dependent on the genetic variety of studied plants and is verified by obtained positive-negative results for some sampled species. Contrary to these results, alkylresorcinols have not been found in the acetone extracts from seeds of *Gleditschia tricanthos*, the only member of Caesalpinioideae subfamily, which can be found in Poland.

The presence of resorcinolic lipids in Papilionoideae seeds seems to be common, but further taxa should be examined. The huge number of species of Fabaceae means that only a small percentage of them has been possible to be screened in this work. However, when the data will be collected from a much wide range of species and genera, it will be possible to make some more detailed comments on the chemotaxonomy of the Fabaceae family. Nowadays it is known that alkylresorcinols are present in the Papilionoideae subfamily and are absent in the Caesalpinioideae. We believe that it is necessary to perform further studies of other phenols and substances that possess biological activities, and that the results might be useful in explanation of their role in the host organism as well as in the modern taxonomy of pea plants.

It has been documented in many papers that alkylresorcinols, like these described in this report, exhibit some biological activities, although the data have been reported for compounds isolated from other plant sources (Barrero et al. 1993). Among these activities, the alkylresorcinols display antibacterial and antifungal activities (Żarnowski et al. 1999). These features and the discovery of resorcinolic lipids in pea plants may support the thesis of the protective role of alkylresorcinolic-type phenols in the biology of seeds. An increased research interest on resorcinolic lipids, will enhance our understanding of the complex biological role of these compounds in plant chemistry and physiology.

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ALKILOREZORCYNOLE W RODZINIE FABACEAE

STRESZCZENIE

Rodzina Fabaceae (Bobowate) jest dużą rodziną, której przedstawiciele są powszechnie występujący na różnych szerokościach geograficznych. Charakterystyczną cechą tych roślin jest symbioza z bakteriami z rodzajów *Rhizobium* i *Bradyrhizobium*.

Alaniza ekstraktów acetonowych uzyskanych z suchych nasion roślin należących do podrodziny Papilionioideae wykazała obecność alkilorezorcynoli w około 85% badanych próbek. Niestety, nie była możliwa analiza przedstawicieli drugiej z podrodzin Fabaceae-Caesalpinioideae. Jednym z powodów był fakt, iż w Polsce praktycznie nie występują przedstawiciele tej podrodziny. Wykazano iż niezależnie od generalnie powszechnego występowania alkilorezorcynoli pośród przedstawicieli Papilinioideae obserwuje się pewne interspecyficzne różnice w poziomie tych związków. Przedstawione wyniki stanowią podstawę do do dalszych badań nad rodziną Fabaceae.

SŁOWA KLUCZOWE: Fabaceae, chemotaksonomia, alkilrezorcynole, Papilinioideae, Caesalpinioideae.