Search for polyprenols in leaves of evergreen and deciduous Ericaceae plants

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Various species and cultivars of Ericaceae family were checked for the presence of long-chain polyprenols in their leaves. In the genus Rhododendron no polyprenols were found in the ever-green species, while they were present in the deciduous type. The polyprenols were of chain-length of 14-20 isoprene residues and they occurred in the form of acetic acid esters. The polyprenol accumulation is discussed with respect to senescence of leaves.

It has been observed that the content of polyprenols in leaves increases with the age of the leaf and that in some species the age-dependent accumulation of polyprenols may attain extremely high values (Wellburn & Hemming, 1966; Swiezewska et al., 1994). The search for polyprenols in the members of the family Ericaceae, reported in the present paper, is a part of our program of research aimed at finding the general rules governing the accumulation of polyprenols.
In our previous studies the family Ericaceae has never been studied thoroughly. There were single observations on the presence of long-chain polyprenols composed of 16–19 isoprene residues in leaves of Vaccinium vitis-idaea (Swiezewska et al., 1994) and on the lack of polyprenols in leaves of some Rhododendron species. Our aim to study plant species of the Ericaceae family in a systematic way came from the observation that one Rhododendron species which occurs in a wild state in Poland and was listed in the group of rare and endangered Polish plants (Rh. luteum) was polyprenol positive (Goles et al., 2001). The genus Rhododendron was found to be attractive for our studies on the occurrence of polyprenols as it is a phylogenetically old group. Some of the species were present in their contemporary form even before 50 million years. The group of rhododendrons is very numerous as it contains over 850 species. They occur mainly in the northern hemisphere. The variety of species offers a great range of forms from tiny prostrate alpines to a tree with enormous leaves.

The present studies could have been made owing to the access to the collection of the Botanical Garden of the Polish Academy of Sciences in Powsin. The rhododendrons present there have been collected since 1978 and they include over 300 taxa. All together 440 taxa from the Heath family (Marczewski, 1995) are cultivated. Most of the collected taxa were obtained from other botanical gardens as seeds.

The great number of species within the genus Rhododendron enabled us to select the most characteristic morphological forms of plants, especially the largest group of evergreen plants with their several varieties, and a representative group of deciduous plant species.

In other group of the species studied belonging to Ericaceae, was confined to various azaleas and other species, some of which are also common in Poland. The total number of Ericaceae is estimated by various authors to contain 130 genera and about 2700 species.

Fig. 1. Structure of polyprenols

MATERIALS AND METHODS

The specimens of leaves of all studied species were collected in the Arboretum of the Botanical Garden of the Polish Academy of Sciences in Powsin near Warsaw. Samples of leaves were collected in the first decade of October 1999 and kept in paper envelopes for about 3 weeks before examination. During that time they became dry.

All chemicals, organic solvents of analytical grade (POCh Gliwice, Poland) and materials for thin-layer chromatography (Merck, Darmstadt, Germany) were the same as previously described (Swiezewska & Chojnacki, 1996).

Extraction of lipids of leaves was done as described previously (Swiezewska & Chojnacki, 1996) with some modifications, namely 100 mg samples of plant material were homogenized in 4 ml of acetone/hexane, 1:1 (v/v). TLC chromatography and semiquantitative assay of the polyprenol content was done as described before (Swiezewska & Chojnacki, 1996; Wellburn & Hemming, 1966). The standards of polyprenols and polypropenyl acetates were from the “Collection of Polyprenols”, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw.

Alkaline hydrolysis of lipid fraction was performed according to Stone et al. (1967) and
the fraction of free polyprenols was isolated by chromatography of unsaponifiable lipids on Silica Gel column with increasing concentrations of ethyl ether in hexane. A 0.8 × 6.0 cm column was used to fractionate the unsaponifiable lipids from up to 5 g of dry leaves. The total volume of 150 ml of the eluent (7.5 ml portions of hexane containing 1,2,3 etc. up to 20% of ethyl ether) was used for elution. The fraction of free polyprenols was eluted with 8–10% ethyl ether.

The fraction of polyprenols was studied by HPLC on reversed phase RP-18 column in a solvent system as described previously (Swiezewska & Chojnacki, 1996) using a Waters dual pump apparatus and a UV detector set at 210 nm. Standard mixture of polyprenols of various chain length (prelogues composed of 9,10,... etc. up to 25 isoprene units) was used to calibrate the HPLC column between each 2-3 analyses. The polyprenol fraction was also examined by $^1$H-NMR spectrometry in deuterochloroform in a Varian 500 MHz apparatus using tetramethylsilane as internal standard.

**RESULTS AND DISCUSSION**

Thirty seven rhododendron species were studied for the content of polyprenols. No detectable amounts of polyprenols or polyprenyl esters were found in any of the following nine evergreen rhododendron species: Rh. auriculatum Hemsl., Rh. brachycarpum D.Don, Rh. brachycarpum subsp. tigerstedti Nitz., Rh. campanulatum D.Don, Rh. carolinianum Rehder, Rh. catawbiense F.Michx., Rh. dauricum L., Rh. degronianum subsp. heptam. (Maxim) Sealy, Rh. fastigiatum Franch., Rh. ferrugineum L., Rh. impeditum Balf.f. & W.W.Sm., Rh. macrophyllum D.Don, Rh. maximum L., Rh. micranthum Turcz., Rh. oreodoxa Franch., Rh. orbiculare DC., Rh. oreotrephes W.W.Sm., Rh. purdomii Rehd. & Wils., Rh. smirnowii Trautv., Rh. yaku shmanum Nakai. The sensitivity of the applied semiquantitative assay enables us to state that if any amount of polyprenol or polyprenyl ester was present in the leaves, its amount was below the limit of detection i.e. less than 0.02% of the dry mass of leaves.


In Table 1 the results for fifteen rhododendrons of deciduous type (1–15) and three of semideciduous type (16–18) were shown. In this group of rhododendrons all 18 species were found to contain detectable though variable amounts of polyprenols (in the form of acetates) from 0.2% to 1.0% dry mass of leaves. The identity of these substances was proved by cochromatography with known amounts of polyprenyl acetates isolated from leaves of Ginkgo biloba (Ibata et al., 1983).

Among 27 plant species of Ericaceae representing 16 genera that were examined for the presence of polyprenols only 5 have been found to be polyprenol positive and the content of polyprenols (in the form of acetates) was in the range between 0.2% and 1.0% of dry mass of leaves. The polyprenol content was not detectable in Andromeda glaucophylla Link, A. polifolia L., Arctostaphyllos uva-ursi (L.) Spreng., Bruckenthalia spiculifolia (Salisb.) Reichenb., Chamaedaphne calyculata (L.) Moench., Enkianthus campanulatus (Miq.) Nichols., Gaultheria cuneata (Rehder et Wilson) Bean, G. itoana Hyata, G. miqueliana Takeda, G. procubens L., G. shallon Pursh., Gaylussacia baccata (Wangenh.) K.Koch, Kalmia angustifolia L., K. latifolia L., Ledum palustre L., Leucothoe walteri (Willd.) Melvin,
Lyonia ligustriana (L.) DC, Pieris floribunda (Pursh) Benth.et Hook.f., P. japonica (Thunb.) D.Don, P. polita W.W.Sm. et J.F.J effrey, P. taiwaniensis Hayata.

The size of the polyprenol molecules in the few polyprenol-positive non-rhododendron Ericaceae was similar to that in the rhododendrons of deciduous type. In each species the polyprenol family was composed of several prenologues ranging from 14 to 20 isoprene residues. The typical representative polyprenol pattern of various plant species studied in this paper is shown in Fig. 2. The dominating polyprenol was built up from either 17, 18 or 19 isoprene units.

In Fig. 3 the 1H-NMR spectrum of polyprenol mixtures isolated from Rhododendron viscosum (listed in Table 1; No.15) is shown. In the record the peaks of the characteristic protons of polyprenol molecule are visible. The assignments of individual peaks are shown in the accompanying legend. The majority of isoprene units are in cis configuration, which is the characteristic feature of the OH-terminal isoprene residue. There seems to be no dolichol component (with the saturated OH-terminal residue) as evident from the absence of the characteristic multiplet at 3.6 ppm. The exact proportion between the cis- and trans-isoprene units in the molecule cannot easily be given as the spectra represent mixtures of molecules of various size. The same type of spectrum, presenting a typical isoprenoid pattern was also obtained for the mixture of polyprenols prepared from another plant Oxydendrum arboreum, (not shown).

The “polyprenol pattern” was characteristic in all so far studied species of Magnoliaceae, Moraceae, etc. (Swiezewska et al., 1994). The main polyprenols in their leaves were prenol-10 and -11. In several other plant families we could observe the domination of prenol-19, -20 e.g. in Rosaceae. In the present paper plants of family Ericaceae have been examined for the presence of long chain polyprenols. The former trials have demon-

### Table 1. Polyprenols in leaves of rhododendrons of deciduous (1-15) and semideciduous (16-18) type

<table>
<thead>
<tr>
<th>Name of species</th>
<th>Content of polyprenols (% dry mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Rh. albrechtii Maxim</td>
<td>0.05–0.2</td>
</tr>
<tr>
<td>2. Rh. arborescens (Pursh) Torr</td>
<td>0.05–0.2</td>
</tr>
<tr>
<td>3. Rh. atlanticum Rehder</td>
<td>0.20–1.0</td>
</tr>
<tr>
<td>4. Rh. calendulaceum Torr</td>
<td>0.20–1.0</td>
</tr>
<tr>
<td>5. Rh. camtschaticum Pall</td>
<td>0.05–0.2</td>
</tr>
<tr>
<td>6. Rh. canadense Torr</td>
<td>0.20–1.0</td>
</tr>
<tr>
<td>7. Rh. gandavense Rheder</td>
<td>0.20–1.0</td>
</tr>
<tr>
<td>8. Rh. japonicum (Gray) Suring</td>
<td>0.20–1.0</td>
</tr>
<tr>
<td>9. Rh. luteum Sweet</td>
<td>0.05–0.2</td>
</tr>
<tr>
<td>10. Rh. prinophyllum (Small) Millais</td>
<td>0.20–1.0</td>
</tr>
<tr>
<td>11. Rh. reticulatum D.Don</td>
<td>0.05–0.2</td>
</tr>
<tr>
<td>12. Rh. schlippenbachii Maxim.</td>
<td>0.05–0.2</td>
</tr>
<tr>
<td>13. Rh. semiobarbatum Maxim.</td>
<td>0.05–0.2</td>
</tr>
<tr>
<td>14. Rh. vaseyi Gray</td>
<td>0.20–1.0</td>
</tr>
<tr>
<td>15. Rh. viscosum Torr.</td>
<td>0.20–1.0</td>
</tr>
<tr>
<td>16. Rh. kaempheri Planch.</td>
<td>0.05–0.2</td>
</tr>
<tr>
<td>17. Rh. obtusum Planch.</td>
<td>0.05–0.2</td>
</tr>
<tr>
<td>18. Rh. poukhanense Level.</td>
<td>0.05–0.2</td>
</tr>
</tbody>
</table>

**Figure 2.** HPLC record of the polyprenols isolated from Oxydendrum arboreum (L.) DC.

The numbers over the peaks mark the position of a given prenologue (17, prenol-17; 18, prenol-18; 19, prenol-19). For other details see Materials and Methods.
strasted that some Ericaceae contained poly-
prenols of the chain length of 16–19 isoprene
units (e.g. Vaccinium vitis idaea) but in the ever-
green rhododendrons the presence of poly-
prenols has never been detected. The de-
ciduous type of rhododendrons as well as other members of Ericaceae were found to
contain polyrenols of similar chain length (in
the form of acetates).

The chromatographic and NMR spectro-
metric characteristics of the polyrenols in
polyrenols positive family of Ericaceae
strongly indicate that they are of the same
structure as those described for other plant
families. It was thought not possible to deter-
mine whether the polyrenols were of di-trans
or tri-trans type. The highest amount of
polyrenols reaching the values of about 1% in
studied plant species was of the same order
observed in several plant species (Swiezewska
et al., 1994). The “polyrenol patterns” of the
studied Ericaceae were found to be similar to
those of Pinaceae family (Ibata et al., 1984;
Swiezewska & Chojnacki, 1988) and of Ginkgo
biloba (Ibata et al., 1983). The similarity of
“polyrenol spectra” in very distant groups of
plants has been observed earlier in the case of
species belonging to Cycadopisida and Rosae-
cae (Chojnacki et al., 1987). One can specu-
late that this similarity may be the reflection
of common function of these substances in the
above mentioned distant groups of plants.

Since a long time it has been known that in
deciduous plants de-greening of chloroplasts
occurs during autumn and finally leads to
death of leaves. A number of physiological fac-
tors are involved in this process. In our de-
ciduous rhododendrons accumulation of poly-
prenols was observed during autumn. This is
in agreement with the observed accumulation
of polyrenols in de-greening leaves of var-
i ous representatives of Rosaceae, Magnoliaceae
and Anacardiaceae (not shown). The accumu-
lation of polyrenols in the material studied
may be due to the enhancement of a bio-
synthetic process, as it is the case of for-
amination of secondary metabolites. The pheno-
menon of accumulation of polyrenols in some
plants (and dolichols in aging animal cells
(Chojnacki & Dallner, 1988) deserves further
investigation.

Our thanks should be expressed to Professor
St. Lewak of the University of Warsaw for

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**Figure 3.** $^1$H-NMR spectrum of polyrenols of Rhododendron viscosum.

As signment of $^1$H-NMR signals

<table>
<thead>
<tr>
<th>ppm</th>
<th>Hydrogen atoms (in italics)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.60</td>
<td>$-\text{CH}_3$ trans, $-\text{CH}_3$ trans (omega)</td>
</tr>
<tr>
<td>1.68</td>
<td>$-\text{CH}_3$ cis, $-\text{CH}_3$ cis (omega)</td>
</tr>
<tr>
<td>1.74</td>
<td>$-\text{CH}_3$ cis (alpha)</td>
</tr>
<tr>
<td>4.10</td>
<td>$=\text{CH}$-$\text{CH}_2$-$\text{OH}$</td>
</tr>
<tr>
<td>5.12</td>
<td>$=\text{CH}$-</td>
</tr>
<tr>
<td>5.45</td>
<td>$=\text{CH}$-$\text{CH}_2$-$\text{OH}$</td>
</tr>
</tbody>
</table>

**Table 2. Polyrenols in leaves of various Ericaceae**

<table>
<thead>
<tr>
<th>Name of species</th>
<th>Content of polyrenols (% dry mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyonia mariana (L.) D.Don</td>
<td>0.4–1.0</td>
</tr>
<tr>
<td>Menziesia pilosa (Michx.) Juss.</td>
<td>0.2–1.0</td>
</tr>
<tr>
<td>Oxydendrum arboreum (L.) DC</td>
<td>0.4–1.0</td>
</tr>
<tr>
<td>Vaccinium vitis idaea L.</td>
<td>0.2–1.0</td>
</tr>
<tr>
<td>Zenobia pulverulenta (W.Bartam ex Wild.) Pollard</td>
<td>0.2–1.0</td>
</tr>
</tbody>
</table>
helpful discussions and to Dr. J. Wójcik for
NMR-analyses.

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