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QUARTERLY

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.

The presence of long-chain polyprenols (Fig. 1) in leaves was documented in the number of botanical systematic groups and was suggested to be a chemotaxonomic criterion (Swiezewska *et al*., 1994) – a species (genus or even family – e.g. *Pinaceae*) specific feature, i.e. the same polyprenol pattern is observed independently of the geographical origin of the plant (Ibata *et al.,* 1984; Swiezewska & Chojnacki, 1988). This phenomenon was confirmed for over 2000 plant species studied in our laboratory (Swiezewska *et al.,* 1994).

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.

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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

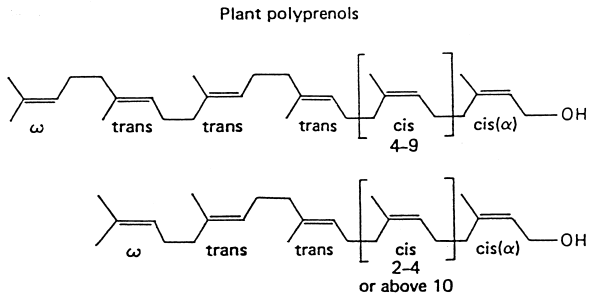

Figure 1. Structure of polyprenols

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 (Golas *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.

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 polyprenyl 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 polyhprenols of various chain length (prenologues 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 nineteen 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. yakushimanum* 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.

The list of names of various local varieties of evergreen type rhododendrons that were found to be polyprenol negative is as follows: 'Alfred', 'America', 'Arno', 'Boursalt', 'Burgemeester Aarts', 'Caractacus', 'Catawbiense Boursault', 'Catharina van Tol', 'Cunningham's White', 'Dora Webbach', 'Dr H.C. Dresselhuys', 'Duke of York', 'Dyr. Frankowski', 'Edward S.Rand', 'Effner', 'Everstianum', 'Godman', 'Fastuosum Plenum', 'Lee's Dark Purple', 'Motyl', 'Parsons Gloriosum', 'Rose Marie', 'Silva Taruoca', 'Van der Hoop', 'Van Weerden Poelman'.

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,

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

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

Lyonia ligustriana (L.) DC, *Pieris floribunda* (Pursh) Benth.et Hook.f., *P. japonica* (Thunb.) D.Don, *P. polita* W.W.Sm. et J.F.Jeffrey, *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 ^{1}H-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).

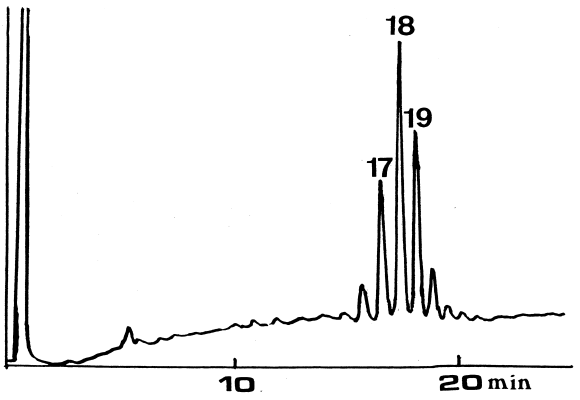

Figure 2. HPLC record of the polyprenols isolated from *Oxydendrum arboreum* (L.) DC.

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.

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-

strated that some *Ericaceae* contained polyprenols of the chain length of 16–19 isoprene units (e.g. *Vaccinium vitis idaea*) but in the evergreen rhododendrons the presence of polyprenols has never been detected. The deciduous type of rhododendrons as well as other members of *Ericaceae* were found to contain polyprenols of similar chain length (in the form of acetates).

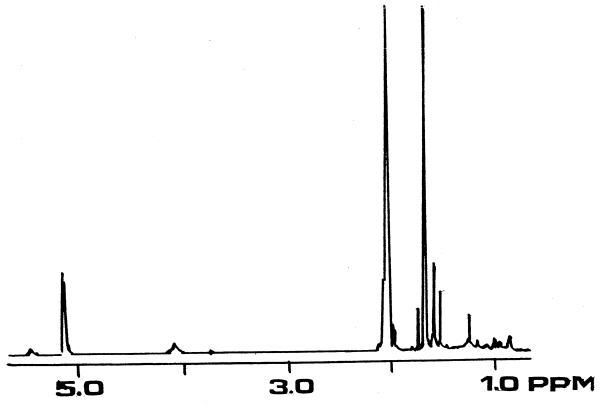

Figure 3. ^{1}H-NMR spectrum of polyprenols of *Rhododendron viscosum*.

Assignment of ^{1}H-NMR signals

ppm	Hydrogen atoms (in italics)
1.60	-C*H*$_3$ *trans*, -C*H*$_3$ *trans* (omega)
1.68	-C*H*$_3$ *cis*, -C*H*$_3$ *cis* (omega)
1.74	-C*H*$_3$ *cis* (alpha)
4.10	=CH-C*H*$_2$ -OH
5.12	=C*H*-
5.45	=C*H*-CH$_2$-OH

The chromatographic and NMR spectrometric characteristics of the polyprenols in polyprenols positive family of *Ericaceae* strongly indicate that they are of the same structure as those described for other plant families. It was though not possible to determine whether the polyprenols were of di-*trans* or tri-*trans* type. The highest amount of polyprenols 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 "polyprenol 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 "polyprenol spectra" in very distant groups of plants has been observed earlier in the case of species belonging to *Cycadopisida* and *Rosaceae* (Chojnacki *et al*., 1987). One can speculate 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 factors are involved in this process. In our deciduous rhododendrons accumulation of polyprenols was observed during autumn. This is in agreement with the observed accumulation

Table 2. Polyprenols in leaves of various *Ericaeae*

Name of species	Content of polyprenols (% dry mass)
Lyonia mariana (L.) D.Don	0.4–1.0
Menziesia pilosa (Michx.) Juss.	0.2–1.0
Oxydendrum arboreum (L.) DC	0.4–1.0
Vaccinium vitis idaea L.	0.2–1.0
Zenobia pulverulenta (W.Bartam ex Wild.) Pollard	0.2–1.0

of polyprenols in de-greening leaves of various representatives of *Rosaceae*, *Magnoliaceae* and *Anacardiaceae* (not shown). The accumulation of polyprenols in the material studied may be due to the enhancement of a biosynthetic process, as it is the case of formation of secondary metabolites. The phenomenon of accumulation of polyprenols in some plants (and dolichols in aging animal cells (Chojnacki & Dallner, 1988) deserves further investigation.

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