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This paper is dedicated to our friend Professor Włodzimierz Ostrowski

On the specific pattern of long chain polyprenols in green needles of *Pinus mugo* Turra*

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In green needles of *Pinus mugo* the most abundant polyprenols occur as a mixture of prenologues in which the dominant alcohol is built of 16 isoprene units. The characteristic spectrum of polyprenols (prenol-15, -16 and -17) was the same irrespective of the location of plant and of distinct morphological differences observed in the various selected forms of this species. The constant pattern of the polyprenols spectrum was preserved throughout the 2-year life span of needles, although the level of polyprenols was increased 2–3-fold. The polyprenol pattern in *Pinaceae* family differs from species to species, thus it may serve as chemotaxonomic criterion within this systematic group.

The long-chain poly-*cis*-prenols in green needles of some representative species belonging to systematic *Pinaceae* family have been described by Zinkel & Evans (1972) and Hannus & Pensar (1974). A more thorough study on the occurrence of these substances in the needles of *Pinaceae* was reported by Ibata *et al.* (1984) and polyprenols in over 100 species of gymnosperm plants were characterized in detail by Świeżewska & Chojnacki (1988). Though the polyprenol pattern in plants seemed to be species specific and independent of the site of plant growth, some discrete intra-species differences in this pattern due to morphological variations of a given plant species could not be excluded. We have earlier noticed very discrete changes in proportions of individual prenologues in the course of a long-term study of some individual plants belonging to the *Pinaceae* and *Taxaceae* families (Świeżewska & Chojnacki, 1988), as well as distinct differences between the data of Japanese authors (Ibata *et al.*, 1984) and our results (Świeżewska *et al.*, 1994; Jankowski *et al.*, 1994).

The aim of the present paper was to ascertain whether the 'intra-species' constancy of the polyprenol pattern is species characteristic, despite evident morphological differences among natural varieties of *Pinus mugo*.

MATERIALS AND METHODS

Plant material. Plants of the species *P. mugo* Turra belonged to a large living population forming a group of 2800 plants 21-

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years old, growing on 3000 m^2 plats in the Botanical Garden of the Polish Academy of Sciences at Powsin, close to Warsaw. They were cultivated from seeds collected in the wild habitats in the Tatra Mountains. After 17 years of cultivation in the abnormal climate conditions plants showed tremendous variability. Over two hundred dwarf and slowly growing plants were transplanted to a special bed in the Arboretum in danger of being dominated by the normally growing plants. From this collection 72 plants of characteristic dwarf size or slow growth were selected.

Morphological and developmental characteristics of the studied shrubs of *P. mugo* are described in Table 1. From all plants one-year-old and two-year-old needles were collected on October 5th, 1995 and stored in dry atmosphere for up to eight weeks before extraction of lipids and chromatographic analysis.

Table 1. Morphological characteristics of *Pinus mugo* plants.

Average values from the number of plants indicated ±S.D.

Growth parameters	Reference plants	Dwarf and slowly growing plants
	n = 100	n = 72
Height (cm)	201.2 ± 35.3	85.3 ± 22.4
Length of 3-year increments (cm)	57.7 ± 11.6	42.6 ± 12.3
Length of needles (mm) on 1-year increments	53.2 ± 9.5	45.6 ± 8.0
Length of needles (mm) on 2-year increments	46.7 ± 8.5	44.5 ± 8.0

The needles of *Pinus aristata, P. montezumae, P. nigra, P. peuce, P. pinaster, P. ponderosa, P. sylvestris* and *P. strobus* and the leaves of *Ginkgo biloba* were collected from plants growing in the same Botanical Garden (Warsaw/Powsin).

Lipid extraction and chromatographic analysis were performed essentially as described earlier (Świeżewska & Chojnacki, 1996). For analysis the 250 mg samples of dry needles were homogenized in 5 ml of acetone/hexane (1:1, v/v). The homogenates were filtered and clear extracts were stored in the dark at 0°C for 1–2 weeks before chromatographic analysis.

Strong alkaline hydrolysis of lipid extracts was carried out as described by Stone *et al.* (1967) in screw-capped tubes at 100°C.

Thin-layer chromatography was run on Kiesel gel G plates and on RP-18 plates (E. Merck, Darmstadt, Germany) with ethyl acetate/toluene (1:19, v/v, solvent A), toluene/hexane (1:1, v/v, solvent B) or with acetone (solvent C). Spots of lipids were visualized by exposure to iodine vapours and chromatograms were recorded as Xerox copies. Semiquantitative analysis of the content of polyprenols and their derivatives was performed as suggested by Wellburn & Hemming (1966) by comparing the size and intensity of chromatographic spots with those formed by known amounts of standard polyprenols and their carboxylic esters supplied by the Collection of Polyprenols of our Institute (Dolichols, Polyprenols and Derivatives, Catalogue 1995, Institute of Biochemistry Biophysics, PAS, Warsaw). The mixture of natural prenyl acetates was prepared from *G. biloba* leaves.

High pressure liquid chromatography was performed on reversed phase ODS-Hypersil column (Knauer 3.9 mm × 60 mm) using the Waters dual pump apparatus, a gradient programmer, UV-detector set at 210 nm and a recorder. For elution, a convex gradient was used: from the initial 2-propanol/methanol/water (8:12:1, by vol.) in the pump system A to 70% hexane/2-propanol (7:3, by vol.) in system B, at a flow rate of 1 ml/min. The procedure was similar to that used previously (Świeżewska & Chojnacki, 1996).

RESULTS AND DISCUSSION

P. mugo species was chosen due to the access to a well established population of dwarf-mountain-pine originating from Tatra Mountains and arranged by one of us (A.M.) in the Botanical Garden in Warsaw (Powsin). Relating a possible variation of the polyprenol spectrum with a distinct morphological feature of a given plant could be helpful in determining factors responsible for the synthesis of long-chain polyprenols producing a prenologue of a particular size. In this regard an exceptional variability of *Pinus mugo* species may throw some light on the systematic position of it in plant taxonomy in view of this species and a possible existence of subspecies or varieties (Szweykowska & Szweykowski, 1993; A. Marczewski, unpublished, 1997).

The very high morphological variability (i.e. height and type of shrubs, length and size of needles served as a criterion for identifying two groups of plants: 1. dwarf and slowly growing plants (group S), and 2. reference plants taken at random from the area of the remaining "normally looking" plants along two separate transects (1 and 2) delineated in an almost perpendicular way through the whole area of the population.

Data on morphology and development of the selected and reference plants are summarized in Table 1. Growth of the dwarf and slowly growing plants was reduced by half as compared to the average height of the reference plants, (data recorded in 1995). It

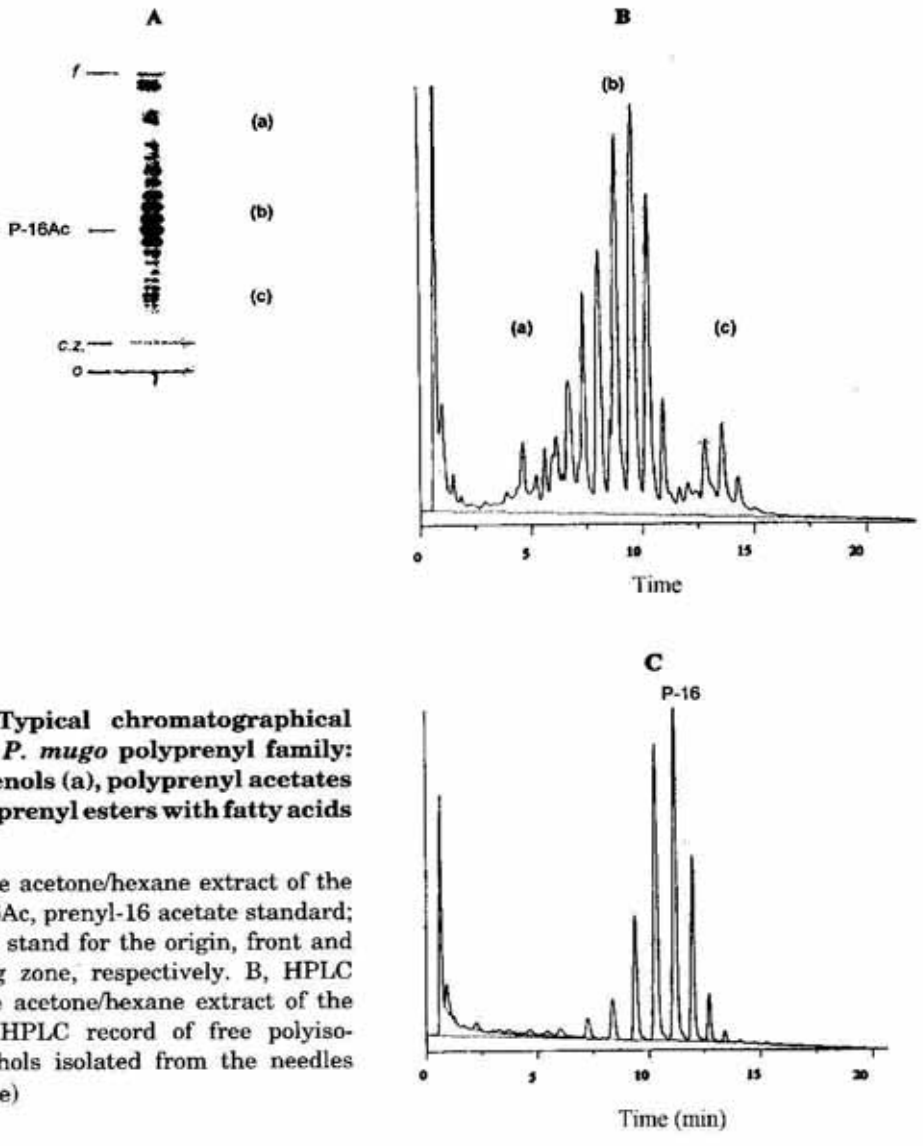

Figure 1. Typical chromatographical patterns of *P. mugo* polyprenyl family: free polyprenols (a), polyprenyl acetates (b) and polyprenyl esters with fatty acids (c).

A, TLC of the acetone/hexane extract of the needles. P-16Ac, prenyl-16 acetate standard; *o*, *f* and *c.z.* stand for the origin, front and concentrating zone, respectively. B, HPLC record of the acetone/hexane extract of the needles. C, HPLC record of free polyisoprenoid alcohols isolated from the needles (16,15,17 type)

should be added that the growing rate of the shrubs, observed during 20 years was not stable, and the growth rate of the slowly growing plants in some years even exceeded the average value of the reference plants. Average length of the needles of both 1-, 2- and 3-year increments was reduced in dwarf plants by 75%.

The pattern of polyprenols and their carboxylic esters in the needles of *P. mugo* is presented as TLC records of the lipid extract from typical plants taken at random from the population. The record of a typical plant shows as a rule the presence of three groups of polyprenoids: the most abundant polyprenyl acetates, 5–10-fold lower levels of polyprenyl esters with long chain fatty acids; and free polyprenols. The amounts of fatty acid esters of polyprenols and of free polyprenols vary, but can always be detected in trace amounts as compared with the dominating polyprenyl acetates (Fig. 1A, B). In the hydrolyzed extract only free polyprenols are present.

The HPLC record of polyprenols in the hydrolyzed extract is shown in Fig. 1C. The short-hand formula of the pattern of polyprenols can be expressed as e.g., 16,15~17, stating that prenol-16 is the dominating prenologue and prenol-15 and prenol-17 are less abundant and are quantitatively equal. In other plants the sequence written as, e.g., 16~15,17~14 means that prenol-16 and prenol-15 are dominating and equally abundant, and prenol-17 and prenol-14 are less abundant and occur in equal amounts. Usually, only the main three or four prenologues were taken into account on formulating the short-hand symbol of the polyprenol pattern in a given plant.

One-year-old and two-year-old needles from the reference plants and from the dwarf type or slowly growing plants were examined (Fig. 2). In the composition of polyprenol families in the dwarf and reference plants some variations were observed. The occurrence of ten most typical patterns and their frequency distribution in the selected and reference plants was calculated (Fig. 2). The data are summarized in Table 2. Four typical groups of prenols were distinguished.

As can be seen the polyprenol pattern in the majority of reference plants was similar. The pattern in the one-year old and two-year-old needles was identical in the majority of reference plants. The level of polyprenols in the two-year-old needles was 2–5 times higher than in the one-year-old needles. As a rule prenol-16 was the dominating prenologue, or two prenologues, prenol-16 and -15 were prevalent together with less frequent prenols. Variations of the occurrence of these accompanying prenols seemed to be of less significance. This observation holds also for the dwarf and slowly growing plants. A slightly different pattern of polyprenols was

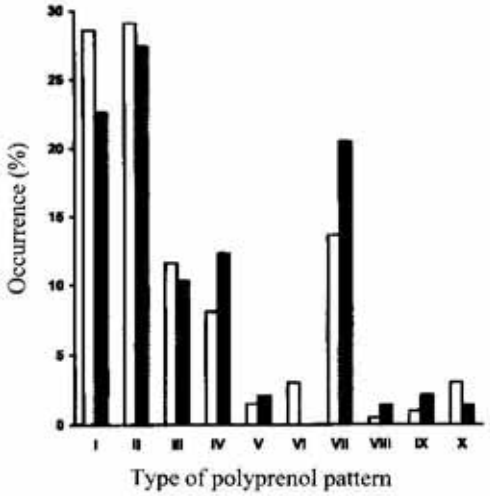

Figure 2. The occurrence of 10 predominant polyprenol sequences in the group of selected (closed bars) and reference plants (open bars).

Roman numbers represent the following sequences: I — 16,15,17; II — 16,15~17; III — 16,17,15; IV — 16~15,14~17; V — 16~15,14,17; VI — 16~15,17,14; VII — 16~15,17; VIII — 16,15,14; IX — 16~15; X — others.

Table 2. The polyprenol patterns in the needles of the reference and the dwarf and slowly growing plants of *P. mugo*.

See text for details.

Pattern of polyprenols	Reference plants (%)	Dwarf and slowly growing plants (%)
16~15	27	37
16,15~17	31	27
16,15,17	30	24
16,17,15	12	12

noted (dominating prenol-17) in a single reference plant.

The polyprenol pattern in the over 200 plant samples studied exhibiting various forms was very stable within the two-years period and did not differ much in spite of striking differences in the morphology of the two groups of plants. A trend toward favouring the pattern 16~15 appears in the reference and the dwarf slowly growing plants (Fig. 2 and Table 2).

The polyprenol pattern in *P. mugo* is different from those recorded for a number of other conifers (Table 3). Only in *Pinus aristata, P. nigra* and *P. sylvestris* the characteristic pattern typical for *P. mugo* was observed. This pattern was very similar to this reported earlier (Ibata *et al.*, 1984; Świeżewska & Chojnacki, 1988).

It seems that the pattern of polyprenols in the various forms of *P. mugo* may serve as a chemotaxonomic criterion since in spite of the distinct morphological differences within this systematic group the characteristic pattern was almost identical in all studied plants. This was shown to be true for the population of reference plants, for that of the dwarf and slowly growing plants, as well as for the needles differing in age. It seems that despite the profound anatomical differences in the size of needles, size of plants and in their morphological appearance, the expression of the genes responsible for the complex process of formation of long chain polyprenols remained unchanged. It can also be concluded that even with the total level of polyprenols increasing in time the proportions of prenologues remain similar, which means that the accuracy of the polyprenols synthesizing machinery was not changed with aging.

Table 3. The polyprenol spectrum in the needles of various species of conifers.

II and IV denote the age of needles (years)

Plant	Content of polyprenols (% dry weight)	Number of isoprene units in prenologues dominating in natural polyprenol mixture
1. *Pinus aristata* **II**	0.8	16,15~17
2. *Pinus cembra* **II**	1.2	17,16,15
3. *Pinus montezumae* **II**	1.2	16,17,15
4. *Pinus nigra* **II**	1.2	16,15~17
5. *Pinus peuce* **II**	1.6	17,18,16
6. *Pinus pinaster* **II**	2.4	18,17,16
7. *Pinus pinea* **IV**	0.8	17,18,16
8. *Pinus strobus* **II**	4.8	17~18,16
9. *Pinus sylvestris* **II**	2.4	16~15,17
10. *Pinus sylvestris van lapponica* **II**	2.0	16,15,17

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