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Polyprenols in leaves of fruit-trees of *Rosaceae* family*

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In leaves of various species of fruit-trees belonging to the *Rosaceae* family, large amounts of polyprenyl acetates (0.5–5.0% of dry weight) were found. Discrete constant differences of polyprenol spectrum characteristic of each genus studied: *Malus, Prunus* and *Pyrus* were observed. In each species poly-*cis*-prenols composed of 19 and 20 isoprene units were predominating. In one of the 23 studied species (*Prunus incisa*) a fraction of long-chain polyprenols composed of 35–45 isoprene units was also present. It seems that this type of unusually long-chain polyprenols could occur also in other plants of the *Rosaceae* family.

Within the last 30 years the occurrence of long-chain poly-*cis*-prenols in leaves of various plants has been the subject of several reports, specifying the presence or a particular type of these substances in different plant species. These studies have been summarized in a review by Świeżewska *et al.* (1994). The complex character of polyprenol spectrum was demonstrated for various representatives of gymnosperms by Japanese authors (Ibata *et al.*, 1984) and further documented by Świeżewska & Chojnacki (1988) who studied a large number of *Cycadopsida* and *Coniferopsida*. Multiple polyprenol families were found also in angiosperms (Świeżewska & Chojnacki, 1989, 1991; Świeżewska *et al.*, 1992). While in the case of gymnosperm plants the specific character of polyprenol spectrum was revealed for more than one hundred species, out of the *Rosaceae* systematic family only one genus of herbaceous plants — Potentilla — has been studied extensively (about 100 species examined) and trees and shrubs were investigated in only a few cases (Chojnacki & Vogtman, 1984). The present paper describes the characteristic polyprenol patterns in 23 common fruit-trees and shrubs: *Malus, Prunus* and *Pyrus* from the collection of the Botanical Garden of the Polish Academy of Sciences in Powsin. The aim of these studies was to find whether there are common features of the polyprenol spectrum within a particular genus and whether detectable differences exist between various genera of *Rosaceae*. In the course of this study it became evident that, apart from the main polyprenol group typical of the particular genus, a group of longer chain polyisoprenoid alcohols can also be present. In the previous studies this group of polyprenols might have been overlooked.

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Abbreviations: TLC, thin-layer chromatography; TMS, tetramethylsilane.

MATERIALS AND METHODS

Leaves of all the fruit-trees studied were taken from plants growing in open air in the Botanical Garden of the Polish Academy of Sciences in Powsin near Warsaw. The date of collection was 20 October 1995, i.e. approximately 2 weeks before the natural yellowing and falling of the leaves. There had been no frost in this part of the Botanical Garden at any time before the moment the leaves were collected. The leaves showed no symptoms of damage or pathology. They were dried and stored in paper envelopes for 2–4 weeks before the experiment.

The homogenization/extraction procedure was performed with a homogenizer with metal blades (Ultra Turrax, Germany). Dry leaves (250 mg), suspended in 5 ml of acetone/hexane (1:1, v/v) were homogenized at top speed for 1 min. The homogenate was stored in the dark at room temperature for 2 days with occasional shaking. During that time the originally green sediment of debris became colorless, thus indicating that all chlorophyll was extracted with the organic solvent.

The dark-green extract (a portion of 10 µl) corresponding to 0.5 mg of dry leaves was subjected to thin-layer chromatography on Kiesel gel plates in solvent I (hexane/toluene, 1:1, v/v). Appropriate standard substances: single prenologues, polyprenols from *P. aurea*, a mixture of polyprenyl acetates and a mixture of polyprenyl palmitates prepared from *P. aurea* polyprenols by chemical procedures (Chojnacki & Vogtman, 1984), were used. Another portion of the extract (corresponding to 250 mg of dry leaves) was subjected to strong alkaline hydrolysis (Stone *et al.*, 1967) and the fraction of unsaponifiable lipids was analyzed by thin-layer chromatography on Kiesel gel plates in solvent II (ethyl acetate/benzene, 1:19, v/v) and on RP-18 plates using aceto- ne/hexane (9:1, v/v, solvent III) or acetone (solvent IV). Chromatography was performed 3 times in the same direction in one of these solvents, each time allowing the solvent to reach the top of the plate and letting it dry for 1–2 min. The amount of lipids spotted on a plate corresponded to 0.5 mg of dry leaves. The spots were stained with iodine. For semiquantitative estimation of polyprenols the size and intensity of the spots were compared with those obtained with variable amounts of standard substances.

Qualitative evaluation of the polyprenol spectrum was performed by comparing on RP-18 plates the chromatographic patterns of the studied polyprenols with those of standard mixtures of polyprenols (Świeżewska *et al.*, 1994).

Preparative separation of groups of polyprenols differing in the size of molecules was performed by column chromatography on Kiesel gel. About 5 mg of unsaponifiable lipids in hexane was applied onto the column (0.8 cm × 12.0 cm) and hexane containing increasing concentrations of ethyl ether (linear gradient of ethyl ether from 3% to 15%) was used as an eluent. The course of elution was checked by TLC on RP-18 plates using solvent III and solvent IV.

HPLC of polyprenol mixtures was performed on a Hypersil ODS 3 µm reversed-phase column (Hewlett-Packard). A dual pump apparatus (Waters Ass., U.S.A.), gradient programmer, UV detector (210 nm), and an integrator were used. For elution, convex gradients were applied from the initial 2-propanol / methanol / water (8:12:1, by vol.) in pump A to 50% or 80% hexane / 2-propanol (7 : 3, v/v) in pump system B at a flow rate of 1.5 ml/min as described earlier (Świeżewska *et al.*, 1992).

^{1}H-NMR (200 MHz) spectra were recorded in $CDCl_3$ using TMS as the internal standard.

All organic solvents used for extraction and chromatography were from Merck (Darmstadt, Germany) either "p.a" or "for chromatography". Kiesel gel TLC plates and RP-18 plates with concentrating zone and Kiesel gel (200–400 mesh) for column chromatography were also from this source.

RESULTS

As presented in Fig. 1, polyprenols predominated in the unpolar lipid fraction of leaves in all the studied fruit-trees of *Rosaceae* family. All but one (*P. incisa* (17)) plant species studied contained a typical polyprenol mixture found as a single spot in different TLC systems (Fig. 1a, b). In the case of *P. incisa* additional compounds (putative family of longer chain polyprenols) formed a second, faster moving spot on the chromatograms.

As shown by TLC of initial, non hydrolyzed lipid extracts (Fig. 1a), in all species studied

polyprenols were present in the form of acetic acid esters. They migrated at R_F of about 0.4 in solvent I. An intensive spot of this mobility was found in all studied species of genus *Malus*, *Prunus* and *Pyrus*. In addition, a second group of typical polyprenyl acetates moving slightly ahead (R_F 0.5) was observed. In *P. incisa* (and only in that species) both the unhydrolyzed and the unsaponifiable fraction gave a faster moving spot which could correspond to the occurrence of a group of longer chain polyprenols other than those present in all the other species studied.

The results of semiquantitative estimation of the polyprenol content and of qualitative evaluation of chain length of polyprenols are shown in Table 1. The total polyprenol content in leaves reached values as high as about 5% of their dry weight. The evaluation of chain length by chromatography on RP-18 plates revealed that in genus *Malus* and *Pyrus* the composition of the polyprenol mixtures was practically the same, with prenol-20 and prenol-21 being the dominating components. However, in all species of genus *Prunus* the spectrum of polyprenols was slightly different; the dominating polyprenols were prenol-19 and prenol-20. Chromatography on RP-18 plate revealed the presence in *P. incisa* of a group of bands that could correspond to polyprenols of chain length longer by about 15 isoprene units than the typical prenol-19 and prenol-20 common to all studied species of *Prunus* (Fig. 1c). These two groups of polyprenols of *P. incisa* were separated by Kiesel gel column chromatography (Fig. 2). Typical polyprenols (prenol-18, -19, -20 and -21) were preceded by substances which on RP-18 plate migrated as prenol-35 and longer prenols in comparison to polyprenol standards from *P. aurea* (Świeżewska *et al.*, 1992). When examined by ^{1}H-NMR spectrometry (cf. Chojnacki *et al.*, 1987), these two groups of polyprenols exhibited the typical poly-*cis* structure (unpublished). However, it was not possible to calculate the exact number of *cis* and *trans* residues due to the low amount of the substance studied.

The composition of the two polyprenol fractions is illustrated more clearly on the HPLC records (Fig. 2b, c). It is evident that both frac-

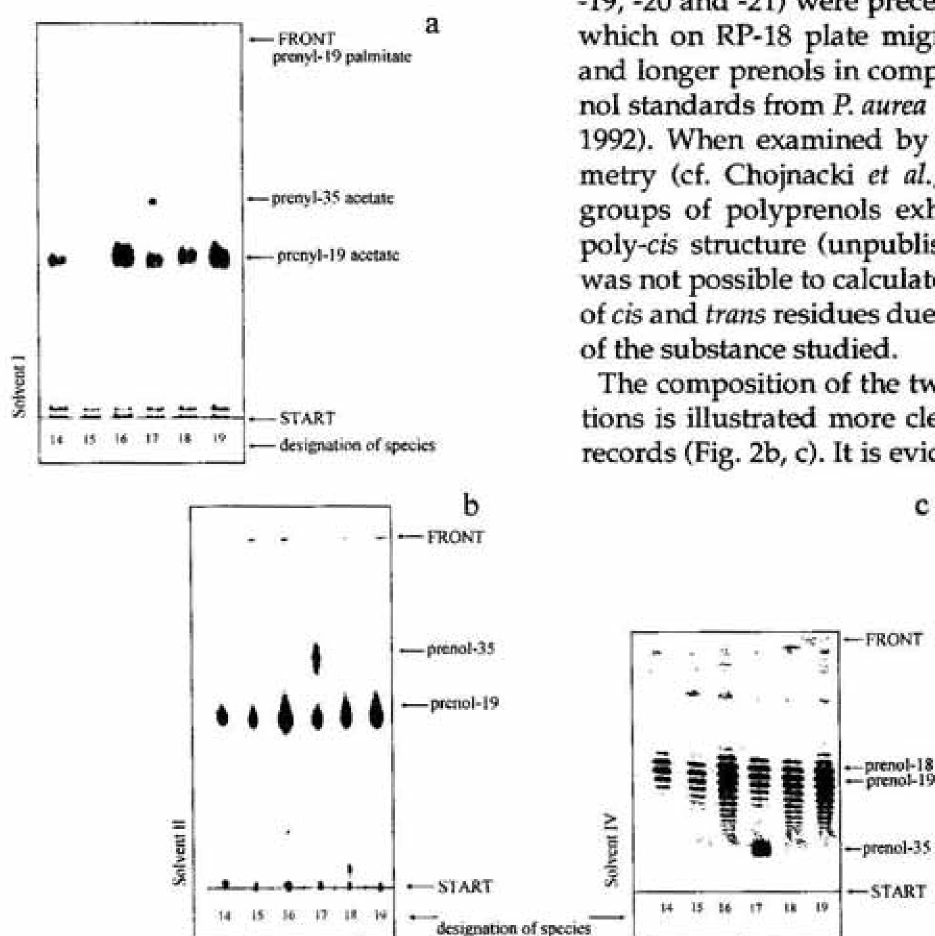

Fig. 1. Chromatography of polyprenols extracted from leaves of various species of Prunus.
Numbers 14, 15, 16, 17, 18 and 19 refer to the species of genus *Prunus* listed in Table 1. Spots were detected with iodine. a, Total lipid extract; TLC on Kiesel gel plate developed with toluene/hexane, 1:1, v/v, solvent I. b, Unsaponifiable fraction of total lipid extract; TLC on Kieselgel plate in ethyl acetate/benzene, 1:19, v/v, solvent II. c, Unsaponifiable fraction of total lipid extract; TLC on RP-18 plate developed with acetone (solvent IV, run 3×). In the extract of *P. incisa* (17) a group of longer polyprenols creating an additional spot of polyprenyl acetates (solvent I) and free polyprenols (solvent II and IV) is observed.

tions are composed of a number of substances which are chromatographically identical to the polyprenols of *P. aurea*. These two groups of polyprenols were incompletely separated and some slight cross-contamination was observed. It can also be seen that there was no complete gap between the dominating parts of the two fractions, and that traces of polyprenols that were not visualized on thin-layer chromatogram (Fig. 2a) were present in the records obtained with the very sensitive UV-detector.

It should be stressed that the presence of the group of longer chain polyprenols was not detectable by thin-layer chromatography in any of the other species of *Prunus* and in any of species of *Malus* and *Pyrus*. On testing again the previously described (Chojnacki & Vogtman, 1984) polyprenol preparations from leaves of *Rosaceae* we did detect traces of longer chain polyprenols in the original preparation from *Sorbus suecica* that was kept in the fridge for about 15 years. The proportion of the longer chain polyprenols was much lower than that found in *P. incisa*, as only a very faint band of unresolved substances was visible on the RP-18 plate developed with acetone (solvent IV), and multiple development in solvent III was required to move this substance by a few millimeters. In the polyprenol preparation from leaves of *S. suecica* a fraction of substances that correspond to polyprenols composed of 40–50 isoprene units is present (Fig. 3). They correspond to less than 1–2% of the total polyprenols constituting the family in which prenol-20, -21 and -22 are the dominating components.

DISCUSSION

The leaves of several fruit-trees of *Rosaceae* family were found to accumulate distinct amounts of polyprenols in the form of acetic acid esters. The yields of polyprenols in several species studied belong to the highest ever noted in the literature (Świeżewska *et al.*, 1994). This is perhaps due to the fact that leaves were taken at the most appropriate period of vegetation (Chojnacki & Vogtman, 1984).

The genera studied exhibited discrete differences in the spectrum of polyprenols. All species belonging to genus *Malus* and *Pyrus* exhibited similar polyprenol patterns in which

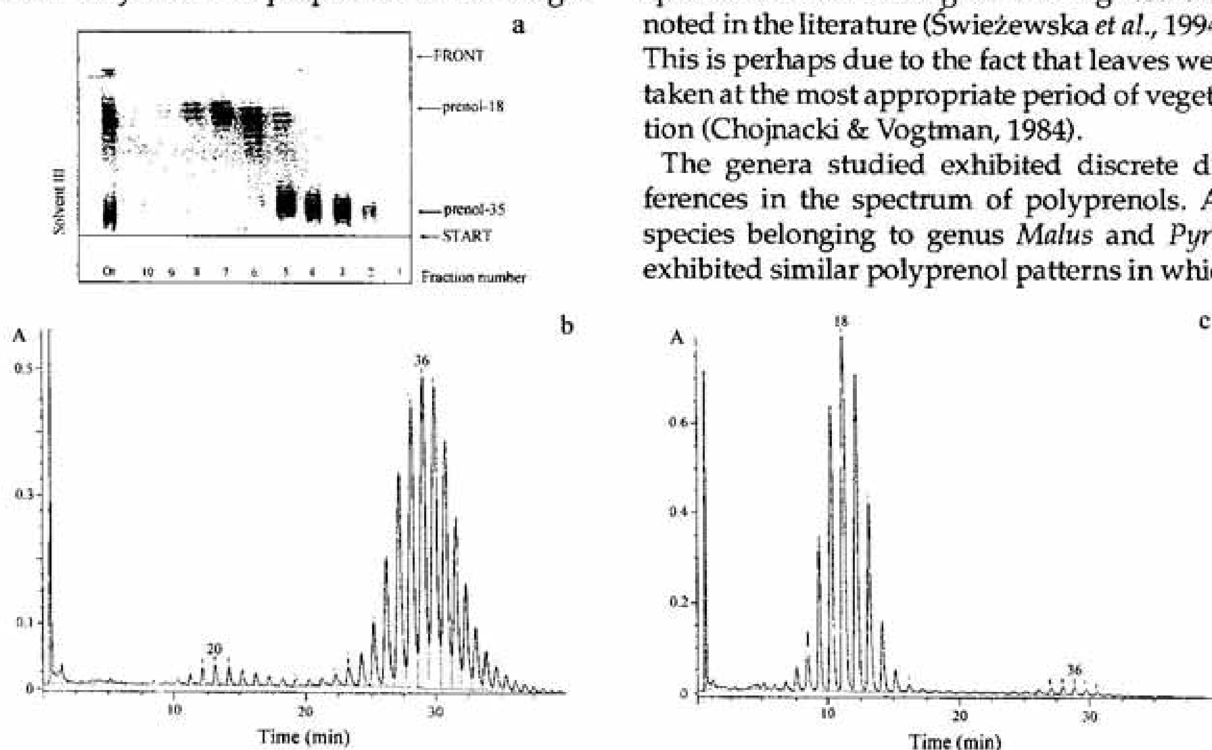

Fig. 2. Chromatography of polyprenols from Prunus incisa.
a, Preparative separation of polyprenols from *P. incisa*. Or, original polyprenol mixture; 1–10, fractions obtained by elution of (Or) from the Kiesel gel column with increasing concentrations of diethyl ether in hexane as described in Methods. Samples of each fraction (1–10) were checked for the presence of polyprenols by TLC on RP-18 plate developed with acetone/hexane, 9:1, v/v mixture. Spots were detected with iodine. b, HPLC of longer chain polyprenols fraction (3+4, cf. Fig. 2a). Peaks of polyprenols detected with the UV detector set at 210 nm. The numbers 19–21 and 34–38 mark the position of elution of prenol-19, -20, etc. prenol-38 as mapped with the aid of polyprenol standards isolated from *P. aurea* (Świeżewska *et al.*, 1992). c, HPLC of the fraction of shorter chain polyprenols (7+8, cf. Fig 2a). Peaks of polyprenols detected with the UV detector set at 210 nm. The position of elution of particular polyprenols is indicated in the same way as described for Fig. 2b.

Table 1

Polyprenols in leaves of fruit-trees.

Approximate content and type of polyprenols was estimated as described in Materials and Methods.

Plant genus and species	Polyprenols	
	content mg/g dry wt	chain length (number of isoprene units)
1. *Malus baccata var. mandshurica*	20–50	19, 20, 21, 22, 23
2. *Malus coronaria*	10–20	19, 20, 21, 22
3. *Malus domestica* "Antonówka zwykla"	5–10	19, 20, 21, 22
4. *Malus domestica* "Grochówka"	5–10	19, 20, 21, 22
5. *Malus domestica* "Kosztela"	10–20	19, 20, 21, 22
6. *Malus domestica* "Królowa Renet" (Z.R.)	10–20	19, 20, 21, 22
7. *Malus domestica* "Reneta Szampanska"	20–50	19, 20, 21, 22, 23
8. *Malus domestica*, "Oliwka Żółta"	20–50	19, 20, 21, 22, 23
9. *Malus domestica* "Węgierczyk"	10–20	19, 20, 21, 22
10. *Malus sargentii*	10–20	19, 20, 21, 22
11. *Malus sieversii*	10–20	19, 20, 21, 22
12. *Malus sylvestris*	5–10	20, 21, 22
13. *Malus tschonoskii*	10–20	19, 20, 21, 22
14. *Prunus avium*	10–20	18, 19, 20
15. *Prunus cerasifera Atropurpurea*	10–20	18, 19, 20
16. *Prunus incisa*	10–20 5–10	18, 19, 20 37, 38, ...41
17. *Prunus mahaleb*	20–50	18, 19, 20
18. *Prunus padus*	10–20	18, 19, 20
19. *Prunus pensylvanica*	20–50	18, 19, 20
20. *Pyrus communis*	5–10	19, 20, 21, 22
21. *Pyrus dasycarpa*	20–50	19, 20, 21, 22, 23
22. *Pyrus korskinskyi*	10–20	19, 20, 21, 22
23. *Pyrus pyrifolia*	20–50	19, 20, 21, 22, 23

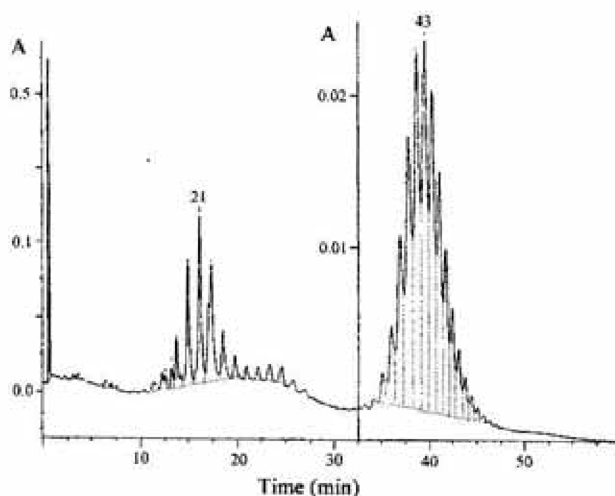

Fig. 3. HPLC of polyprenol mixture from Sorbus suecica.

Peaks of polyprenols were recorded with the UV detector set at 210 nm. The sensitivity of the detector was increased 100-fold in the 32nd minute, that is after the bulk of polyprenols (prenol-19, -20, -21, -22 and -23) had been eluted. The numbers 21 and 43 mark the position of elution of prenol-21 and prenol-43, respectively.

prenols composed of 20, 21 and 22 isoprene units were predominating, while in genus *Prunus* the dominating prenologues are prenol-19, -20 and -21. Within each genus the polyprenol patterns of particular species are indistinguishable. These types of polyprenol patterns are similar to those already recorded in few cases of trees (Chojnacki & Vogtman, 1984) and in shrubs and herbaceous plants of *Rosaceae* family (Świeżewska *et al.*, 1992). A large body of evidence has been accumulated supporting the suggestion that the polyprenol pattern constitutes a chemotaxonomic marker in gymnosperms (Świeżewska & Chojnacki, 1988) and in various systematic families of angiosperms, e.g. *Sapindaceae* (Jankowski & Chojnacki, 1995), *Magnoliaceae* (Sasak & Chojnacki, 1973) and several others (Świeżewska *et al.*, 1994).

The occurrence of a group of polyprenols of chain length considerably exceeding that of the typical polyisoprenoid alcohols (by about 15 isoprene units) in *P. incisa* appeared to be a unique phenomenon as none of the other species studied so far exhibited the presence of such substances in detectable amounts. This fact suggested to us to look for traces of such very long-chain polyprenols in lipid extracts of leaves of some *Rosaceae* plants that have been previously reported to contain typical polyprenol mixtures with dominating prenol-19, -20, -21 and -22. Traces of very long-chain polyprenols (composed of 40–50 isoprene residues) were detected in this material. The complexity of polyprenol mixtures in various species of *Rosaceae* has been described in our previous report (Świeżewska *et al.*, 1992) though the presence of a separate group of polyprenols of much longer chain length than observed in the main fraction, has not been encountered. On the other hand, in genus *Potentilla* and *Rosa* a group of somewhat longer-chain polyprenols was also usually present, but their length was smaller than that reported in this paper. It should be noted that out of the studied 23 representatives of *Malus*, *Prunus* and *Pyrus* only in one species such a pattern was observed.

The presence of polyisoprenoid alcohols greatly exceeding the length of the commonly found polyprenols might have been overlooked in the previous studies, as it was, e.g., in the case of polyprenols of *S. suecica* which were present in very low amounts in the preparations then obtained.

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