DENDROBIOLOGY
2002, vol. 47: 41–50

Magdalena Roślińska, Krystyna Walińska, Ewa Świeżewska, Tadeusz Chojnacki*

Plant long-chain polyprenols as chemotaxonomic markers

Abstract: The occurrence of long-chain polyprenols in leaves of plants of *Lauraceae, Tiliaceae* and *Magnoliaceae* families was studied. Separation of groups of polyprenols differing in the size of molecules was performed by thin-layer chromatography. In all studied species long-chain polyprenols were detected. The total polyprenol content in leaves reached values as high as 3% of their dry weight. In the studied species belonging to *Lauraceae* family a fraction of polyprenols composed of 10–14 isoprene units was present. In species of *Tiliaceae* and *Magnoliaceae* polyprenols composed of 9–12 and 10–12 isoprene units were present, respectively. Differences in polyprenol profile characteristic for each family studied were observed. These results were confirmed by HPLC. The structure of these polyprenols was examined by NMR spectrometry and in representative species studied polyprenols exibited the typical poly-*cis* structure. The effect of insolation on the rate of accumulation of polyprenols was documented.

Additional key words: *Lauraceae, Tiliaceae, Magnoliaceae*

Addresses: M. Roślińska, K. Walińska, Department of Biology, Institute of Biotechnology and Environmental Sciences, University of Zielona Góra, Monte Cassino 21 B, 65-561 Zielona Góra, Poland
E. Świeżewska, T. Chojnacki, Department of Lipid Biochemistry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, A. Pawińskiego 5a, 02-106 Warszawa, Poland

Introduction

Polyisoprenols (poly-*cis*-prenols) are natural products being derivatives of a common C_5 isoprene unit (Bach 1995). Many natural membrane fractions from prokaryotic and eukaryotic cells contain polyisoprenols and their phosphate esters (Chojnacki et al. 1987; Hemming 1983). Unicellular eukaryotes, fungi, animal and some plant tissuses contain α-saturated polyisoprenols (dolichols) (Bach 1995; Jankowski et al. 1994). Bacterial membranes and leaves of some plants contain α-unsaturated polyisoprenols (polyprenols) (Jankowski et al. 1994; Świeżewska et al. 1994). A polyprenol molecule consists of a hydrophilic part – a hydroxyl group at the α-end, and a relatively large hydrophobic part – a long unsaturated, mainly of poly-*cis* configuration, isoprenyl chain (Chojnacki and Świeżewska 1995). Long-chain polyprenols have been found in a large number of plants (Jankowski et al. 1994; Zinkel and Evans 1972). Poly-*cis* prenols with the longest chain are those known as natural rubber (Tanaka 1989). Two types of long-chain polyprenols can be distinguished with respect of their conformation: di-*trans*- and tri-*trans* poly-*cis* prenols (Fenney and Hemming 1967; Tanaka and Takagi 1979)**. Most of the polyprenols isolated from the leaf tissues of angiosperms have consisted of three internal *trans*-

* Corresponding author: Krystyna Walińska, fax (+48-68-3234-080), e-mail: sekretariat@ibos.uz.zgora.pl

** Chemical literature uses letters E and Z for marking the *t* (*trans*) and *c* (*cis*) isoprene units

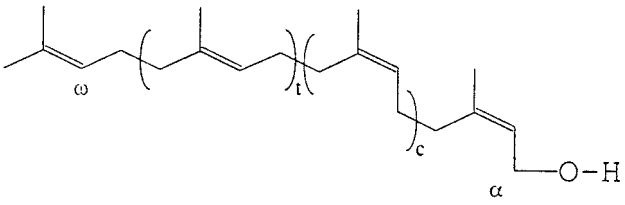

Fig. 1. The general formula of polyprenols; ω – ω-terminal isoprene residue, t – number of internal trans-isoprene residues, c – number of internal *cis*-isoprene residues, α – α-terminal *cis*-isoprene residue

isoprene units and all other residues including α-terminal unit in the *cis*-configuration. The structure of these polyprenols with general formula $\omega t_3 c_n OH$, is presented in Fig. 1, where ω is an isoprene residue farthest from the hydroxyl group, t is a *trans*-isoprene residue, c is a *cis*-isoprene residue and OH is the hydroxyl group and the number of isoprene units n varies usually from 6 to 30–40, depending on the plant species (Chojnacki et al. 1987; Zinkel and Evans 1972).

Large amounts of polyprenyl acetates were found in leaves of various species of genus: *Malus*, *Prunus* and *Pyrus* belonging to *Rosaceae* family. In each species poly-*cis*-prenols composed of 19 and 20 isoprene units were predominating (Świeżewska and Chojnacki 1996). The longest polyprenol molecules composed of 25–35 isoprene units were isolated from leaves of *Potentilla* and *Rosa* genus (Świeżewska et al. 1992). Polyprenols of chain length of 20–60 isoprene units or longer were found in leaves of plants belonging to *Combretaceae* family. The polyprenols of this type were found in half of the 20 species studied (Kulcitsky et al. 1996). Long-chain poly-*cis*-prenols composed of 15–100 isoprene units were found in leaves of *Lumnitzera racemosa* belonging to *Combretaceae* family, grown in the mangrove type forest. These long-chain polyprenols can be considered as a group of intermediate compounds between prenyl lipids and polyisoprene rubber polymer (Skoczylas et al. 1994). Multiple polyprenol families were found also in gymnosperms. The complex character of polyprenol spectrum was demonstrated for various representatives of gymnosperms (Ibata et al. 1984). A group of long chain polyprenols were found and documented in large number of *Cycadopsida* and *Coniferopsida* (Świeżewska and Chojnacki 1988). The information on the occurrence and multiplicity of polyprenol families in plants might be useful as a chemotaxonomic marker in the taxonomy of plants.

Polyprenyl phosphates function mainly as carriers of glycosyl units across membranes in the prokaryotic peptidoglycan and lipopolysaccharide biosynthesis (Bugg and Brandish 1994). Similar role is played by dihydropolyprenyl phosphates (dolichylphosphates) in glycoprotein synthesis (Bugg and Brandish 1994; Chojnacki and Dallner 1988).

Some of polyprenols are attached to proteins during the prenylation (Grünler et al. 1994; Shipton et al. 1994; Zhang and Casey 1996; Casey and Seabra 1996). Prenylation is a type of posttranslational modification of proteins *via* covalent linking of isoprenoid groups. Prenylated proteins play crucial roles in vital cellular processes. The prenylation serves as a hydrophobic membrane anchor and the function of prenylated proteins is connected with the control of cellular functions such as protein maturation and cell growth, signal transduction and intracellular trafficking pathways (Casey and Seabra 1996). About 2% of total cellular proteins are prenylated, i.e. approximately 60–80 different proteins per cell (Epstein et al. 1991). Known prenylated proteins include fungal mating factors, nuclear lamins, Ras and Ras-related GTP-binding proteins (G-proteins), the subunits of trimeric G proteins, protein kinases, and at least one viral protein (Zhang and Casey 1996). Isoprenylation is not restricted to proteins, but also has been described to occur in case of certain species of tRNA and heme (Grünler et al. 1994).

The lipid bilayers modified by long-chain polyprenols and their phosphoryl derivatives exhibit different properties from phospholipid bilayers (Mc Closkey and Troy 1980; Walińska et al. 1997; Janas et al. 2000a; Janas et al. 2000b). Polyprenols increase the membrane elasticity and modulate the surface curvature of the membranes by the formation of fluid microdomains (Walińska et al. 1997; Janas et al. 2000b). Contrary to the behavior of polyprenols, polyprenyl phosphates decrease the membrane specific conductance, increase activation energy of ion migration, breakdown voltage and membrane thickness (Janas et al. 2000a).

The terpenoid theory of the origin of the primitive membranes proposed that di-polyprenyl phosphates and other simple terpenoids might form spontaneously on the surface of a clay or other minerals and self-organize into vesicles (Ourisson and Nakatani 1994). These vesicles may evolve into progressively more complex units, similar to protocells. A vesicle is not a cell, but the formation of a vesicle is by itself a far reaching event (Morovitz 1992). Terpenoids (archeal lipids, hopanoids, carotenoids, sterols, dolichols, polyprenols, polyprenyl phosphates, etc.) are involved in the formation or reinforcement of all known biological membranes (Nakatani et al. 1993).

The previous data shown that there exists species-specific pattern of polyprenol mixture and that it can serve as a chemotaxonomic marker for a given species, and to some extent for a larger systematic groups (genera and families) (Chojnacki et al. 1987; Świeżewska and Chojnacki 1988; Świeżewska et al. 1992; Jankowski et al. 1994; Świeżewska et al. 1994).

The individual variations within a given group that have been occasionaly noticed have never been published. In the present paper the observations of such variations are given. We investigated the occurence of long-chain polyprenols in plants of *Lauraceae, Tiliaceae* and *Magnoliaceae* families. Separation and examination of groups of polyprenols might be useful as chemotaxonomic markers.

Materials and methods

Plant material

Specimens of leaves of species of *Lauraceae* family except *Laurus azorica* and *Laurus nobilis* were from the Kunming Institute of Botany, Chinese Academy of Sciences (September 2000). *Laurus azorica, Laurus nobilis* and specimens of species of *Magnoliaceae* family were from the Powsin Botanical Garden of the Polish Academy of Sciences (September 2000). Specimens of species of *Tiliaceae* family were from the Botanical Garden of Stockholm University and from the Institute of Dendrology of the Polish Academy of Sciences in Kórnik (November 2000).

Isolation and identification of polyprenols from plant tissuses

Dried leaves (200 mg) were homogenized in an Ultra-Turrax T25 with 4 ml acetone:hexane (1:1, v/v) mixture (at top speed for 1 min) and the extract was subjec-ted to alkaline hydrolysis (Wellburn et al. 1967). Analytical separation of polyprenols was performed by TLC on Silica gel plates in ethyl acetate : toluene (5:95, v/v) and on RP-18 plates in acetone. Spots of lipids were detected with iodine vapors and compared with standard compounds.

The unsaponifiable lipids were chromatographed on a Silica gel 60 (Merck) column and eluted with hexane, containing increasing concentration of diethyl ether (0–18%). The course of elution was monitored by TLC and HPLC.

All organic solvents used for extraction and chromatography were from Merck (Darmstadt, Germany). Silica gel TLC plates and RP-18-plates with concentrating zone and Silica gel for column chromatography were also from this source.

Isolation and identification of individual prenologues from polyprenol mixture

Isolation of individual prenologues from polyprenol mixture was performed by column chromatography on hydroxyalkoxypropyl-Sephadex (Packard-Becker) and equilibrated with acetone:water (85:15, v/v). The column was eluted with acetone containg decreasing concentration of water (15%–0%) (Chojnacki et al. 1975). The course of elution was monitored by TLC and HPLC.

HPLC was performed by the method of Eggens *et al.* (Eggens et al. 1983). HPLC of polyprenols mixtures and individual prenologues was performed on Hypersil-ODS column, 4.6 × 60 mm, corn size 3 μm (Knauer). 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 flow rate of 1,5 ml/min as described earlier (Świeżewska et al. 1992).

Semiquntitative estimation of polyprenol content

Semiquntitative determination of polyprenols was performed by visual comparing the size and intensity of spot observed on adsorption chromatography with that of a known amount of standard substance (Wellburn and Hemming 1966).

Measurements

^{1}H-NMR and ^{13}C-NMR spectra were recorded with GX-500 JEOL apparatus at 500 MHz and 125 MHz, respectively. Measurements were made in $CDCl_3$, with TMS (tetramethylsilane) as an internal standard, at room temperature.

Results

The occurrence of long-chain polyprenols in angiosperm plants described in this paper was evidenced in several species of *Lauraceae, Tiliaceae* and *Magnoliaceae* families. As presented in Fig. 2a and 2b, polyprenols predominated in the lipid fraction of leaves in all studied species of *Lauraceae* family.

All studied plant species contained a typical polyprenol mixture found as a single spot upon silica gel TLC (Fig. 2a). The prenologues composed of 11, 12 isoprene units were the dominating ones in this family (Fig. 2b).

The results of qualitative evalution of chain length of polyprenols and semiquantitative estimation of polyprenol content in leaves of species belonging to *Lauraceae* family are shown in Table 1. In nine of the studied plants, prenologues of longer chain lenght, composed of 10–12 isoprene units could be detected, with dominating prenol-11. Only in one plant species (*Lindera angustifolia*) polyprenols composed of 10–14 isoprene units were present. In four plant species polyprenols composed of 11–13 of isoprene units were detectable, with dominating prenol-12 and in three studied plants poly-prenols composed of 11–13 isoprene units were present, with dominating prenol-11. Among the 16 species studied, *Machilus longipedicellata* (No.1) had the highest polyprenol content (3.0–4.0%, dry wt.). These results confirm the

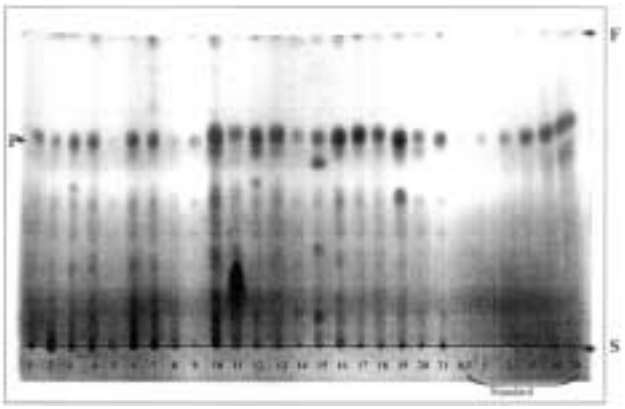

Fig. 2a. Chromatography (TLC on Silica gel plates) of polyprenols extracted from leaves of various species of *Lauraceae* family. Unsaponifiable (after alkaline hydrolysis) fraction of total lipid extract. Solvent: ethyl acetate : toluene, 5:95, v/v. Numbers 10–21 refer to the species of *Lauraceae* listed in Table 1. Standard: polyprenols extracted from *Tilia henryana* (Pr-10, -11, -12). P – polyprenols, S – start, F – front

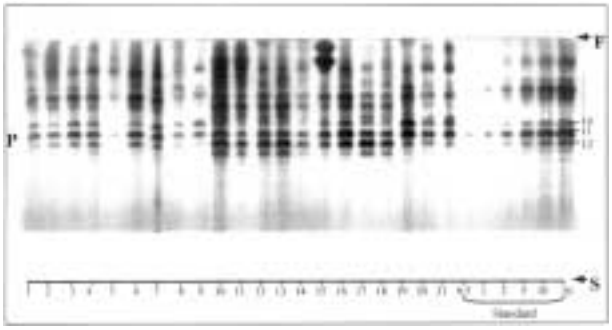

Fig. 2b. Chromatography (TLC on RP-18 plate) of polyprenols extracted from leaves of various species of *Lauraceae* family. Unsaponifiable (after alkaline hydrolysis) fraction of total lipid extract. Solvent: acetone. Numbers 1–21 refer to the species of *Lauraceae* listed in Table 1. Standard: polyprenols extracted from *Tilia henryana* (Pr-10, -11, -12)

existence of a characteristic pattern of chain length of polyprenols in leaves of *Lauraceae* in which the polyprenols composed of 11 and 12 isoprene units were dominating.

The composition of the polyprenol fractions is illustrated more clearly on HPLC records (Fig. 3a, b). The HPLC record of polyprenol mixture from *Neolitsea chuii* is presented in Fig. 3a. One group of polyprenols can be observed with polyprenols composed of 10–13, with dominating prenol-11. Figure 3b shows the HPLC spectrum of polyprenol mixture from *Phoebe forrestii*. This spectrum confirms results obtained in the analytical separation of polyprenols by TLC. One polyprenol fraction composed of 10–13 isoprene units, with dominating prenol-12 is visible.

The next studied species belonged to *Tiliceae* family. As presented in Fig. 4a and 4b, polyprenols composed of 9–12 isoprene units predominated in the

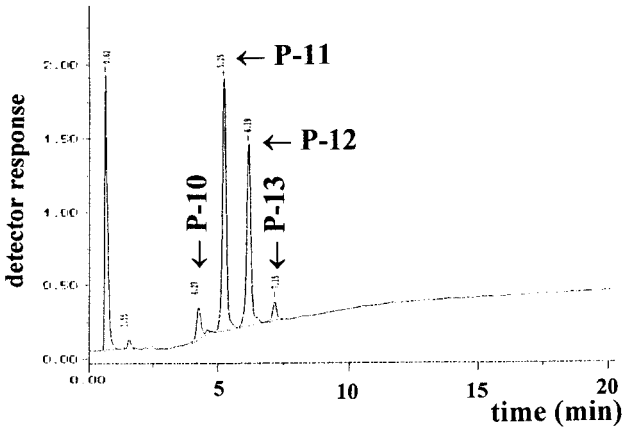

Fig. 3a. Chromatography HPLC of the natural polyprenol mixture of *Neolitsea chuii*. Peaks of polyprenols detected with the UV detector set at 210 nm. The numbers 10–13 mark the position of elution of prenol-10,-11, etc. The position of elution of the studied prenologues was mapped using standard of the mixture of polyprenols composed of 9, 11, ..., 23, 25 isoprene units

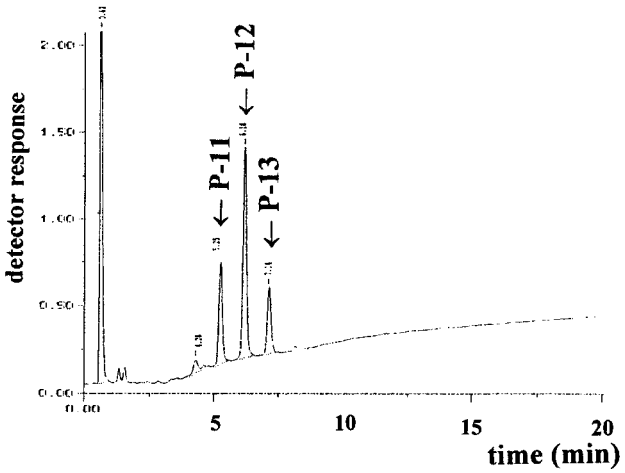

Fig. 3b. Chromatography HPLC of the natural polyprenol mixture of *Phoebe forrestii*. Peaks of polyprenols detected with the UV detector set at 210 nm. The numbers 10–13 mark the position of elution of prenol-10,-11, etc. The position of elution of the studied prenologues was mapped using standard of the mixture of polyprenols composed of 9, 11, ..., 23, 25 isoprene units

lipid fraction of leaves in all the studied species of the this family. This polyprenol mixture moved as a single spot in TLC.

The results of qualitative evalution of chain lenght of polyprenols and semiquantitative estimation of polyprenol content in leaves of species belonging to *Tiliceae* family are shown in Table 2. In six plant species polyprenols composed of 9–11 isoprene units were detectable, with dominating prenol-10. Four studied species contained polyprenol mixture, without dominating prenol. In the case of species *Tilia x vulgaris* and *Tilia henryana* polyprenols composed of 10–11 of isoprene units were present. The polyprenol

Table 1. Polyprenols in leaves of various species of *Lauraceae* family

Species of Lauraceae family	Content (% dry wt.)	Number of isoprene units (with dominating polyprenol)
1. *Cinnamomum bodinieri*	0.3–0.4	10, 11, 12
2. *Cinnamomum burmannii*	0.2–0.3	10, 11, 12
3. *Cinnamomum glanduliferum*	0.6–0.7	10, 11, 12
4. *Lindera angustifolia*	0.6–0.7	10, 11, 12, 13, 14
5. *Lindera communis*	0.06–0.1	10, 11, 12
6. *Lindera megaphylla*	1.0–2.0	10, 11, 12
7. *Lindera thomsonii* (No. 1)	0.6–0.7	10, 11, 12
8. *Lindera thomsonii* (No. 2)	0.06–0.1	10, 11, 12
9. *Lindera auriculata*	0.1–0.2	10, 11, 12
10. *Machilus longipedicellata* (No. 1)	3.0–4.0	11, 12, 13
11. *Machilus longipedicellata* (No. 2)	0.5–1.0	11, 12, 13
12. *Machilus yunnanesis var. dichouxii* (No. 1)	1.0–2.0	11, 12, 13
13. *Machilus yunnanesis var. dichouxii* (No. 2)	1.0–2.0	11, 12, 13
14. *Neocinnamomum delavayi*	0.2–0.3	11, 12, 13
15. *Neolitsea chuii* (No. 1)	0.5–1.0	10, 11, 12
16. *Neolitsea chuii* (No. 2)	2.0–3.0	10, 11, 12
17. *Phoebe forrestii* (No. 1)	2.0–3.0	11, 12, 13
18. *Phoebe forrestii* (No. 2)	1.0–2.0	11, 12, 13
19. *Sassafras tzumu*	2.0–3.0	10, 11, 12, 13
20. *Laurus azorica*	0.5–1.0	10, 11, 12, 13
21. *Laurus nobilis*	0.6–1.0	10, 11, 12, 13

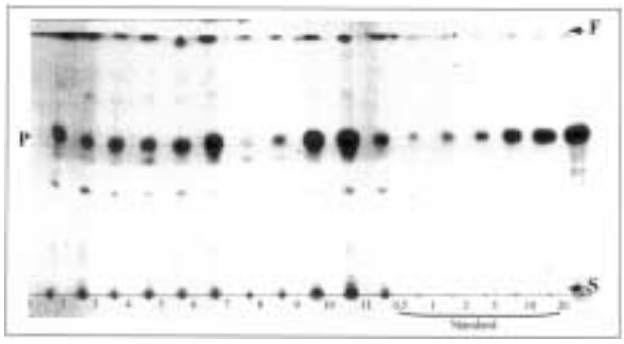

Fig. 4a. Chromatography (TLC on Silica gel plates) of polyprenols extracted from leaves of various species of *Tiliaceae* family. Unsaponifiable (after alkaline hydrolysis) fraction of total lipid extract: solvent: ethyl acetate : toluen, 5:95, v/v. Numbers 1–11 refer to the species of *Tiliaceae* listed in Table 2. Standard: polyprenols extracted from Tilia henryana (Pr-10, -11, -12). P – polyprenols, S – start, F – front

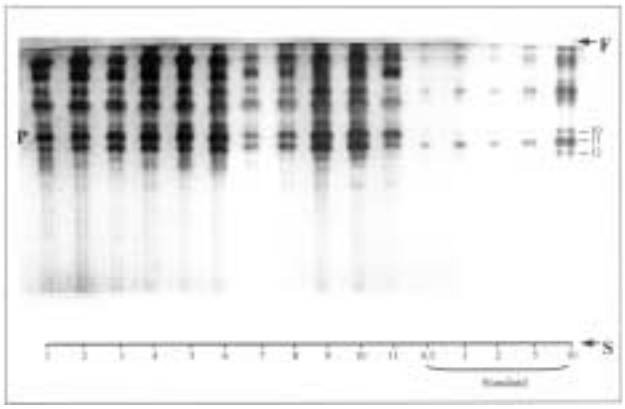

Fig. 4b. Chromatography (TLC on RP-18 plate) of polyprenols extracted from leaves of various species of *Tiliaceae* family. Unsaponifiable (after alkaline hydrolysis) fraction of total lipid extract. Solvent: acetone. Numbers 1–11 refer to the species of *Tiliaceae* listed in Table 2. Standard: polyprenols extracted from Tilia henryana (Pr-10, -11, -12). P – polyprenols, S – start, F – front

mixture composed of 9–12 of isoprene units was found in the case of species *Tilia insularis* and *Tilia tomentosa*. Among the 9 species studied, *Tilia tomentosa* had the highest polyprenol content (2.0–3.0% dry wt.). These results were confirmed by HPLC (Fig. 5). The HPLC record obtained with the very sensitive UV-detector visualized the dominating prenol-11 in the polyprenol mixture from *Tilia henryana*. It was not illustrated so clearly on thin-layer chromatography.

The total polyprenol content in leaves of plants belonging to these two studied families reached values as high as about 3% of their dry weight. In the case of the *Combretaceae* family the amount of long-chain polyprenols varied with the plant species and the richest source was *Combretum molle* (about 4% of dry mass of leaves) (Kulcitsky et al. 1996). Large

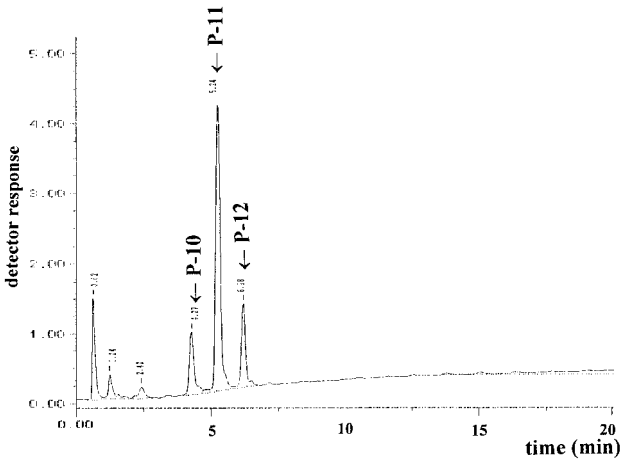

Fig. 5. Chromatography HPLC of the natural polyprenol mixture of *Tilia henryana*. Peaks of polyprenols detected with the UV detector set at 210 nm. The numbers 10–12 mark the position of elution of prenol-10,-11, etc. The position of elution of the studied prenologues was mapped using standard of the mixture of polyprenols composed of 9, 11, ..., 23, 25 isoprene units

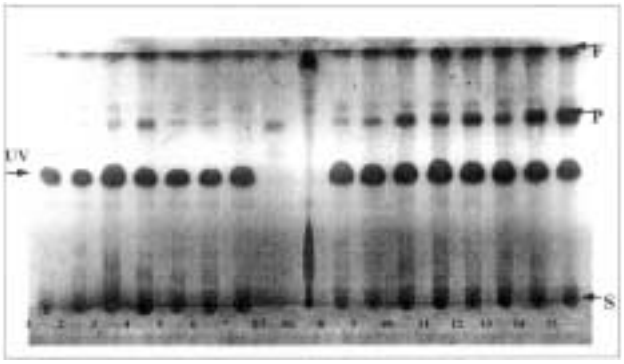

Fig. 6. Chromatography (TLC on Silica gel plates) of polyprenols extracted from leaves of *Magnolia*. Unsaponifiable (after alkaline hydrolysis) fraction of total lipid extract: solvent: ethyl acetate : toluene, 5:95, v/v. Numbers 1–15 refer to *Magnolia* species listed in Table 3. Standards: ST – standard of polyprenols extracted from *Tilia henryana* (Pr-10, -11, -12), SG – Standard of polyprenyl (Pr-15...–19) acetates extracted from *Ginkgo biloba*. P – poyprenols, S – start, F – front, UV- unidentified UV absorbing spot

amounts of polyprenyl acetates (0.5 – 5%) were found in leaves of various species of fruit-trees belonging to *Rosaceae* family (Świeżewska and Chojnacki 1996).

In *Magnolia tripetala* polyprenols composed of 10–12 (Fig. 6) isoprene units were present. As shown by TLC on Silica gel plates polyprenols were located at R_f = 0.7, above a massive spot of UV absorbing substance (R_f = 0.6). The leaves were either from shadowed or from insolated place. The collection was made throughout the season. The seasonal differences of the polyprenol content in leaves of *Magnolia* species is presented in Table 3. The increase of the content of polyprenols in *Magnolia* with the maturation of leaves was observed. Higher amounts of polyprenols were accumulated in leaves collected in insolated place. Leaves of *Magnolia* collected on 26 of September 2000 had the highest polyprenol content (0.2–0.3%, dry wt.).

The structure of these polyprenols were examined by ^{1}H-NMR and ^{13}C- NMR spectrometry. The examination of the polyprenol-10 extracted from leaves of *Tilia henryana* revealed the characteristic domination in its structure of *cis*-isoprene units over the *trans*-residues, and the presence of other characteristic features previously reported for fully unsaturated polyprenols from leaves of a number of angiosperm and gymnosperm plants (Chojnacki et al. 1987; Fenney and Hemming 1967; Tanaka and Takagi 1979).

By comparison of well-known polyprenol spectra, *e.g.* especially chemical shifts of methyl protons and carbon-13 atoms signals it seems possible to assign *cis/trans* isometric structure of studied polyprenol-10. Methyl protons in polyprenol are expected to show ^{1}H-NMR signals reflecting triad sequences of dimethylallyl, *cis* and *trans* isoprene units as well as *cis*

Table 2. Polyprenols in leaves of various species of *Tiliaceae* family

Species of *Tiliaceae* family	Content (% dry wt.)	Number of isoprene units (with dominating polyprenol)
1. *Tilia* × *euchlora*	0.3–0.4	9, 10, 11
2. *Tilia europea (T. platyphyllos + T. cordata)*	0.3–0.4	9, 10, 11
3. *Tilia heterophylla*	0.5–1.0	9, 10, 11
4. *Tilia platyphyllos* (No. 1)	0.3–0.4	9, 10, 11
5. *Tilia platyphyllos* (No. 2)	0.5–1.0	9, 10, 11
6. *Tilia cordata*	0.8–1.0	9, 10, 11
7. *Tilia* × *vulgaris*	0.03–0.1	10, 11
8. *Tilia henryana*	0.2–0.3	10, 11
9. *Tilia insularis*	1.0–2.0	9, 10, 11, 12
10. *Tilia tomentosa*	2.0–3.0	9, 10, 11, 12
11. *Tilia tuan*	0.3–0.4	11, 12

Table 3. The seasonal differences of the polyprenol content in leaves of *Magnolia tripetala*

The number of sample	The place of plant collection	Dates of collecting leaves	Total content (% dry wt.)
1.	Shaded place	04.07.2000	0.0
2.		18.07.2000	<0.002
3.		02.08.2000	<0.01
4.		16.08.2000	0.05-0.1
5.		29.08.2000	<0.01
6.		12.09.2000	<0.01
7.		26.09.2000	0.0
8.	Insolated place	20.06.2000	<0.01
9.		05.07.2000	0.05–0.1
10.		18.07.2000	<0.1
11.		02.08.2000	<0.1
12.		16.08.2000	<0.1
13.		29.08.2000	0.08–0.1
14.		12.09.2000	0.1–0.2
15.		26.09.2000	0.2–0.3

isoprene unit that links to the hydroxyl group. The splitting of these signals according to the triad sequences allows to differentiate the structure of examined polyprenol. One can see from the ^{1}H-NMR and ^{13}C-NMR spectra that polyprenol-10 extracted from *Tilia henryana* contained three *trans* and five *cis* isoprene units.

The relative intensities of signals in prenol-10 is good agreement with the theoretical ones for this type of structure. This polyprenol belongs to the group of medium-chain-length prenologues (composed from 9 to 14 isoprene units).

As presented in Fig. 7 and 7a plant polyprenol-10 exibited theoretical proportion of *cis*- and *trans*-isoprene residues: *cis* (α) : *cis* : *trans* (ω) : *trans*; 1 : 6 : 1 : 3

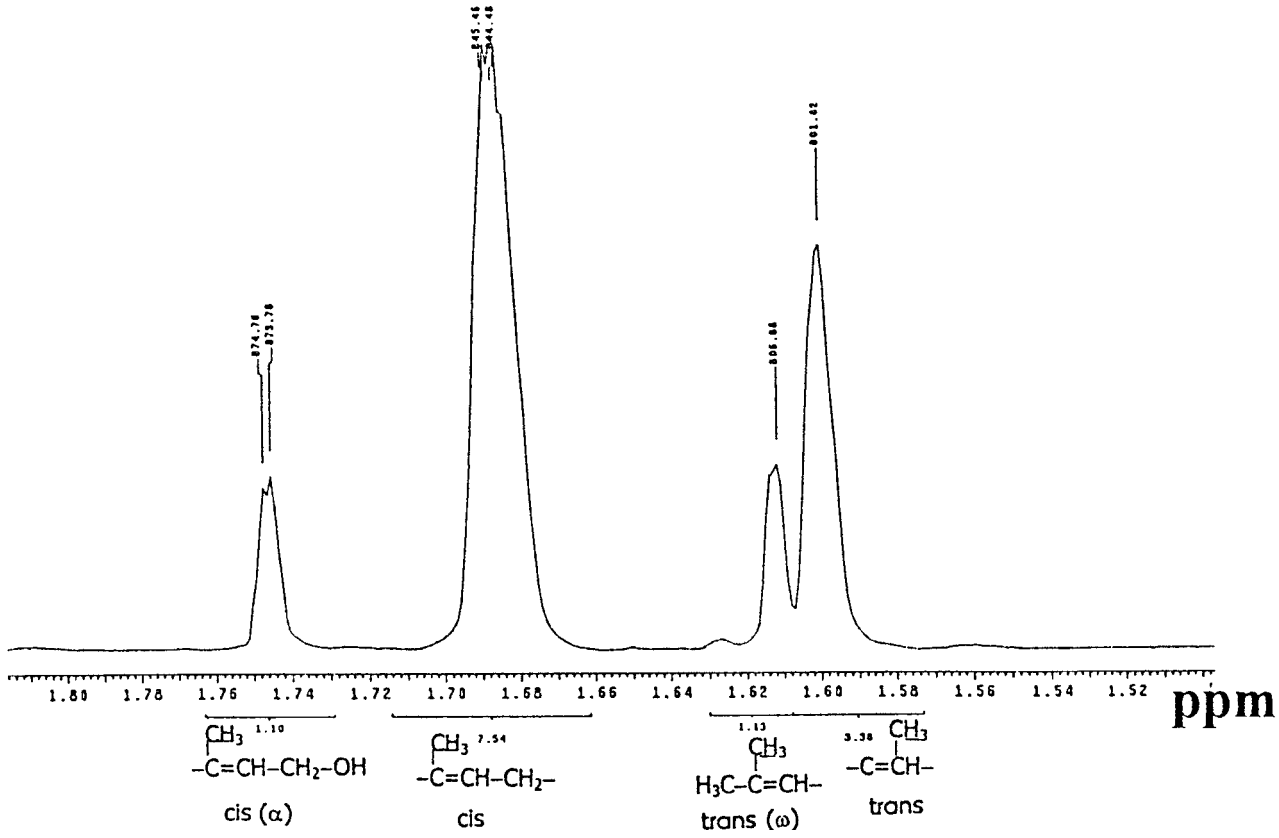

Fig. 7. ^{1}H-NMR records of polyprenol-10 extracted from *Tilia henryana* using GX-500 JEOL apparatus

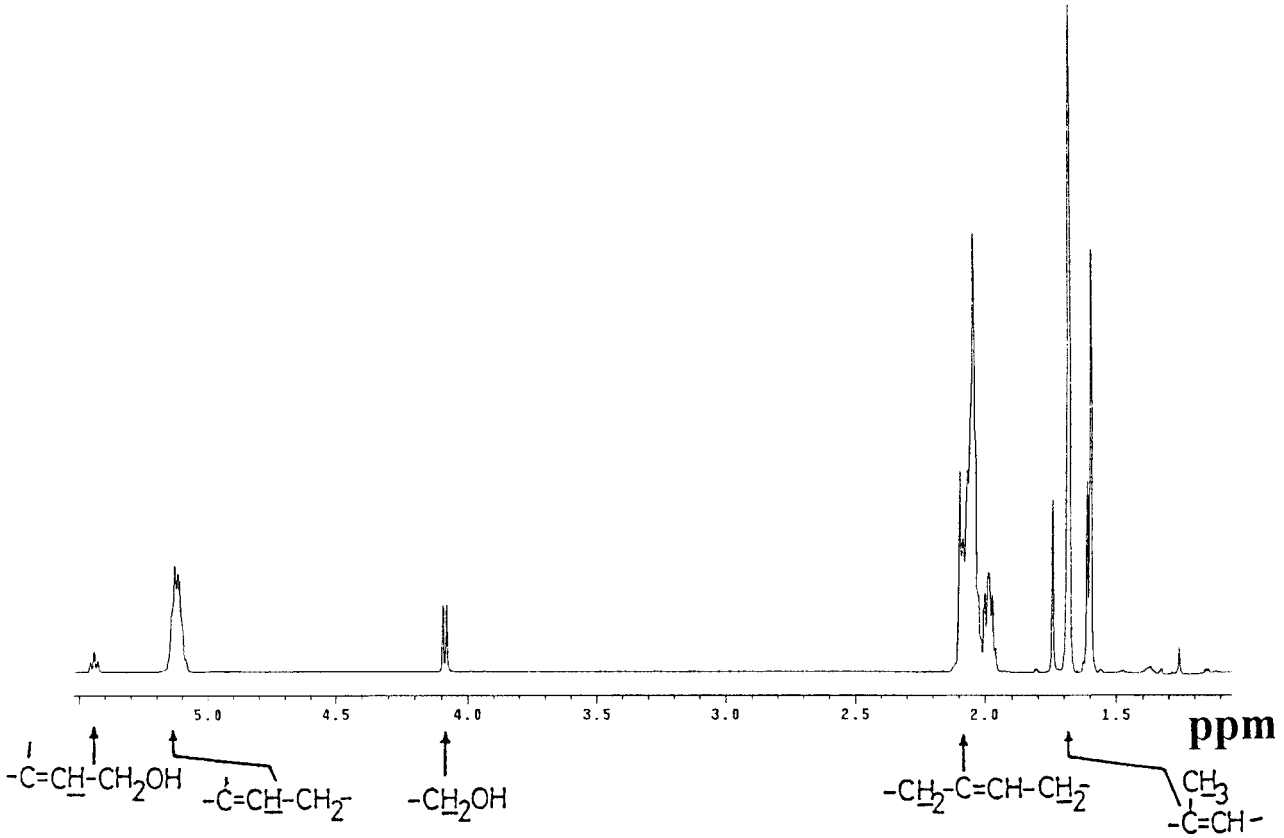

Fig. 7a. The part of ^{1}H- NMR spectrum (Fig. 6) close to 1.5 ppm of polyprenol-10 extracted from *Tilia henryana* using GX-500 JEOL apparatus

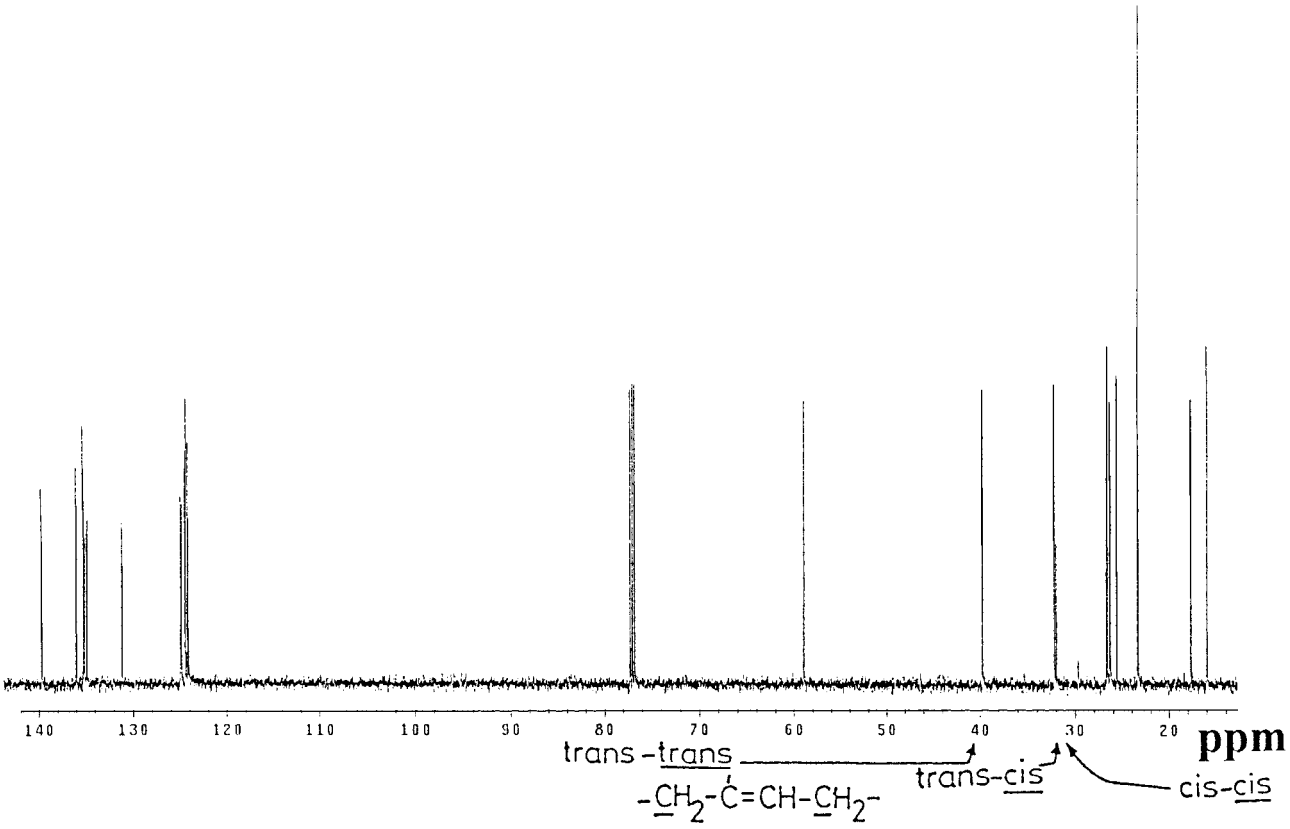

Fig. 8. ^{13}C-NMR records of polyprenol-10 extracted from *Tilia henryana* using GX-500 JEOL apparatus

and as presented in Fig. 8, theoretical proportion of *trans-trans* : *cis-cis* : *trans-cis*; 3 : 5 : 1.

Discussion

In our present studies it was shown that several species of *Lauraceae* and *Tiliaceae* family are rich sources of long-chain polyprenols. The polyprenols in few species of *Lauraceae* family have been characterized before (Chojnacki et al. 1987; Świeżewska et al. 1994) and the typical pattern of their polyprenol mixture was almost the same, i.e. it was composed from prenols 11–12. In the present studies we had the opportunity to screen larger group of unique *Lauraceae* species of the Kunming living collection. Some of them have never been studied before. The new original finding was that in individuals of the same species the content of polyprenols was different, though its qualitative pattern was the same.

The results of studying large groups of various species of a given systematic family confirmed the validity of polyprenol spectrum as a chemotaxonomic marker. However our observation concerning *Magnolia tripetala* that plants of the same species accumulate in leaves different quantities of polyprenols depending on environmental conditions should draw the attention also on the physiological factors which may determine the rate of formation of polyprenols in leaves. This finding has not been taken into account before (Chojnacki et al. 1987) and its exploration may be helpful in elucidating the phenomenon of accumulation of polyisoprenoids in the process of aging.

On observing the polyprenol content in two individuals of *Magnolia tripetala* we were able to suggest the reason of the dramatic differences of the polyprenols content which was most probably due to the exposure of the polyprenol-rich individual to the sun light. The finding that in *Magnolia tripetala* the dominating polyprenol was composed from 11 isoprene units was in accord with the earlier results of our studies on *Magnoliaceae* (Świeżewska et al. 1994).

Also large quantitative differences were noticed among closely related members of genus *Tilia*. Few members of *Tiliaceae* have been studied by us before. These observations have shown that only the qualitative aspect of the presence of polyprenol mixture in leaves of plants (mainly prenol-10 and -11) may be considered as a chemotaxonomic marker and that negative result in screening for polyprenols may not be relevant. The observations of the variations of polyprenol content while keeping its qualitative pattern may limit only to some extent its validity.

The proton NMR spectrum recorded on a 500 MHz instrument of a pure individual prenol-10 isolated from leaves of *Tilia henryana* may serve as the most recent document of its identity as having been taken of a sample thoroughly purified using the newest isolation techniques. This record documents the new features of the molecule in that a complex character of what was previously known as single peaks was revealed and that could not have been observed on using older instruments. This discrete character ot this record awaits further more detailed interpretation.

In conclusion in most of studied plant species the polyprenol families exhibited a distinct character and we suggest that the observed polyprenol pattern may serve as chemotaxonomic marker for systematic families in botanic taxonomy.

These substances are analoguous and/or substituents of dolichols in the formation of glycoproteins and bacterial polysaccharides (Chojnacki et al. 1987; Chojnacki and Świeżewska 1995; Bugg and Brandish 1994; Chojnacki and Dallner 1988).

Acknowledgements. The authors would like to thank the Kunming Institute of Botany, Chinese Academy of Sciences for the cooperation, mgr A. Marczewski from the Botanical Garden-Center of Biological Diversity, Polish Academy of Sciences, Warszawa, for collecting samples of leaves of *Magnolia tripetala* and fruitful suggestions and Dr J. Wójcik from the Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warszawa, for help in NMR spectroscopy.

We dedicate this paper to Professor Bolesław Suszka to appreciate 50 years of his successful scientific activity.

References

Bach T.J. 1995. Some New Aspects of Isoprenoid Biosynthesis in Plants – A Review. Lipids 30: 191–202.

Bugg T.D.H, Brandish P.E. 1994. From peptidoglycan to glycoproteins: Common features of lipid-linked oligosaccharide biosynthesis. FEMS Microbiol. Lett. 119: 255–262.

Casey P.J., Seabra M.C. 1996. Protein Prenyltransferases. J. Biol. Chem. 271: 5289–5292.

Chojnacki T. and Dallner G. 1988. The biological role of dolichol. Biochem. J. 251: 1–9.

Chojnacki T., Jankowski W., Mańkowski T., Sasak T. 1975. Preparative separation of naturally occurring mixtures of polyprenols on hydroxyalkoxypropyl-Sephadex. Anal. Biochem. 69: 114–119.

Chojnacki T., Świeżewska E. 1995. Fully unsaturated mainly-*cis* poly-prenols. in: Dolichols Polyprenols and Derivatives, Collection of Polyprenols, 1st edition, Warszawa, pp. 17–23.

Chojnacki T., Świeżewska E., Vogtman T. 1987. Polyprenols from Plants – Structural Analogues of Mammalian Dolichols. Chemica Scripta 27: 209–214.

Eggens I., Chojnacki T., Kenne L., Dallner G. 1983. Separation, quantitation and distibution of dolichol and dolichyl phosphate in rat and human tissuses. Biochim. Biophys. Acta 751: 355–369.

Epstein W.W., Lever D., Leining L.M., Bruenger E., Rilling H.C. 1991. Quantitation of prenylcysteines by a selective cleavage reaction. Proc. Natl. Acad. Sci. USA 88: 9668–9670.

Fenney I., Hemming F.W. 1967. Nuclear magnetic resonance spectrometry of naturally occurring polyprenols. Anal. Biochem. 20: 1–15.

Grünler J., Ericsson J., Dallner G. 1994. Branch-point reactions in the biosynthesis of cholesterol, dolichol, ubiquinone and prenylated proteins. Biochim. Biophys. Acta 1212: 259–277.

Hemming F. W. 1983. Biosynthesis of Dolichols and Related Compounds. in: Biosynthesis of Isoprenoid Compounds. Porter J.W., Spurgeon S.L. (eds.), John Wiley, New York, pp. 305–354.

Ibata K., Kageyu A., Takigawa T., Mizuno M., Tanaka Y. 1984. Polyprenols from conifers: multiplicity in chain length distribution. Phytochemistry 27: 2517–2521.

Janas T., Janas T., Walińska K. 2000 A. The effect of hexadecaprenyl diposphate on phospholipid membranes. Biochim. Biophys. Acta 1464: 273–283.

Janas T., Walińska K., Chojnacki T., Świeżewska E., Janas T. 2000 B. Modulation of properties of phospholipid membranes by the long-chain polyprenol (C_{160}). Chem. and Phys. of Lipids 106: 31–40.

Jankowski W.J., Świeżewska E., Sasak W., Chojnacki T. 1994. Occurrence of Polyprenols and Dolichols in Plants. J. Plant Physiol. 143: 448–452.

Kulcitsky V., Hertel J., Skoczylas E., Świeżewska E., Chojnacki T. 1996. The occurrence of long-chain polyprenols in leaves of plants of *Combretaceae* family. Acta Biochim. Polon. 43: 707–712.

Mc Closkey M.A., Troy F.A. 1980. Paramagnetic isoprenoid carrier lipids. 2. Dispersion and dynamics in lipid membranes. Biochemistry 19: 2061–2066.

Morovitz H.J.1992. in: Beginnings of Cellular Life. Yale University Press, New Haven.

Nakatani Y., Plobeck N., Eifler S., Brisson A., Ourisson G. 1993. Polyprenyl phosphates: Do they form vesicles, like natural phospholipids ? Phosphorus, Sulfur, and Silicon 77: 125–128.

Ourisson G., Nakatani Y. 1994. The terpenoid theory of the orgin of cellular life: the evolution of terpenoids to cholesterol. Chemistry & Biology 1: 11–23.

Shipton C.A., Parmryd I., Świeżewska E., Andersson B., Dallner G. 1994. Isoprenylation of Plant Proteins in *vivo*. J. Biol. Chem. 270: 566–572.

Skoczylas E., Świeżewska E., Chojnacki T., Tanaka Y. 1994. Long-chain rubber-like polyisoprenoid alcohols in leaves of *Lumnitzera racemosa*. Plant Physiol. Biochem. 32: 825–829.

Świeżewska E., Chojnacki T. 1988. Long-chain polyprenols in gymnosperm plants. Acta Biochim. Polon. 35: 131–145.

Świeżewska E., Chojnacki T. 1996. Polyprenols in leaves of fruit-trees of *Rosaceae* family. Acta Biochim. Polon. 43: 701–706.

Świeżewska E., Chojnacki T., Jankowski W.J., Singh A.K., Olsson J. 1992. The occurrence of long-chain polyprenols in leaves of *Rosaceae* family and their isolation by time-extended liquid chromatography. Biochem. Cell Biol. 70: 448–454.

Świeżewska E., Sasak W., Mańkowski T., Jankowski W., Vogtman T., Krajewska I., Hertel J., Skoczylas E., Chojnacki T. 1994. The search for plant polyprenols. Acta Biochim. Polon. 41: 221–260.

Tanaka Y. 1989. Structure and biosynthesis mechanism of natural polyisoprene. Prog. Polym. Sci. 14: 339–371.

Tanaka Y., Takagi M. 1979. Structural characterization of ficaprenol-11 by ^{13}C Nuclear Magnetic Resonance. Biochem. J. 183: 163–165.

Walińska K., Janas Teresa, Chojnacki T., Świeżewska E., Janas Tadeusz 1997. Properties of lipid bilayers modified by long-chain polyprenols. Cell. Mol. Biol. Lett. 2: 89–100.

Wellburn A.R., Hemming F.W. 1966. The occurrence and seasonal distribution of higher isoprenoid alcohols in the plant kingdom. Phytochemistry 5: 969–975.

Wellburn A.R., Stevenson J., Hemming F.W., Morton R.A. 1967. The Characterization and Properties of Castaprenol-11, -12 and -13 from the Leaves of Aesculus hippocastanum (Horse Chestnut). Biochem. J. 102: 313–324.

Zhang F.L., Casey P.J. 1996. Protein prenylation: Molecular mechanisms and functional consequences. Annu. Rev. Biochem. 65: 241–269.

Zinkel D., Evans B.B. 1972. Terpenoids of *Pinus strobus* cortex tissues. Phytochemistry 102: 3387–3389.