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Minireview

The search for plant polyprenols*

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This review on plant polyprenols illustrates that part of the research carried on in the Department of Lipid Biochemistry of the Institute of Biochemistry and Biophysics in Warsaw (Poland) which grew up on the boundary of botany as a side-line of our main researches, i.e., the studies on biosynthesis and biological role of lipid-linked sugars. These studies were initiated in late 60-ies by the works of M.J. Osborn, P.W. Robbins and J. Strominger in the U.S.A. [1–3]. At that time they had a strong scientific and methodological background in the fundamental studies of the scientists working in Liverpool (England) where the long-chain polyprenols had been discovered [4]. We decided to join this line of research already in late sixties. From the very beginning our research on bacterial lipid-linked sugars was ac-

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companied by studies concerning the content and the structure of polyisoprenoid alcohols in several tropical plants. Later we began to study animal dolichol-dependent transglycosylations simultaneously extending our research to several groups of plants of moderate and cold climate.

The studies on biosynthesis and biological role of lipid-linked sugars were carried out at the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences, simultaneously with the search for rich plant sources of poly-*cis*-prenols, by the same colleagues who had biochemical training and had to become acquainted also with phytochemistry and botany. A number of plants grown in natural and seminatural conditions were selected as potential sources of polyprenol molecules of a desired size. The "Collection of Polyprenols", was established originally as a sort of hobby. Later on our "Collection of Polyprenols", which originated from our research and from two "plant oriented" doctoral dissertations (W. Sasak [5] and E. Świeżewska [6]), has become known due to the fact that we were always ready to supply many biochemists and coworkers in other disciplines with our unique compounds, especially after publication of our first catalogue (Dolichols, Polyprenols and Derivatives, Warszawa, February 1984). It should be added that from the point of view of botanical systematics, the data on occurrence of specific polyprenols in plant leaves may serve as a valuable taxonomic tool.

The aim of the present paper is to present and review the result of the search for long-chain polyprenols performed on a large number of plants over the years 1968–1993 in the Department of Lipid Biochemistry of the Institute of Biochemistry and Biophysics in Warsaw. That part of research concerning plant polyprenols is dedicated to Professor Frank Hemming whose fathership to polyprenols and friendliness to our research group are greatly appreciated. Perhaps the name of Carl Linné should also be mentioned here as he was our excellent guide in the labirynth of the plant kingdom.

There is both a long distance of time and a large difference in the efficiency of the methods applied between the studies performed, on the one hand, in late sixties or in early seventies, i.e. at the beginning of development of thin-layer chromatography and the domination of 60- and 80-MHz NMR spectrometry, and on the other hand, the present time with its most sensitive, accurate HPLC equipment and almost common use of 500-MHz NMR spectrometers. Thus, it is only now that it is possible to carry out structural studies on milligram quantities of polyprenols with the aid of sophisticated two-dimensional C-13 — H-1 COSY technique.

The structure of plant polyprenols is shown in Fig. 1. There are two main types of long-chain linear polymers constructed by plant organisms: the tri-*trans* poly-*cis* prenols and di-*trans* poly-*cis* prenols. It is assumed that they are formed either from tri-*trans* geranylgeranyl pyrophosphate or from di-*trans* farnesyl pyrophosphate, respectively, and isopentenyl pyrophosphate by the action of a *cis*-prenyl transferase [7]. The di-*trans* polyprenols are either of the size of 6–9 isoprene residues, or of the size of usually more than 14, 15 isoprene residues. The tri-*trans* polyprenols have usually the size of 10–13 isoprene residues. The structure of polyisoprenoid alcohol of the dolichol type, with its characteristic saturated α-terminal isoprene unit is also shown (Fig. 1). Dolichols occur in mammalian tissues and yeast, they were also found in some plants (see below).

A number of the earlier data obtained with the use of more primitive chromatographic and NMR techniques would now require confirmatory evidence, especially as concerns polyprenols of atypically large size. However, these earlier data opened the way for the taxonomic approach which even now, nearly 25 years later, seems to be a valid and valuable solution of the problem of the diversity of long-chain polyprenols accumulated in plant leaves.

It seems that there was a natural interesting logic in the chronology of the research performed over those years in that, at first, our attention was focused on shorter chain-length polyprenols, and the methods used at that time were quite satisfactory for dealing with molecules of M_r = 600–800, as it was the case with the most common polyprenol of bacterial origin — bactoprenol, composed of 11 isoprene units (undecaprenol; M_r = 766) [3]. It was only 6–8 years later that the interest in dolichols (α-dihydropolyprenols) from animal tissues and from yeast, composed of 16–22 isoprene units, stimulated the studies on the longer chain-length polyprenols [8, 9]. This, in turn,

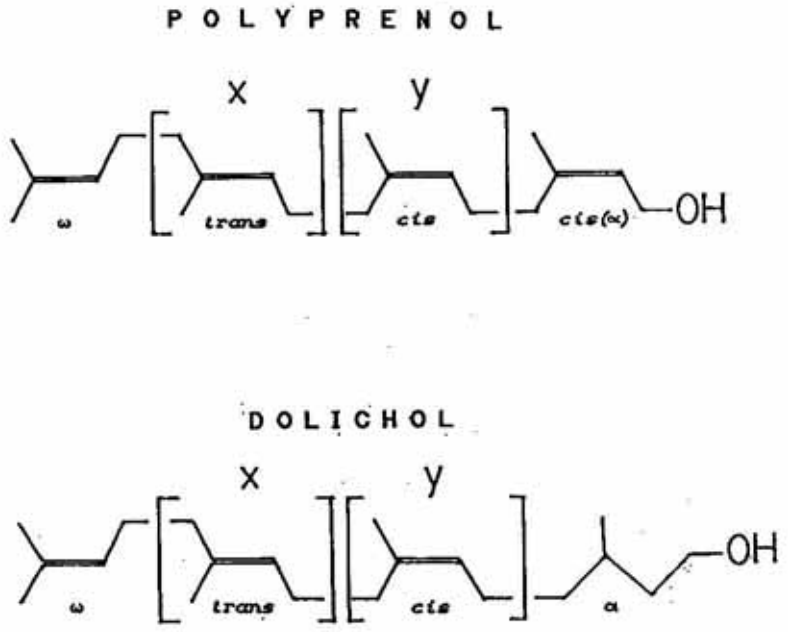


Fig. 1. Structures of polyprenol and dolichol.
Polyprenol: X = 2, Y = 2–5; X = 2, Y > 10; X = 3, Y = 5–9. Dolichol: X = 2, Y > 12.

led to new methodological approaches which made possible studies on such large lipid biomolecules as the most typical for mammalian tissues, polyisoprenoid alcohols composed of 19 and more isoprene units.

We present the research concerning plant polyprenols performed in our Institute in chronological order and supplement it with some data illustrating the general trends in such research taken from publications from other laboratories in various parts of the world. Such an approach is all the more justified as the achievements of other workers helped us in formulating new questions and suggested new lines of research.

As mentioned above, with the development of chromatographic methods, it became possible to detect larger and larger polyprenol molecules. Another trend which appeared, was the focusing of attention on the relationship between the type of polyprenols in plants and their taxonomy rather than on the dependence of the polyprenol pattern on the geographical and environmental factors. Another important factor was the observation that the accumulation of polyprenols in plants was dependent on physiological ageing [10]. This line of our research and the results of studies of the Liverpool group have stimulated more recent studies on human tissues, in which dramatic changes were found to occur concurrently with ageing. This phenomenon was especially evident in brain cortex on examination of autopsy specimens of persons from 0 to 80 years old [11].

BACTOPRENOL AND THE SEARCH FOR SIMILAR POLYPRENOLS IN TROPICAL PLANTS

The so called "ficaprenol" was first isolated in 1967 by the Liverpool group in the course of work on the Ph.D. Thesis of K.J. Stone, and was described in fundamental, elegant papers [12, 13]. The preparation of ficaprenol and the newly graduated doctor (J.K.S.) played an important role in designing and performing studies on formation of bacterial peptidoglycan (in the laboratory of J.L. Strominger in Boston, in the studies of the group of M.J. Osborn in Connecticut on the biosynthesis of bacterial O-antigen, and those of P.W. Robbins and coworkers at the MIT in Boston [1–3]).

It was due to the well established structure of plant polyprenols that the identification of bacterial undecaprenol was possible. The "ficaprenol" from *Ficus elastica* was taken as the standard substance in many biochemical studies on the biosynthesis of microbial sugar heteropolymers. One should also mention here

some other preparations of plant polyprenols that served a similar purpose and played an equally important role, e.g. "moraprenol" of the Russian group in Moscow [14]. Moraprenol was isolated from leaves of *Morus alba* grown in Uzbekhistan; it was almost identical with "ficaprenol" in that, in both preparations, the mainly-*cis*-undecaprenol (C_{55}-prenol) was the main component. It should be added that undecaprenol has been called various local names taken from the plant source available in a particular laboratory. Thin-layer chromatography shows that the polyprenol fraction isolated by column chromatography is rather abundant in leaves both of *F. elastica* and of other plant sources. This type of procedure *via* large scale adsorption chromatography on aluminum oxide or on silica gel is still in use for obtaining from various biological sources both milligram and gram amounts of many different polyisoprenoid alcohols. The course of the chromatographic isolation of ficaprenol, performed in our laboratory in 1970 [15], is shown in Fig. 2. Since in the early seventies it was believed that plants which are the richest sources of polyprenols grew in tropical regions, a thorough screening of a large number of tropical and subtropical plant species available from botanical gardens was undertaken. At that time it was known that *F. elastica*, *Hevea brasiliensis* and other rubber producing plants are the best sources of undecaprenol and similar polyprenols [16]. Our search involved tropical and subtropical plants, both those cultivated in the hot house and those growing in open air. As shown in Table 1, among the 22 plant species included in our first screening [17] not only several new sources of polyprenols were found, but it was established that particular plant species tended to accumulate polyprenols differing in chain length. Thus, C_{45}-prenol (prenol-9) of mainly-*cis* structure (common to all polyprenols described in this article), was found to be the dominating component in leaves of *Exoecaria bussei*, a tree growing in tropical regions of East Africa. Other plants were found to contain as dominating prenologues C_{50}-prenol, or C_{60}-prenol, though most common was the domination of C_{55}-prenol. The high content of polyprenols was not related to the production of rubber, e.g. plants like *Hura crepitans*, in which the leaf juice did not have the appearance characteristic of rubber-producing plants, were rich in polyprenols (Table 1).

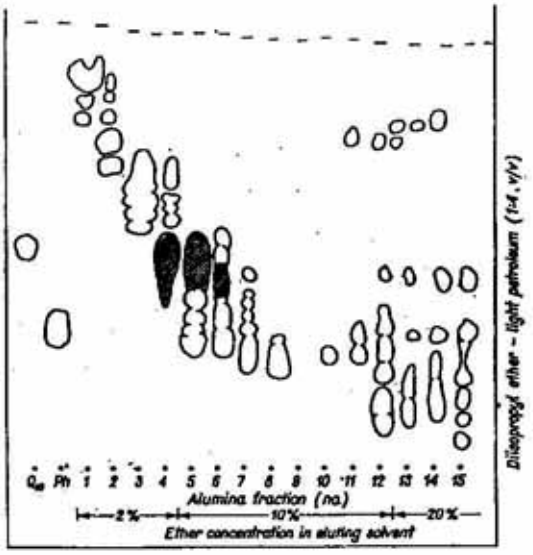


Fig. 2. Thin-layer chromatography on silica gel G of lipids from Ficus elastica separated by alumina column chromatography.
Detection with anisaldehyde reagent. Q_{10}, ubiquinone; Ph, phytol; hatched areas, ficaprenol. Fractions (1–15) eluted with light petroleum containing increasing concentrations of diethyl ether. Diisopropyl ether/light petroleum (1:4, v/v) was used for TLC. Reproduced from [15].

Since almost all the chosen plants studied were of tropical or subtropical origin, this geographical factor was thought for a long time to be of importance for accumulation of long-chain polyprenols. However, further research proved that polyprenols were accumulated also in some plant species growing in other climatic spheres.

Some tropical and subtropical plants which also accumulate polyprenols, are presented in Table 2. All these plants are trees and shrubs grown in natural conditions in an open air botanical garden (Batumi at the Black Sea, Georgia) and in the natural environment in this region. Table 3 gives semiquantitative data for another large group of tropical and subtropical plants growing in this geographical region. It is evident that in almost all the plants which were taken for analysis with the geographical criterion in view, prenol-11 or prenols of similar chain length were the main prenologues.

It should be stressed that the specimens listed in Table 3 are those which were strongly polyprenol-positive in the TLC test (Kiesel gel G, ethyl acetate:benzene, 1:19; staining with

Table 1

Long-chain polyprenols in leaves of angiosperm plants.

Free isoprenoid alcohols were estimated. The plants studied were obtained from the Botanical Garden of the Academy of Sciences of the USSR in Moscow (Ostankino) thanks to Dr Stanisław Razumowski. Data from [17]. In all the tables the dominating polyprenols were considered those which constituted at least 10% of the total polyprenol mixture.

Systematic group Species	Number of isoprene units in prenologues dominating in natural polyprenol mixture	Content of polyprenols (% wet weight)
Annonaceae		
Anaxagorea brevipens	10, 11, 12, 13, 14	0.124
Annona reticulata	9, 10, 11, 12	0.043
Apocynaceae		
Plumeria rubra	10, 11, 12	0.026
Eucommiaceae		
Eucommia ulmoides	8, 9, 10, 11	0.014
Euphorbiaceae		
Codiaeum variegatum	10, 11, 12, 13	0.089
Euphorbia splendens	10, 11, 12	0.011
Euphorbia tirucallii	10, 11	0.028
Exoecaria bussei	8, 9, 10, 11, 12	0.096
Hura crepitans	10, 11, 12, 13, 14	0.239
Mallotus barbatus	9, 10, 11, 12	0.132
Putranjiva roxburghii	9, 10, 11, 12	0.009
Guttiferae		
Mammea americana	8, 9, 10, 11	0.035
Moraceae		
Ficus altissima	9, 10, 11, 12	0.194
Ficus bengalensis	9, 10, 11, 12	0.156
Ficus craterostoma	9, 10, 11, 12, 13	0.229
Ficus elastica	9, 10, 11, 12, 13	0.161
Ficus lyrata	9, 10, 11, 12	0.082
Ficus religiosa	9, 10, 11, 12	0.010
Ficus retusa	9, 10, 11, 12, 13	0.164
Ficus subrepanda	9, 10, 11, 12	0.283
Ficus triangularis	9, 10, 11, 12	0.193

iodine vapours). They represent approx. 30% of the total number of samples examined; leaves of plant species which gave only faint spots of polyprenols or polyprenyl esters with carboxylic acids (less than 50 mg per kg of dry leaves) are not shown in Figures and Tables.

In Table 4 are listed 69 plant species grown in seminatural environment in northern India, which on TLC examination exhibited distinct amounts of polyprenols (or polyprenyl esters). They are listed in two geographical groups: A, the area of the National Botanical Research Institute in Lucknow with its open air botanical garden, and B, the area of the National Forest Research Institute in Dehra Dun together with the Musoori region north of Dehra Dun in the mountain area, about 2500 m above sea level. It should be mentioned that the plants listed in

Table 2

Long-chain polyprenols as free alcohols and carboxylic esters in leaves of (angiosperm) plants.
The plants were obtained from the Botanical Garden of USSR Academy of Sciences, Batumi (Georgia), thanks to Dr N.M. Sharashidze.

Systematic group Species	Number of isoprene units in prenologues dominating in natural polyprenol mixture	Content of polyprenols (% of wet weight)	
		Total	(free + esterified)
Annonaceae			
Asimina triloba	10, 11, 12, 13	0.087	(0.078 + 0.009)
Calycanthaceae			
Meratia yunnanensis	9, 10, 11	0.019	(0.016 + 0.003)
Euphorbiaceae			
Mallotus japonicus	9, 10, 11, 12	0.175	(0.165 + 0.010)
Aleurites cordata	10, 11, 12, 13	0.083	(0.080 + 0.003)
Mallotus apelta	9, 10, 11, 12	0.066	(0.056 + 0.010)
Hamamelidaceae			
Corylopsis spicata	10, 11	0.011	(0.009 + 0.003)
Lauraceae			
Cinnamonum camphorae	10, 11, 12	0.104	(0.090 + 0.014)
Cinnamonum glanduliferum	9, 10, 11, 12	0.075	(0.070 + 0.005)
Cinnamonum lourelli	10, 11, 12, 13	0.063	(0.052 + 0.011)
Laurus nobilis	10, 11, 12, 13	0.240	(0.231 + 0.009)
Magnoliaceae			
Liriodendron chinensis	9, 10, 11, 12	0.129	(0.107 + 0.022)
Liriodendron tulipifera	10, 11, 12, 13	0.275	(0.264 + 0.011)
Magnolia campbellii	10, 11, 12, 13	0.082	(0.079 + 0.003)
Magnolia grandiflora	9, 10, 11, 12, 13	0.083	(0.064 + 0.019)
Magnolia kobus	9, 10, 11, 12, 13	0.270	(0.260 + 0.010)
Magnolia liliflora	9, 10, 11, 12, 13	0.084	(0.080 + 0.004)
Magnolia soulangiana	10, 11, 12	0.023	(0.020 + 0.003)
Magnolia watsonii	9, 10, 11, 12	0.187	(0.161 + 0.026)
Michelia fuscata	10, 11, 12	0.030	(0.026 + 0.004)
Musaceae			
Musa basjoo	10, 11, 12	0.082	(0.077 + 0.005)
Oleaceae			
Osmatus aquifolium	10, 11	0.006	(0.005 + 0.001)
Saxifragaceae			
Itea japonica	9, 10, 11, 12	0.120	(0.114 + 0.006)

Table 4 were examined in early May, i.e. at the time when leaves were rather old, before falling from the tree in the hot summer period (in most of the studied species). The total number of plants examined in all three geographical regions was 230, thus the percentage of "polyprenol-positive" species was about 30%. In a few cases the plant species reported in Tables 1–3 were also collected in India and examined. The accumulation of polyprenols was very similar

Table 3

Free polyprenols in leaves of tropical plants.

The plants were obtained from the Batumi (Georgia) Botanical Garden by W. Sasak. The estimate of the presence of polyprenols was based on the intensity of spots on thin-layer chromatography following stainir.g with iodine vapours [10].

Plant studied	Approximate content of polyprenols (% of dry weight)	Chain length of polyprenols (number of isoprene units)
Aquifoliaceae		
Ilex latifolia	0.1–0.2	11, 12
Buxaceae		
Buxus microphylla	0.1–0.2	10, 11, 12
Calycanthaceae		
Calycanthus fluoridus	0.1–0.2	11, 12
Calycanthus glaucus	0.2–0.5	10, 11, 12, 13
Calycanthus occidentalis	0.2–0.5	10, 11, 12, 13
Meratia praecox	0.2–0.5	10, 11, 12, 13
Caprifoliaceae		
Abelia chinensis	0.1–0.2	10, 11, 12, 13
Cornaceae		
Aucuba japonica	0.1–0.2	10, 11, 12
Cucurbitaceae		
Cucurbita pepo	0.2–0.5	9, 10, 11, 12, 13
Ebenaceae		
Diospyros kaki	0.2–0.5	10, 11, 12
Euphorbiaceae		
Aleuritis cordata	0.2–0.5	10, 11, 12, 13
Aleuritis fordii	0.2–0.5	10, 11, 12, 13
Bischofia javanica	0.2–0.5	11
Exoecaria bicolor	0.2–0.5	8, 9, 10, 11, 12
Euphorbia bubalina	0.2–0.5	8, 9, 10, 11
Hamamelidaceae		
Corylopsis spicata	0.1–0.2	10, 11
Lauraceae		
Cinnamonum iners	0.2–0.5	9, 10, 11, 12, 13
Cinnamonum japonicum	0.2–0.5	10, 11, 12, 13
Cinnamonum yunnanensis	0.5–1.0	10, 11, 12, 13
Persea gratissima	0.2–0.5	10, 11, 12, 13
Magnoliaceae		
Ilicium floridanum	0.1–0.2	10, 11, 12
Magnolia denudata	0.2–0.5	10, 11, 12
Magnolia glauca	0.2–0.5	10, 11, 12
Magnolia stellata	0.2–0.5	10, 11, 12, 13
Magnolia tripelata	0.2–0.5	11, 12
Michelia compressa	0.2–0.5	10, 11, 12

Table 3 (continued)

Plant studied	Approximate content of polyprenols (% of dry weight)	Chain length of polyprenols (number of isoprene units)
Malvaceae		
Gossypium barbadense	0.2–0.5	10, 11, 12, 13, 14
Moraceae		
Cudrania tricuspidata	0.2–0.5	10, 11, 12
Ficus carica	0.1–0.2	11
Ficus eximia	0.2–0.5	9, 10, 11, 12, 13
Ficus scandens	0.2–0.5	9, 10, 11, 12
Myrtaceae		
Eucalyptus globus	0.1–0.2	9, 10
Oleaceae		
Ligustrum lucidum	0.2–0.5	10, 11, 12
Osmantus aquifolium	0.1–0.2	10, 11, 12
Osmantus fragrans	0.1–0.2	10, 11, 12
Syringa vulgaris	0.1–0.2	11, 12
Pittosporaceae		
Pittosporum tenuifolium	0.1–0.2	9, 10, 11
Podocarpaceae		
Podocarpus nageia	0.2–0.5	10, 11, 12, 13
Rubiaceae		
Coffea arabica	0.2–0.5	9, 10, 11, 12
Coffea canephora	0.2–0.5	6, 7, 8, 9, 10, 11
Gardenia jasmonoides	0.1–0.2	11, 12
Rutaceae		
Zanthoxylium bungei	0.1–0.2	10, 11, 12,13
Zanthoxylium piperitum	0.2–0.5	10, 11, 12,13
Saxifragaceae		
Hydrangea paniculata	0.1–0.2	11
Sterculiaceae		
Sterculia platanifolia	0.2–0.5	10, 11, 12
Theobroma cacao	0.5–1.0	10, 11, 12, 13, 14, 15
Styraceae		
Pterostyrax corymbosa	0.1–0.2	11
Theaceae		
Camelia sasanqua	0.1–0.2	11, 12
Camelia sinensis	0.2–0.5	6, 7, 8, 9
Transtroemia japonica	0.1–0.2	11, 12

Table 4

Long-chain polyprenols in leaves of plants collected in Lucknow Botanical Garden and in Dehra Dun area (India, May 1979)

Plant studied	Approximate content (% of fresh weight)	Chain length (number of isoprene units or presence of prenyl esters — E)
Magnoliophyta (Angiospermae)		
Magnoliopsida (Dicotyledons)		
Alangiaceae		
Alangium sp.	0.2–0.5	10, 11
Anacardiaceae		
Mangifera indica L.	0.2–0.5	10, 11
Pleiogynium sp.	0.2–0.5	10, 11
Annonaceae		
Annona reticulata L.	0.5–1.0	11, 12
Polyalthia longif. Bet H.	0.2–0.5	11, 12
Polyalthia longif. var. *pend.*	0.2–0.5	11, 12
Apocynaceae		
Acocanthera vanenata G. Don.		E
Holarrhena sp.	0.2–0.5	10, 11
Asteraceae (Compositae)		
Viguiera helianthoides "coronaria" Kuntz	2.0–3.0	10, 11, 12
Berberidaceae		
Mahonia sp.*	0.1–0.2	8, 9
Capparaceae		
Capparis zeylanica L.*	1.0–1.5	14, 15
Cochlospermaceae		
Cochlospermum goss. (L.)DC	0.2–0.5	12, 13
Dilleniaceae		
Dillenia indica L.		E
Euphorbiaceae		
Acalypha godseffiana Baill.	1.0–1.5	10, 11, 12
Acalypha macr. H. B. et K.	0.5–1.0	11, 12
Exoecaria bicolor Hassk.	0.2–0.5	10, 11
Gelonum multifl. A. Juss.	0.2–0.5	10, 11
Macaranga denticul. Muell.	1.0–1.5	11, 12
Mallotus philippin. Muell.	0.5–1.0	10, 11, 12
Fabaceae (Leguminosae)		
Acacia sp.	0.5–1.0	11, 12, 13, 14
Acacia sp.	0.2–0.5	11, 12
Adenanthera pavonina	0.1–0.2	10, 11
Albizia lebbek Benth.	0.2–0.5	11, 12

Table 4 (continued)

Plant studied	Approximate content (% of fresh weight)	Chain length (number of isoprene units or presence of prenyl esters — E)
Bauhinia purpurea L.	0.2–0.5	8, 9, 10, 11, 12
Bauhinia vahlii W. et Am.	0.5–1.0	11, 12
Caesalpinia coriaria	0.2–0.5	11, 12
Cassia siamea Lam.	0.2–0.5	11, 12
Erythria sp.	0.2–0.5	10, 11
Inga dulcis Willd.	0.5–1.0	11, 12
Mimosa pudica	0.1–0.2	11, 12, 13
Saraca cauliflora Baker	0.1–0.2	11, 12, 13
Saraca indica L.	1.0–1.5	11, 12, 13
Tamarindus indica L.	1.0–1.5	11, 12, 13
Fagaceae		
Quercus sp.	0.1–0.2	11, 12
Kiggelariaceae		
Hydnocarpus sp.	0.2–0.5	11, 12
Lauraceae		
Cinnamonum camphorae T. Ness et Eberm.	1.5–2.0	10, 11
Macholus sp.	0.1–0.2	11, 12, 13
Lythraceae		
Lawsonia inermis L.		E
Magnoliaceae		
Michelia champaca L.	0.1–0.2	10, 11
Malvaceae		
Achania (syn. *Malvaviscus, Hibiscus)*	0.1–0.2	10, 11, 12
Kydia calycina Roxb.	0.5–1.0	11, 12
Hibiscus liliiflorus Gar.	1.0–1.5	10, 11, 12
Meliaceae		
Toona ciliata M. Roemer*	0.1–0.2	11, 12
Dysoxylum binectariferum Hook. f. ex. Bedd.	0.1–0.2	9, 10, 11
Swetonia mahoganii Jacq.	0.2–0.5	11, 12
Moraceae		
Ficus bengalensis L.	0.1–0.2	E, 10, 11
Ficus elastica Roxb.	0.2–0.5	10, 11
Morus alba L.	1.5–2.0	10, 11, 12
Morus laevigata Wall.	0.2–0.5	10, 11, 12
Streblus asper Lour.	1.0–1.5	11, 12
Myrtaceae		
Callistemon lanceolatus Sweet.	0.2–0.5	7, 8, 9

Table 4 (continued)

Plant studied	Approximate content (% of fresh weight)	Chain length (number of isoprene units or presence of prenyl esters — E)
Rosaceae		
Spiraea corymbosa		E
Rubiaceae		
Ixora coccinea L.		E
Rutaceae		
Glycosomis pentaphylla Correa	0.2–0.5	12, 13
Sapindaceae		
Blighia sapida K. Koenig	0.2–0.5	12, 13
*Nephelium litchii**	1.5–2.0	12, 13, 14, 15
Solanaceae		
Cestrum album (diurn) Ferrens	0.5–1.0	10, 11, 12
Cestrum hirsutum (noct.) Jacq.	1.0–1.5	10, 11, 12
Solanum macranthum Dun.	0.1–0.2	12, 13
Sterculiaceae		
Dombeya mastersii Hook. f.	0.2–0.5	11, 12, 13, 14
Eriolaena hookeriana Wight et Arn.*	0.2–0.5	11, 12, 13, 14
Pterospermum acerifolium Willd.	0.5–1.0	E, 12, 13, 14
Sterculia alata Roxb.*	1.5–2.0	E, 11, 12, 13, 14
Sterculia colorata Roxb.	1.0–1.5	10, 11, 12, 13
Sterculia diversifolia G. Don.	0.2–0.5	11, 12, 13, 14
Tiliaceae		
Berrya ammonilia Roxb.	3.0–5.0	11, 12
Ulmaceae		
Celtis australis L.	1.0–1.5	12, 13
Verbenaceae		
Tectona grandis L.		E
Liliopsida (Monocotyledons)		
Arecaceae (Palmae)		
Livistona sp.	0.1–0.2	11, 12
Coniferopsida (Gymnospermae)		
Araucariaceae		
Araucaria cooki R. Br ex D. Don.		E
Cycadopsida (Gymnospermae)		
Cycadaceae		
Cycas revoluta Thunb.		E
Cycas rumphii Miq.		E

*Samples from Dehra Dun region and Musoori.

within the species, irrespective of the origin of the plant. It is evident from the previous data (Table 1, 2 and 3) and similar data of Table 4, that prenol-10, -11 and -12 are the most common polyprenols in the plants studied, irrespective of the systematic group. In a few cases slightly longer polyprenol molecules were dominating, e.g. prenol-13, -14 and -15 were found in *Nephelium litchii*, in *Sterculia alata* var. *diverisfolia* and in *Capparis zeylanica*. In only a few species a distinct spot of polyprenyl esters (marked with letter E) was observed. In the case of *Araucaria coockii*, *Cycas rumphii* and *Cycas revoluta* the substances were further identified as acetic acid esters of prenol-19 and of longer chain prenologues. In a few cases a distinct spot of polyprenyl esters (E) was accompanied by large quantities of free polyprenols, e.g. in *Cinnamonum camphorae* and in *S. alata*. The alcohol component of the esters was that polyprenol which was present also as a free isoprenoid alcohol (usually prenol-11 or prenol-12; cf. also Table 2).

In the course of further studies the distinct spot of esters found in *Spirea species* collected in India was found to be a substance common to leaves of almost all the *Rosaceae* species. The polyprenyl ester in *Spirea* was found to contain mainly prenol-19, prenol-20 and longer chain prenologues (see below). In our further studies great attention was paid to the presence of large amounts of still longer chain polyprenols in the *Rosaceae* family which is more common to cold and moderate climate. One can see from the data of Tables 1–4, in which the results for tropical and subtropical plants are shown, that the domination of prenol-11 and polyprenols of similar size seems to be a common feature of several systematic groups. The semiquantitative results of estimation of the content of polyprenols, based on comparing the size and intensity of chromatographic spots on TLC pointed to the presence of extremely high amounts of polyprenols in some of the species studied (e.g. approx. 5% in *Berrya ammonilla*, approx. 3% in *Cinnamonum camphorae* and *Morus alba*, etc.). We could expect that these and other plants listed in Table 4 may show still higher quantities of polyprenols upon further ageing.

On checking leaves of several plant species (shrubs and trees) growing in the neighbourhood of our laboratory and our homes in the cities of Lublin, Przasnysz and Warsaw (Table 5) we found a similar frequency of the occurrence of plants with dominating prenol-10, -11 and -12 in the majority of plants growing in our public parks, courtyards, by the pavements, etc. There was, however, also quite a large group of trees and shrubs that showed the presence of much longer isoprenoid molecules of prenol-19, -20 and longer ones, in most cases esterified with a fatty acid or with acetic acid. All these plants belonged to the *Rosaceae* family; some of them were characteristic of orchards and natural woods like various types of *Malus*, *Prunus*, etc. In those "within-city-spots" of trees and shrubs we have met only angiosperm plants. One should realize that this group of plants created by our anthropopressing activity was purely artificial and had nothing in common with any plant sociological scheme. At one time it was even declared by one of us (W.J.) that the "factor of elegance" played a role in making the plant an effective accumulator of polyprenols. It was really stricking that the plants cultivated in the city areas that had an elegant shape, colours of leaves and fruits, and, in general attractive appearance contained large quantities of polyprenols. Of course, the feature of elegance is something subjective and almost mysterious as concerns a plant. Later on we found (that was E.Ś.) that plants of inconspicuous, modest appearance synthesized and stored long-chain polyprenols most effectively. This was the case of *Potentilla aurea*, which was a warm-green, but still grey and modest Cinderella among the many colourful, elegant plants growing in Tatra mountains. But we also like Cinderella and it was thus more than an usual elegance-factor that was responsible for the unusual phenomenon of accumulation of long-chain polyprenols in its leaves.

For a long time, until late eighties, it seemed that plants did not produce in large quantities polyprenols composed of 13 and 14 isoprene units. The data available for numerous angiosperm species showed the accumulation mainly of prenol-11 (which was called "plant polyprenol") and prenol-12, and numerous examples were known of the occurrence of prenol-15, -16 and longer ones in gymnosperm plants (described below), while there was a gap concerning prenols-13 and -14. This seemed to suggest the absence in the plant kingdom of domination of prenologues of this particular chain

Table 5

Polyprenols in leaves of most typical shrubs and trees in city parks and courtyards in Lublin, Przasnysz and Warsaw (Poland), September 1978 and 1979.

The content of polyprenols was estimated by comparing the size of TLC spot with that of known amount of standard. (E) Polyprenols in the form of esters.

Plant studied	Approximate content of polyprenols	Chain length (number of isoprene units)
Aceraceae		
Acer platanoides L.	0.2–0.5%	10, 11, 12
Acer pseudoplatanus L.	0.5–1.0%	11, 12, 13
Anacardiaceae		
Cotinum coggygria Scop.	1.0–2.0%	11, 12
Rhus sylvestris Sieb et Zucc.	0.5–1.0%	11, 12
Rhus typhina L.	1.0–2.0%	11, 12
Betulaceae		
Alnus glutinosa (L.) Gaertner	0.5–1.0%	10, 11
Carpinus betulas L.	0.2–0.5%	10, 11
Calcycanthaceae		
Calycanthus floridus L.	0.2–0.5%	9, 10
Euphorbiaceae		
Codiaeum variegatum (L.) Bl.	0.5–1.0%	9, 10, 11
Fagaceae		
Fagus sylvatica L.	0.1–0.2%	10, 11
Juglandaceae		
Juglans nigra L.	0.5–1.0%	10, 11
Juglans regia L.	1.0–2.0%	10, 11
Lauraceae		
Laurus nobilis	1.0–2.0%	10, 11
Leguminosae		
Ceratonia siliqua	0.2–0.5%	9, 10, 11
Gleditsia triacanthos L.	0.2–0.5%	12, 13
Robinia pseud.	0.1–0.2%	9, 10, 11
Moraceae		
Morus alba L.	1.0–2.0%	10, 11
Nyctaginaceae		
Bougainvillea glabra Choisy	0.2–0.5%	9, 10, 11
Oleaceae		
Fraxinus excelsior L.	0.2–0.5%	11, 12
Palmae		
Jubaea chilensis Baill.	0.2–0.5%	9, 10, 11
Styraceae		
Halesia tetraptera	0.2–0.5%	11, 12

Table 5 (continued)

Plant studied	Approximate content of polyprenols	Chain length (number of isoprene units)
Tiliaceae		
Tilia cordata Miller	0.2–0.5%	9, 10, 11
Tilia euchlora K. Koch	0.2–0.5%	10, 11
Tilia platyphyllos Scop.	0.2–0.5%	10, 11, 12
Tilia tomentosa Moench	0.2–0.5%	9, 10
Ulmaceae		
Celtis audibersiana	0.5–1.0%	12, 13
Ulmus laevis Pallas	0.5–1.0%	10, 11
Vitaceae		
Vitis voineriana Baltet	0.1–0.2%	10, 11
Rosaceae		
Cotonaster dielsiana Pritz.	0.2–0.5%	19, 20, 21 (E)
Malus domestica Borkh.	0.2–0.5%	19, 20, 21 (E)
Prunus avium L.	0.2–0.5%	18, 19, 20 (E)
Prunus cerasus L.	0.2–0.5%	18, 19, 20 (E)
Prunus laurocerasus L.	0.2–0.5%	18, 19, 20 (E)
Prunus spinosa L.	0.2–0.5%	18, 19, 20 (E)
Pyracantha coccinea Roem.	0.5–1.0%	19, 20, 21 (E)
Sorbus aria (L.) Crantz	0.2–0.5%	19, 20, 21 (E)
Sorbus decora Schneid.	0.2–0.5%	19, 20, 21 (E)
Sorbus thuringiaca Fritsch	0.2–0.5%	19, 20, 21 (E)

length. This view was due to the rare occurrence in natural flora of those species in which such polyprenols are abundant, and which are characteristic exclusively to tropical and to less known geographical regions. One of the systematic groups in which prenols-13 and -14 are the dominating components is the *Capparidaceae* family (Table 6); in one of the *Capparis* species, *C. coriacea* (Fig. 3), we found also dolichol-type polyprenols [18]. The domination of prenols composed of 13 and 14 isoprene units was found also in another systematic group, the family *Sapindaceae* [19]. The few representative species of this family belonging to the genus *Nephelium* accumulate mainly prenol-13. Also in this systematic group, characteristic for accumulation of prenols composed of 13 and 14 isoprene units, we observed a heterogeneity of the isolated single polyprenols. The nature of this heterogeneity is determined most probably by the presence of a small proportion of isomeric forms differing in the amount of *cis* and *trans* isoprene units in the molecule (cf. subsequent chapters in this article), or due to another modification of the polyprenol molecule. Trace amounts of heterogeneous forms of C_{55}-polyprenol have been observed in our studies of a polyprenol mixture from leaves of *Magnolia campbellii* [20].

POLYPRENOLS OF LARGER SIZE, SIMILAR TO MAMMALIAN AND YEAST DOLICHOLS

In plants, polyprenols with a chain longer than 11, 12 isoprene residues were first detected in 1972 in needles of coniferous trees. Prenols composed of 16 or 17 isoprene residues were found in representatives of the *Pinaceae* family by Zinkel & Evans [21] and Hanus & Pensar [22]. In 1976 we described still longer chain polyprenols in *Juniperus communis* [23]

Table 6

Polyprenols in leaves of various species of the genus Capparis.

The content of polyprenols was estimated by HPLC [31]. The plants were from the Botanical Garden in Caracas (Venezuela).

Species	Number of isoprene units in dominating polyprenol*	Content of polyprenols (% of dry weight)
Capparis afzelii	14, 15, 16	0.07
*Capparis coriacea***	13, 14, 15, 20, 21	0.27
Capparis decidua	11, 12, 13, 14	0.07
Capparis linearis	13, 14, 15	0.38
Capparis odoratissima	12, 13, 14	0.12
Capparis pachaco	14, 15, 16, 21	0.19
Capparis pubiflora	14, 15, 16	0.07
Capparis separia	15, 16, 17, 22	0.02
Capparis verrucosa	13, 14, 15, 16, 21, 22	0.10

*For polyprenols longer than 20 isoprene units content not exceeding 5% of total; **prenologues 13, 14 and 15 are prenols + dolichols

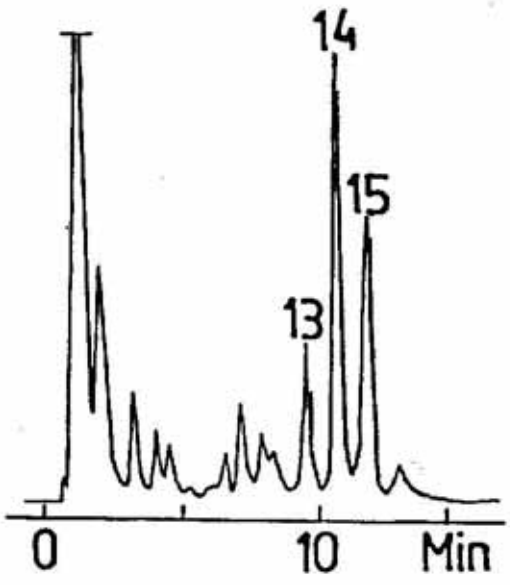


Fig. 3. High pressure liquid chromatography of polyprenol fraction from leaves of Capparis coriacea. The chain length of double peaks (polyprenol + dolichol) marked with numbers 13, 14 and 15 (number of isoprene units). A Waters Ass. dual pump apparatus, gradient programmer, UV detector (set at 210 nm) and the Resolve-Bondapak C18 (5 microns, 12.5 cm × 0.4 cm) column were used. The elution system was: methanol/isopropanol/water (60:40:5, by vol.) with isopropanol/hexane (30:70, v/v) from 0 to 60% added according to gradient "5". The solvent flow was 1.5 ml/min and the end of the gradient was reached in 45 min. Reproduced from [18].

and demonstrated that, unlike the species belonging to *Pinaceae*, *J. communis* accumulated polyprenols in the form of fatty acid esters (in many other conifers polyprenols are accumulated in the form of acetic acid esters). We also found that two groups of polyprenols are present in *J. communis*, one with dominating prenologues composed of 16 and 17 isoprene units, and another, in which the dominating prenologue was prenol-20. This finding was the first demonstration of complexity of the polyprenol mixture in gymnosperm plants.

Complex mixtures of very long-chain polyprenols in gymnosperm plants

Ibata *et al.* [24] found a number of cases of multiplicity in chain length distribution among coniferous plants. From the results of their studies it seemed that this multiplicity was a rather common feature in this systematic group. An almost complete picture of the pattern of polyprenols in gymnosperm plants was made in 1988 by Świeżewska & Chojnacki [25], who examined over 100 species of gymnosperms of well documented origin; a part of them are presented in Table 7 and the summary of these studies is shown in Table 8. One can see that in all systematic families within the group of *Coniferopsida* the multiplicity is a rule. Only in *Pinaceae* there is without exception, only one group of polyprenols. Most of the studies shown in Table 7 and 8 were performed on

plants grown in natural (or close to natural) conditions in the well known arboretum at Kórnik (Poland); samples of several unique species of *Cycadopsida* originated from the collection of these plants grown in the hot house of the Jagiellonian University in Kraków (Poland). In most of the coniferous plants taken for examination large quantities of polyprenols were found. It looks as if the ability to accumulate long-chain polyprenols is more common in gymnosperm plants than it was observed in angiosperms. The large number of gymnosperms that were studied by us gives strong support to our final conclusions about the occurrence of polyprenols in this systematic group. One should emphasize that the total number of gymnosperm species is estimated to be about 400, and we recorded the polyprenol content in approximately 150 species.

For a long time we were engaged in examining various species of wildly grown ferns for polyprenol content; as a rule ferns were polyprenol-negative in the preliminary TLC test. A more thorough search for polyprenols in the group of *Pteridophytina* was done on a large number of various species listed in Table 9, but in none of them long-chain polyprenols were detected.

Unique position of the *Rosaceae* family with respect to the pattern of polyprenols

A polyprenol pattern similar to that found in *Cycadopsida* was found in leaves of several representatives of the *Rosaceae* family. Typical representatives of the genera *Cotonoaster, Crataegus, Prunus* and *Sorbus* are listed and the content of typical polyprenols in their leaves are given in Table 10. In all of these plants prenol-19 or -20 were the dominating prenologues, and the spectrum of prenologues was rather wide. To illustrate the similarity of polyprenol spectrum in *Rosaceae* and *Cycadopsida* we show in Fig. 4 the original HPLC records of *Sorbus suecica* and *Zamia integrifolia* together with three others [26]. The "polyprenol spectra" of *S. suecica* and *Z. integrifolia* — taxonomically distant species are indistinguishable. On the other hand, the contemporary *Rosaceae* originate most probably from one of the groups of gymnosperms, and the validity of this suggestion of botanists can be supported by our data on the similarity of their polyprenols.

The presence of long-chain polyprenols in *Rosaceae* was further confirmed in our research on several members of the genus *Potentilla*. A large number of herbaceous plants belonging to this genus and investigated in our laboratory were found to be rich sources of extremely long polyprenol molecules, longer than any previously known substances isolated from conifers or from other species of *Rosaceae*. *Potentilla* species have as a rule two groups of polyisoprenoid alcohols: one with dominating prenol-19 or prenol-20 and another, with the dominating polyprenols composed of 24, 25 and even up to 28 isoprene units [27, 28]. In Table 11 are shown the results of quantitative and semiquantitative estimation of polyprenols in leaves of a number of *Potentilla* species taken from the open air collection of the University Botanical Garden in Wrocław (Poland) and in the Botanical Garden in Edinbourgh (Great Britain). It seems that seasonal variations in the content of polyprenols in *Potentilla* are not so critical as those observed in the case of shrubs and trees both of the gymnosperm and angiosperm type (see below). Most of the results for *Potentilla* species represent a typical polyprenol pattern composed of two families, though in some of the plants (e.g. *P. ambigua, P. rupestris)* only one polyprenol family was observed. The most characteristic types of polyprenol mixtures in leaves of five species of *Potentilla* are visualized in Fig. 5. While in most of the species polyprenols form two families very similar to the pattern found in *P. aurea*, a more complex spectrum was found in *P. rigoana*, where three families of polyprenols were observed, with dominating prenol-18 and -19, prenol-26 and prenol-38. Also in *P. crantzii* and in *P. flabeliformis*, in addition to two main families of polyprenols with dominating prenol-19 and -20 and prenol-26–29, there was a group of polyprenols composed of 36–39 isoprene units, however, this last group was less distinct than in *P. rigoana*. Altogether over 80 species of *Potentilla* were examined in our laboratory (E. Świeżewska & E. Skoczylas, unpublished) and in most of them the typical multiple character of polyprenol spectrum was observed. It was also found (in *P. anserina*) that polyprenols occur exclusively in leaves; they were not detected in stems and in roots.

Leaves of the species belonging to the genus *Rosa* have also been found to contain two

Table 7 (continued)

1	2	3														
Coniferopsida																
Araucariaceae																
Agathis robusta																
Free	1.08	0.6	1.2	2.6	2.3	2.0	3.5	3.5	6.8	9.1	13.3	17.3	19.4	13.9	3.3	0.9
Acetylated	0.31			1.6	3.3	3.0	3.3	5.5	9.1	17.4	21.8	17.7	10.2	4.1	1.9	0.8
Araucaria bidwilla	0.20				3.7	6.7	8.3	6.5	6.0	7.4	13.3	19.9	17.1	8.6	2.4	0.3
Araucaria excelsa	0.17			0.5	1.6	4.7	7.6	7.6	9.1	19.6	26.4	17.3	4.4	1.1		
Araucaria imbricata																
Free	0.51	0.7	4.9	1.4	1.3	0.7	6.7	7.9	9.8	12.6	12.8	11.7	11.2	14.1	3.2	0.7
Acetylated	0.45			0.7	2.4	6.9	8.6	6.8	6.0	8.4	14.6	20.6	16.3	6.6	1.6	0.4
Araucaria angustifolia	0.17			1.3	9.8	12.9	6.1	3.7	3.1	5.8	13.8	22.7	15.3	3.7		
Cupressaceae																
Chamaecyparis lawsoniana	0															
Chamaecyparis nootkatensis	0															
Chamaecyparis obtusa	0															
Chamaecyparis pisifera	0															
Chamaecyparis plumosa	0															
Chamaecyparis thioides	0															
Chamaecyparis leylandii	0															
Juniperus chinesis	0															
Juniperus communis																
Free	0.45		1.4	5.6	24.6	24.6	11.6	10.6	13.6	8.1						
Acetylated	0.45		3.1	10.8	25.2	22.5	11.4	11.4	10.4	5.2						
Juniperus converta	0															
Juniperus horizontalis	0															
Juniperus procumbens	0															
Juniperus rigida	0															
Juniperus sabina	0															
Juniperus squamata	0.13			4.9	20.3	30.0	17.4	11.6	9.5	5.8	0.5					

Juniperus virginiana	0.28		1.3	4.6	21.4	33.7	20.0	12.4	6.2	0.4				
Microbiota decussata	0													
Thuja koreaensis	0.21		1.3	7.3	25.2	24.1	13.0	9.1	9.4	8.8	1.9			
Thuja occidentalis	0.21		0.9	8.3	17.4	17.9	15.0	10.2	12.8	12.5	4.8	0.1		
Thuja orientalis	0													
Thuja plicata	0.20		0.4	6.1	22.8	20.9	9.2	8.8	10.4	15.4	5.8	0.2		
Thuja standishi	0													
Thujopsis delabrata	0.46	0.2	0.8	4.0	13.5	21.1	15.1	14.3	12.7	10.1	5.7	1.9	0.6	0.1
Taxodiaceae														
Cryptomeria elegans	0													
Cryptomeria japonica	0													
Metasequoia glyptostroboides	0.19		1.1	1.5	8.1	13.5	10.0	7.2	24.4	23.9	9.0	1.2		
Sequoia sempervirens	0													
Sciadopitys verticillata	0													
Taxodium distichum	0													
Taiwania cryptomerioides	0.13			2.5	7.8	13.9	15.5	24.8	20.6	9.5	3.5	0.9		
Pinaceae														
Abies alba	0.21	0.6	9.3	29.0	40.3	15.3	4.2	1.3						
Abies balsamea	0													
Abies cephalonica	0													
Abies concolor	0.05		1.8	15.4	45.7	32.9	4.2							
Abies grandis	0.10				11.4	41.9	35.8	9.0	1.8					
Abies homolepis	0.61	0.5	4.4	20.0	43.6	27.9	3.7							
Abies holophylla	1.24	0.5	3.2	16.0	42.9	30.7	6.6							
Abies koreana	1.33	0.9	5.1	20.2	43.0	25.7	4.9	0.2						
Abies normandiana	0													
Abies pinsapo	0													
Abies procera	0.08			2.0	18.5	45.2	27.6	6.0	0.8					
Abies sachalinensis	0.54		0.8	12.5	45.2	34.3	5.2	1.9						
Abies veitchi	0.78		2.2	13.4	44.7	32.0	7.4	0.3						

Table 7 (continued)

1	2	3								
Cedrus atlantica	0.57		1.2	4.9	21.6	40.3	23.0	6.6	2.0	0.4
Cedrus libani	0									
Cedrus decurrens	0									
Larix kempferi	0									
Larix lariciana	0.11	1.5	9.6	39.0	40.7	7.7	1.5			
Picea abies virgata	0.55	11.5	37.4	33.3	13.5	4.3				
Picea sperata	0									
Picea breweriana	0.12	2.4	18.7	46.7	27.7	4.6				
Picea omorica	0.18	1.7	13.1	40.5	32.6	9.6	2.4			
Picea orientalis	0.11	3.8	19.9	37.0	27.5	9.2	2.6			
Picea pungens	0									
Picea wilsoni	0									
Pinus aristata	0.03	4.1	33.6	39.9	18.9	3.4				
Pinus armandi	0.35	0.8	6.4	23.3	38.9	21.3	6.9	2.0	0.4	
Pinus banksiana	0.33	1.5	18.0	43.9	28.3	7.4	1.0			
Pinus cembra	0.25	2.2	10.1	32.3	37.0	15.5	2.9			
Pinus contorta	0.19	0.6	6.4	28.7	44.2	17.4	2.6			
Pinus flexis	0.08	5.8	32.8	45.8	15.5					
Pinus heldreichii	0.18			16.6	41.6	35.3	6.5			
Pinus jeffreyi	0									
Pinus koreaensis	0.08	3.2	20.6	47.0	24.4	4.0	0.8			
Pinus mugo	0.13	0.4	4.3	25.9	48.0	19.5	1.8			
Pinus nigra	0.22	0.6	5.7	26.4	43.1	20.6	3.5			
Pinus parviflora	0.33	0.7	3.3	14.7	25.0	27.1	19.4	6.7	2.0	1.0
Pinus peuce	0.22			5.7	21.3	41.5	24.9	5.4	1.3	
Pinus ponderosa	0.23	0.8	3.8	25.5	46.8	19.5	3.1	0.4		
Pinus rigida	0									
Pinus strobus	1.09		0.5	3.8	17.2	35.2	32.0	8.2	2.4	0.6	0.2
Pinus silvestris	0.56	0.4	4.4	23.2	42.0	24.9	4.7	0.4		
Pinus uncinata	0.10	1.3	8.7	31.3	42.9	13.9	2.0			
Pseudotsuga menziesi	0									
Tsuga canadensis	1.00	0.6	2.7	16.7	42.8	30.3	6.3	0.6		

Tsuga diversifolia	0.28		5.3	23.1	39.5	23.5	6.8	1.6	0.2							
Podocarpaceae																
Podocarpus neriformis	0.06				2.1	2.8	2.1	0.7	1.4	4.2	17.0	34.0	23.4	8.5	3.5	
Taxopsida																
Taxus baccata	0.37			3.6	18.0	29.5	15.8	6.5	7.4	9.6	6.4	2.4				
Taxus cuspidata	0				14.6	29.3	10.7	5.7	12.5	8.9	2.2	0.7				
Taxus media	0.13															
Cephalotaxus drupacea	0															
Cephalotaxus harringtoni	0															
Torreya nucifera	0															
Gnetopsida																
Ephedra equisitina	0															
Euphera fragilis	0															

*The values given by Ibata *et al.* [24].

Table 8
Polyprenols in leaves of gymnosperm plants.
Summary of the data from [25].

Systematic group Family Number of species studied Representative species	Number of isoprene units in prenologues dominating in natural polyprenol mixture	Content (% of wet weight)
Cycadopsida (11)		
Ceratozamia mexicana	18, 19, 20	3.28
Encephalartos horridans	19, 20, 21	1.42
Ginkgopsida (1)		
Gingko biloba	17, 18, 19	1.00
Taxopsida (6)		
Taxus baccata	17, 18, 19, 23, 24, 25	0.37
Coniferopsida		
Araucariaceae (6)		
Agathis robusta	16, 17, 18, 22, 23, 24	1.39
Cupressaceae (24)		
Juniperus communis	16, 17, 18, 19, 20, 21	0.90
Taxodiaceae (7)		
Metasequoia glyptostroboides	16, 17, 18, 19, 20, 21, 22	0.19
Pinaceae (46)		
Abies koreana	15, 16, 17	1.33
Cedrus atlantica	17, 18, 19	0.57
Picea omorica	14, 15, 16	0.18
Pinus silvestris	15, 16, 17	0.56
Pinus strobus	16, 17, 18	1.09
Tsuga canadensis	15, 16, 17	1.00
Podocarpaceae (1)		
Podocarpus neriformis	17, 18, 19, 23, 24, 25	0.06

groups of polyprenols, similarly as observed in many representatives of the genus *Potentilla* [28]. The spectrum of longer polyprenols in various species of the genus *Rosa* was rather narrow (W. Jankowski, unpublished) compared with that observed in many species belonging to the genus *Potentilla*. In Table 12 are shown the results of semiquantitative estimations of polyprenols in leaves of various species of *Rosa* grown in the University Botanical Garden in Poznań (Poland). In most of them one can see a polyprenol family with dominating prenol-19 and a second family of very long-chain polyprenols built up of about 30 isoprene units.

Another thoroughly examined genus of the *Rosaceae* family was the genus *Rubus* in which 11 species were examined. In all of them accumulation of polyprenols was noticeable and in all of them the type of polyprenols was identical, i.e. prenol-18, -19 and prenol-20 were the dominating prenologues (Table 13). Polyprenols occurred in *Rubus* in the esterified form.

The systematic family *Rosaceae* contains about 3000 species which are grouped in a number of distinguishable subfamilies. It seems that in this systematic group which is one of the evolutionary youngest group of plant species, polyprenols exceed the chain length of 18, 19 isoprene units and an additional group of poly-

Table 9

List of Pteridophytina checked (with negative results) for the presence of long-chain polyprenols in leaves

A. Plants from the Collection of the Jagiellonian University Botanical Garden in Kraków (Poland)	
1. *Adiantum* sp.	14. *Platycerium chili*
2. *Adiantum cuncunatum*	15. *Platycerium elisi*
3. *Adiantum hispidulum*	16. *Platycerium steromic*
4. *Acrostichum aureus*	17. *Platycerium superate*
5. *Asplenium nidus*	18. *Platycerium varreyi*
6. *Cyrconitum falcata*	19. *Platycerium veitchii*
7. *Blechnum brasiliense*	20. *Platycerium wilimcher*
8. *Divalia solidar*	21. *Polypodium* sp.
9. *Lygodium japonica*	22. *Polypodium aurea*
10. *Nephrolepis exalt.*	23. *Pityrocysomma sulfurea*
11. *Nephrolepis cordifolium*	24. *Pteria* sp.
12. *Pessopteris crassifolia*	25. *Tectaria cicutaris*
13. *Platycerium bifurcatum*	26. *Woodwardia orientalis*
B. Plants from the Tatra Field Research Station of the Polish Academy of Sciences in Zakopane (Poland)	
1. *Athyrium philix femina*	7. *Dryopteris spinulosa*
2. *Dryopteris affinis*	8. *Matheutis sthrutiopteris*
3. *Dryopteris "bushiana«*	9. *Polystichum browni*
4. *Dryopteris dilatata*	10. *Polystichum lobatum*
5. *Dryopteris oreopteris*	11. *Phegopteris lobatum*
6. *Dryopteris philix-mass.*	

Table 10

Proportion of individual polyprenols in the natural mixtures of polyprenyl esters in leaves of trees of Rosaceae family.

Estimations were made at the age of 21 or 24 weeks after terminal bud unfolding (number of weeks indicated in brackets).

Plant	% of prenologues									
	P-16	P-17	P-18	P-19	P-20	P-21	P-22	P-23	P-24	P-25
Cotonoaster lucida (24)	1.1	3.7	15.5	27.9	20.7	12.6	7.9	5.0	3.4	2.2
Crataegus crus-galli (24)	1.5	2.7	7.4	20.8	23.1	16.7	11.3	7.7	5.2	3.6
Prunus serratia (24)	1.8	6.1	23.1	35.6	18.6	8.1	3.7	1.7	0.9	0.4
Sorbus suecica (21)	1.0	2.2	6.9	16.3	42.6	14.9	7.9	4.3	2.4	1.5

prenols composed of approx. 25–55 isoprene units is often present. A similar pattern of polyprenols was found in a number of gymnosperm plants, e.g. in *Cycadopsida* in which the type, the amounts and the proportions of polyprenols were similar or the same as in various *Rosaceae* like *Sorbus suecica, Prunus serratia*, etc. (cf. Fig. 4). In many gymnosperm species belonging to *Araucariaceae, Cupressaceae* or *Taxodiaceae*, the pattern of polyprenols with two distinct groups of prenologues is similar to that found in various species of the genus *Potentilla* or genus *Rosa*. The studies performed so far indicate that the longest chain prenologues are a charac-

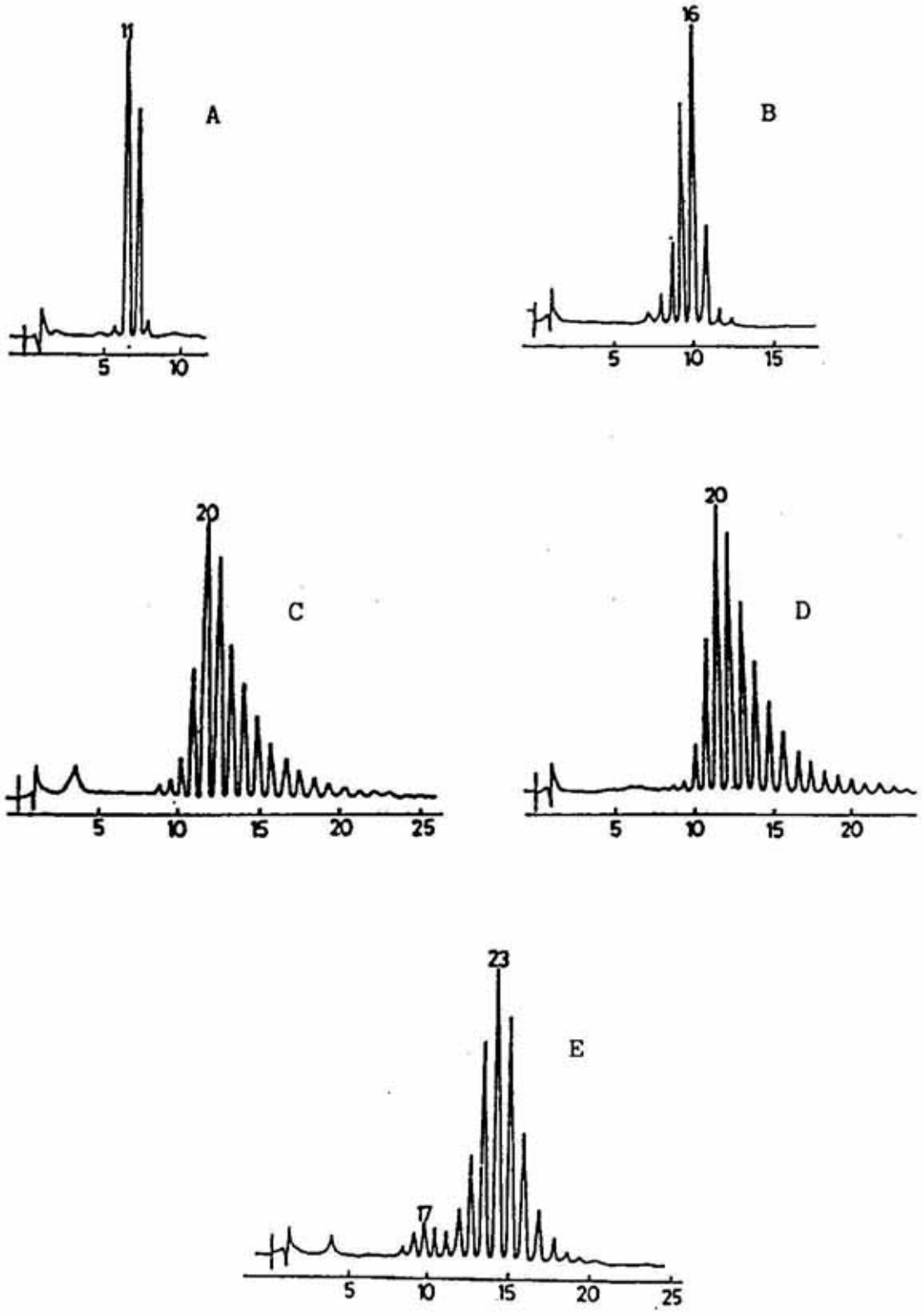


Fig. 4. Chromatographic records of the most typical natural mixtures of polyprenols extracted from leaves of (A) Rhus typhina, (B) Picea engelmanii, (C) Zamia integrifolia, (D) Sorbus suecica, and (K) Agathis robusta. Details of chromatography as in Fig. 3. Reproduced from [26] and [31].

Table 11

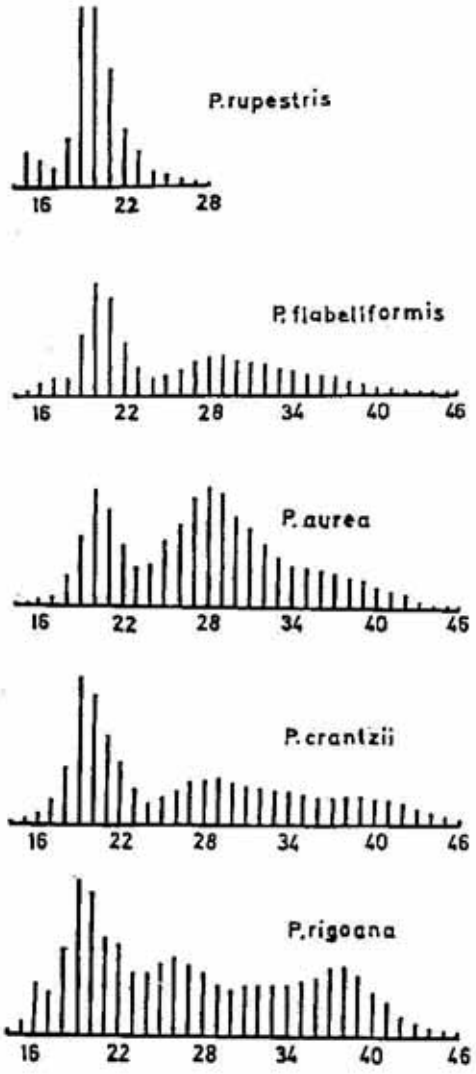
Polyprenols in leaves of various species of genus Potentilla.

(W) Samples of leaves were collected from plants in the University Botanical Garden in Wrocław (Poland), in June 1989. Other samples were from the Botanical Garden in Edinbourg (Scotland), September 1991. The range of polyprenol concentration was evaluated by TLC on silica gel G; ethyl acetate:benzene (19:1, v/v), spots were detected with iodine and their intensity was compared with that of known amounts of standards. Chain length of polyprenols was estimated by RPTLC in acetone on RP-18 plate.

Plant species	Approximate content (% of dry weight)	Dominating prenologues (number of isoprene units)
P. agrophylla	1.0–2.0	19, 20, 21, 27, 28, 29
P. alba	1.0–2.0	18, 19, 20, 23, 24, 25
P. alchemiliodes	0.2–0.5	19, 20, 21
P. ambigua	0.2–0.5	18, 19, 20
P. anserina (W)	0.5–1.0	19, 20, 21, 24, 25, 26
P. arbuscula	< 0.1	18, 19, 20
P. argentea	< 0.1	18, 19, 20, 29, 30, 31, 38, 39, 40
P. argyrophylla	1.0–2.0	18, 19, 20, 26, 27, 28
P. atrosanguinea Sc.	1.0–2.0	18, 19, 20, 25, 26, 27
P. aurea (W)	0.2–0.5	19, 20, 21, 27, 28, 29
P. chrysanthea	0.5–1.0	18, 19, 20, 26, 27, 28
P. crantzii (W)	< 0.1	17, 18, 19, 26, 27, 28
P. crinita	< 0.1	19, 20, 21, 28, 29, 30, 38, 39, 40
P. cuneata	< 0.1	18, 19, 20
P. drummondii	1.0–2.0	18, 19, 20
P. erecta	–	20, 21, 22, 27, 28, 29
P. fissa	–	18, 19, 20
P. flabeliformis	< 0.1	19, 20, 21, 27, 28, 29
P. fragiformis	0.1–0.2	19, 20, 21
P. fragarioides	< 0.1	18, 19, 20
P. fruticosa	0.2–0.5	17, 18, 19, 24, 25, 26
P. heptaphylla	0.5–1.0	19, 20, 21, 25, 26, 27
P. hippiana	–	19, 20, 21, 30, 31, 32, 38, 39, 40
P. hyparctica	0.2–0.5	19, 20, 21, 27, 28, 29
P. impolita	–	19, 20, 21, 27, 28, 29
P. intermedia	0.5–1.0	19, 20, 21, 25, 26, 27
P. macrobiana	–	18, 19, 20, 26, 27, 28
P. megalantha	0.5–1.0	18, 19, 20, 25, 26, 27
P. montenegrina	< 0.1	19, 20, 21, 27, 28, 29
P. nepalensis	0.2–0.5	19, 20, 21, 26, 27, 28
P. nitida	–	17, 18, 19
P. norvegica	< 0.1	19, 20, 21, 27, 28, 29
P. palustris	–	17, 18, 19
P. pulcherrima	–	19, 20, 21, 29, 30, 31

Table 11 (continued)

Plant species	Approximate content (% of dry weight)	Dominating prenologues (number of isoprene units)		
P. recta	–	19, 20, 21,	28, 29, 30,	38, 39, 40
P. reptans	–	17, 18, 19		
P. rigoana	< 0.1	19, 20, 21,	26, 27, 28, 33, 34,	39, 40
P. rupestris	0.1–0.2	18, 19, 20		
P. russeliana	–	21, 22, 23	29, 30, 31,	
P. speciosa	–	19, 20, 21		
P. tabernaemotnani	–	19, 20, 21,	28, 29, 30	37, 38, 39
P. thuringiaca	–	19, 20, 21,	28, 29, 30	
P. umbrosa	–	19, 20, 21,	28, 29, 30	



teristic feature of species of the *Rosaceae* family and that in none of them prenol-11, which is the most common plant polyprenol, or prenol-12, was found. It should be pointed out, however, that from the total number of approx. 3000 species of the *Rosaceae* family the number of species examined by us did not exceed 200. A thorough search for polyprenols in this systematic group may result in finding shorter or still longer polyprenols. It should be recalled that very rarely in angiosperm families other than the *Rosaceae* one could find polyprenols longer than prenol-14, -15. Świeżewska & Chojnacki [27] reported the occurrence of moderate amounts of prenol-18 and similar prenologues in *Ericaceae*. The studies of Carroll [30] documented the presence of polyisoprenoid alcohols composed of 15, 16 and 17 isoprene units in leaves of monocotyledon plants; they were mixtures of both fully unsaturated polyprenols and α-dihydroprenols (dolichols). Polyprenols of the same size, i.e. composed of 14, 15, 16, 17 residues and a low amount of longer ones were found in leaves of a few species of the *Capparidaceae* family [18]. It seems that in earlier studies the presence of the lon-

Fig. 5. Schemes of chromatographic records of polyprenol fractions from leaves of various species of the genus Potentilla.
Numbers 16–46 indicate the chain length of polyprenol (number of isoprene residues). The height of each bar represents the proportion of the prenologue calculated from the size of the HPLC peak. High pressure liquid chromatography was performed as in Fig. 2.

Table 12

Polyprenols in leaves of various species of the genus Rosa (Rosaceae family).

Approximate content of polyprenols was estimated by TLC [10]. Plants were from the University Botanical Garden in Poznań (Poland).

Species	Number of isoprene units in dominating polyprenol	Content of polyprenols (% of wet weight)
Rosa arvensis	18, 19, 20, 33, 34, 35	0.10–0.20
Rosa blonda	18, 19, 20, 28, 29, 30	0.05–0.10
Rosa gallica	18, 19, 20, 28, 29, 30	0.05–0.10
Rosa glauca	18, 19, 20, 28, 29, 30	0.05–0.10
Rosa multiflora	18, 19, 20, 30, 31, 32	0.05–0.10
Rosa nitida	18, 19, 20, 28, 29, 30	0.05–0.10
Rosa nutkana	18, 19, 20	0.05–0.10
Rosa paulii	18, 19, 20, 31, 32, 33	0.05–0.10
Rosa pimpinelif.	18, 19, 20, 30, 31, 32	0.10–0.20
Rosa rugosa	18, 19, 20, 31, 32, 33	0.10–0.20
Rosa sericea	18, 19, 20	0.05–0.10
Rosa setigera	18, 19, 20, 31, 32, 33	0.20–0.50
Rosa tomentosa	18, 19, 20, 28, 29, 30	0.05–0.10
Rosa virginiana	18, 19, 20, 30, 31, 32	0.20–0.50
Rosa vichural.	18, 19, 20, 31, 32, 33	0.20–0.50

Table 13

Polyprenols in leaves of various species of the genus Rubus (Rosaceae family).

Approximate content of polyprenols was estimated by TLC [10]. Plants were from the Arboretum in Kórnik (Poland).

Species	Number of isoprene units in dominating polyprenol	Content of polyprenols (% of wet weight)
Rubus bellardi	18, 19, 20	0.10–0.20
Rubus corylifolium	18, 19, 20	1.00–2.00
Rubus grabovskii	18, 19, 20	0.05–0.10
Rubus hirtus	18, 19, 20	0.10–0.20
Rubus nessensis	18, 19, 20	0.10–0.20
Rubus plicatus	18, 19, 20, 21	0.50–0.10
Rubus radula	18, 19, 20	0.05–0.10
Rubus schleicherii	18, 19, 20	1.00–2.00
Rubus selmerii	18, 19, 20	0.10–0.20
Rubus villicaulis	18, 19, 20	0.05–0.10

gest polyprenols might have been overlooked due to the lack of appropriate chromatographic methods. The recent data from our laboratory (Skoczylas *et al.*, in preparation) point to the occurrence of still longer polyprenols (up to 80 isoprene residues) in leaves of a halophytic tropical tree *Lumnitzera racemosa* belonging to the *Combretaceae* family.

On comparing the above mentioned results concerning many various species of the *Rosaceae* family with their very long-chain (more than 19 isoprene residues) polyprenols, and the

presence of mainly prenol-10, -11 and -12 in a variety of species belonging to other families of angiosperms the uniqueness of the polyprenol pattern in *Rosaceae* is evident. The other angiosperm families like e.g. *Magnoliaceae* or *Moraceae* (cf. Tables 1–3) are characteristic (and similar to each other) in that in all of them a domination of prenol-10, -11 or -12 was always observed.

Physiological variations in the content of polyprenols

The phenomenon of age-dependent accumulation of polyprenols in leaves of some plants (mainly *Aesculus hippocastanum*) was described by Wellburn & Hemming [10]. While the earlier studies concerned mainly the accumulation of prenol-11 and -12, our research dealt also with the accumulation of polyprenols composed of 19, 20 and more isoprene units, which occur in plant leaves in the form of acetic esters and fatty acid esters. In Fig. 6 are shown seasonal variations in polyprenol content in 8 plant species growing in the open air areas of the city of Warsaw [31]. The age of the trees was 20–50 years. An increase in the content of polyprenols with the age of leaves was observed in all the plants studied. The highest amounts were noted in late August in the case of plants accumulating prenol-11 and prenols of similar size, while in plants accumulating prenol-19, -20, etc. (in the form of esters), the maximum accumulation was observed in late September and in October. It was found that, following the peak of accumulation, there was a drop in the polyprenol content. This decrease was more dramatic in the case of *Rosaceae* in which the content dropped rapidly to very low values. It is not known whether the disappearance of polyprenols from leaves is due to their metabolism *in situ* or to their evacuation from leaves to other parts of the plant. Fluctuations in the content of polyprenols were also observed on studying green needles of nine coniferous trees in December, March and May. This experiment (Fig. 7) was performed on a set of 2-year-old plants which were specially grown from seedlings for one year before the actual experiment was started, and then half of them was kept in

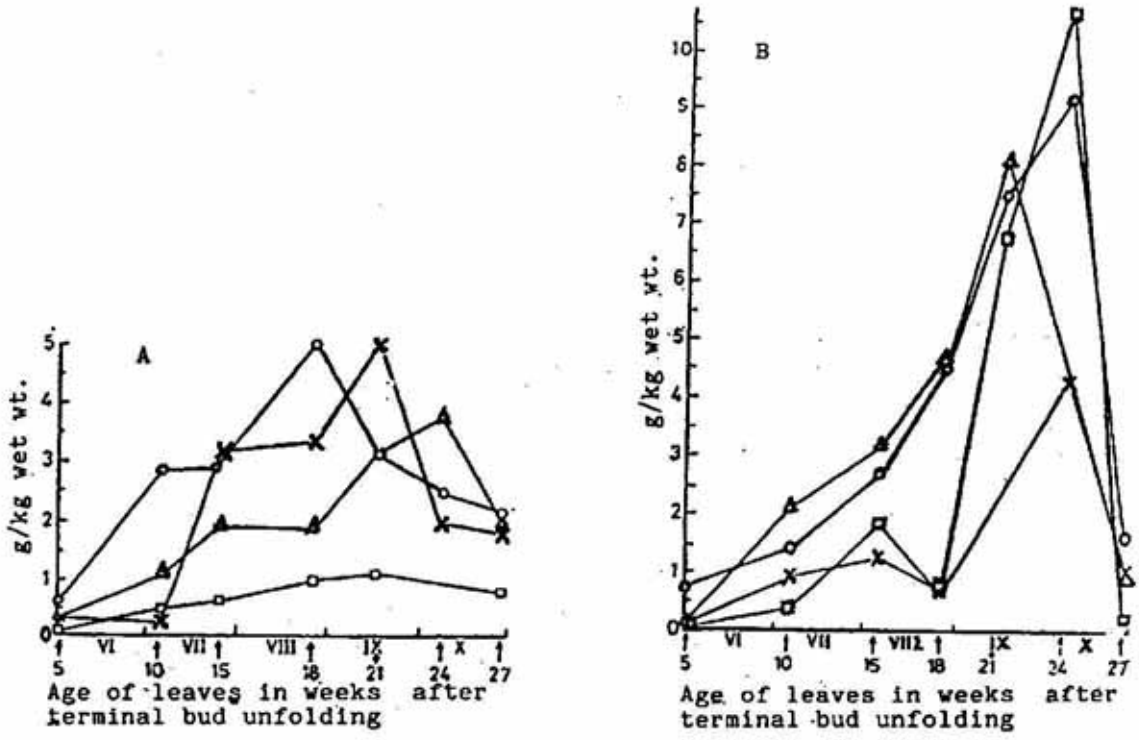


Fig. 6. Seasonal changes in polyprenol content.
A, of total prenol-10–12 in leaves of plants: ×, *Carya cordiformis*; Δ, *Juglans regia*; □, *Magnolia liliflora*, and ○, *Rhus typhina*. B, of total prenols-16–25 in leaves of plants: ○, *Cotonoaster lucida*; □, *Cratageus crus-galli*; ×, *Prunus serratia*, and Δ, *Sorbus suecica*.

natural open air conditions (the Botanical Garden in the Powsin suburb of Warsaw) and they were affected by all the drastic climatic changes that are characteristic of middle Europe for the period between December and May. The second half of the plants was transferred, 5 months before starting the analyses of polyprenols, to a thermostated all-glass house situated in the same area. They were kept there under the same conditions of lighting but at the temperature maintained within the limits of 8–15°C. While being kept in artificial conditions the plants studied did not show any pathological changes during the 11 months of the experiment.

In plants growing in the open air seasonal changes in polyprenol content were evident in all the species studied. It seems that there exists a rhythm of fluctuations, and that the content of polyprenols is rather low in late autumn (December) and increases at least until May. However, the course of fluctuations was somewhat different in *Taxus* sp. (a decrease between December and March) and in *Pinus peuce* (a decrease between March and May) in which changes seemed to be delayed. In the set of plants growing in the controlled conditions changes in polyprenol content were also visible though their rhythm was somewhat modified, and in some species between December and May even a decrease was observed.

The long term fluctuations in the type and content of polyprenols are evident from the studies performed on needles or leaves of evergreen plants of various age (1–3 years) in spring (June) and in autumn (October) in four plant species (Fig. 8). One can see both the seasonal variations in the polyprenol content and also the steady increase in the amount of polyprenols during the 3 years period. The highest amounts of polyprenols were observed in autumn, the lowest in spring.

Although the content of polyprenols decreased over the period October–June, in late spring (June) it was consistently higher in each consecutive year. One may assume the existence of a "basic level" of polyprenols which remains in the green parts during winter despite seasonal elimination of polyprenols. The nature of this elimination in winter is still unknown. In the needles of coniferous trees, e.g. *Abies koreana, Picea abies virg.* and *Pinus strobus* (Fig. 8) the "basic level" of polyprenols, which becomes the starting level in late spring, tends to increase each year. This observation is consistent with the results of examining green needles of *Pinus aristata, Pinus heldreichii* and *Taxus baccata* (Table 14) in the middle of winter (January 15). The age of needles in these studies was up to 6 years. While the total content increased with age in all three species studied there was not much change in the proportions of individual prenologues in the polyprenol mixture. Only in *T. baccata* a shift towards longer chain prenologues was observed in the 2- and 3-year-old needles. Thus it is evident that considerable amounts of polyprenols remained in green needles over winter.

The age-dependent variations in the content of polyprenols may lead to erroneous conclusions when classifying the plant as a rich or poor source of polyprenols. Single analyses could be misleading and we are aware of the possibility that on studying at random numerous samples of various species some of the very rich sources might have been not registered by us. On the other hand, even if our search for polyprenols was fragmentary with respect to the above mentioned seasonal variations, our studies in their part performed on gymnosperm plants growing in Middle Europe (e.g. Table 7 and 8) gave similar results to those reported by Ibata *et al.* [24, 32, 33] on the local flora of Japan. Only small discrepancies concerning the polyprenol family were noted that could arise from the fact that the two studied specimens of a given species were grown in very distant geographical regions or that, in fact, they may have represented non-identical species; one should take into account that erroneous botanical classification may happen in the case of specimens of two species exhibiting only discrete morphological differences. In general, the polyprenol pattern of green needles of gymnosperm plants is a characteristic feature of a given species. It was observed that even genetic mutants of *Pinus mugo* and *Pinus uliginosa* could be distinguished by examination of their polyprenol pattern (E. Świeżewska, unpublished).

The occurrence of different derivatives of polyprenols in plants may reflect the physiological state of the organism. It is known from the studies on lipid-linked sugar intermediates in bacteria and in animal tissues that phosphate esters of polyprenols and dolichols and their

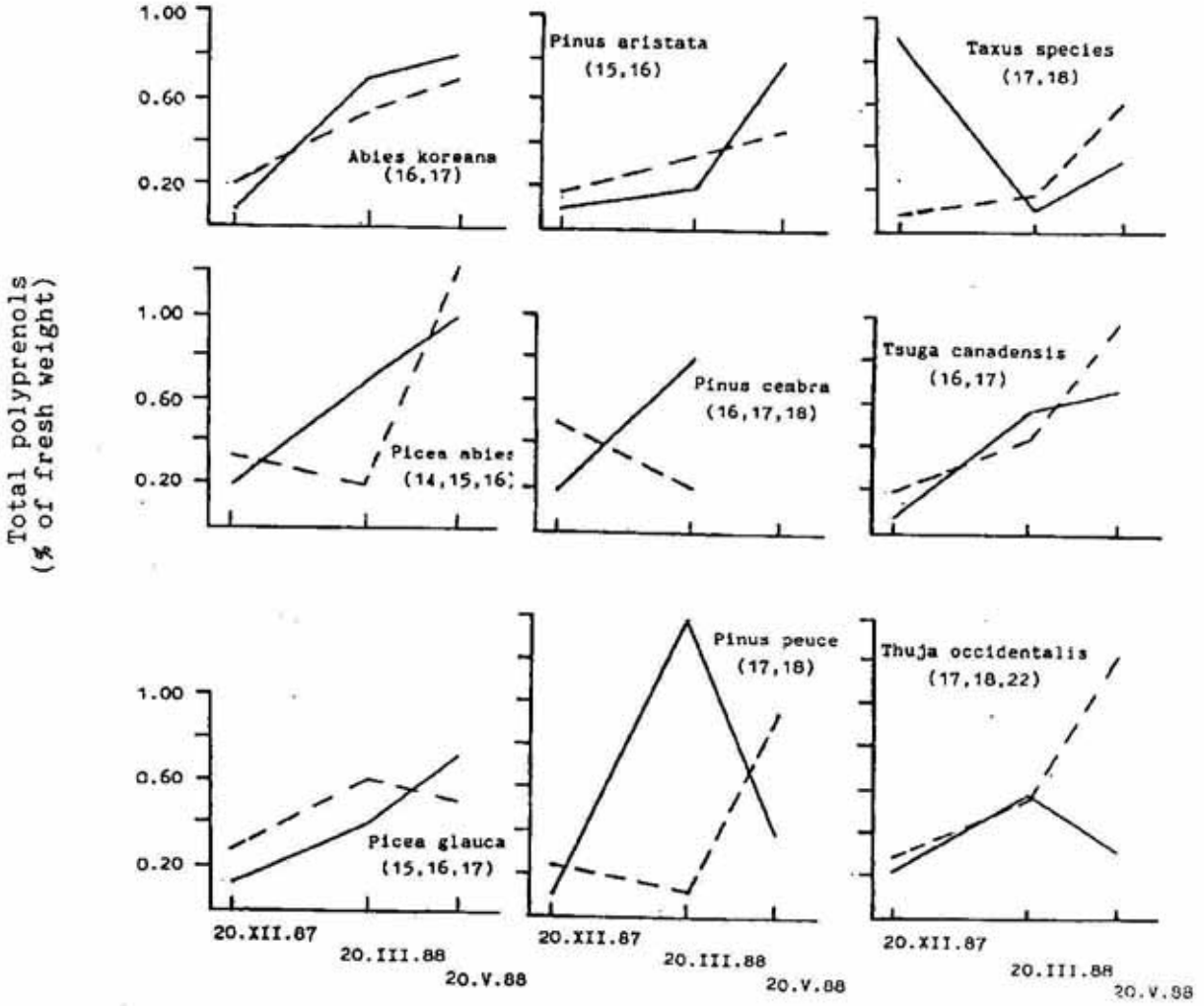


Fig. 7. Seasonal fluctuations of polyprenol content in leaves (needles) of plants cultivated in natural environment (—) and in isolated space at 8–15°C (- - -).

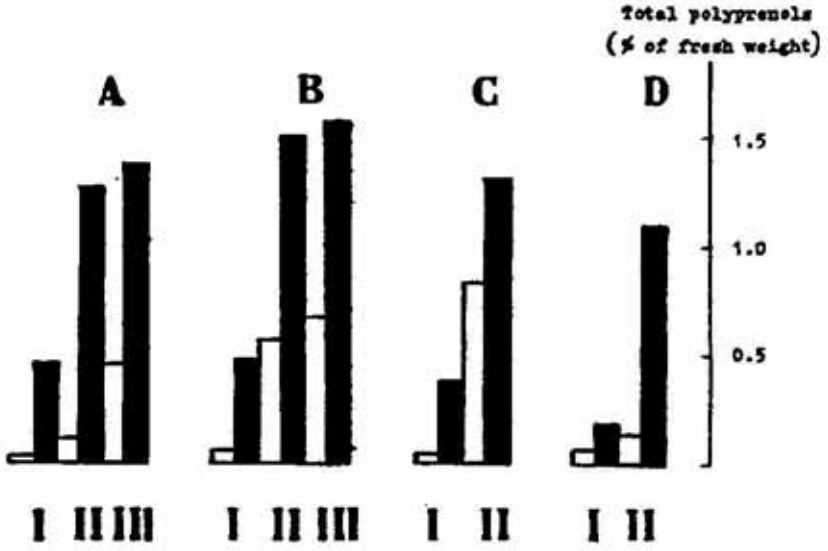


Fig. 8. Seasonal changes in the content of polyprenols in the 1, 2 and 3-year old leaves of evergreen plants in spring (open columns) and autumn (dark columns).
I, II, III — age of leaves. A, *Abies koreana*; B, *Picea abies virg.*; C, *Pinus strobus*; D, *Cotonoaster Ursynów*. Reproduced from [25].

Table 14

The content of polyprenols in coniferous trees in the needles of various age

Plant	Age of needles (year)	Total polyprenols (% of fresh weight)	Content of prenologues (%)											
			13	14	15	16	17	18	19	20	21	22	23	24
Pinus aristata	I	0.06		10.1	22.3	31.5	23.0	13.2						
	II	0.42		12.1	23.2	34.9	19.6	10.3						
	III	0.48	2.6	8.0	25.9	35.7	19.5	6.0	2.2					
	IV	0.32	3.1	9.4	28.5	36.8	17.7	4.5						
	V	0.32	2.8	8.5	26.5	37.7	19.2	5.3						
Pinus heldreichii	I	0.07			14.6	21.9	30.1	25.3	8.1					
	II	0.30				13.6	38.5	35.7	12.1					
	III	0.46			2.2	11.2	36.4	40.0	8.9	1.2				
	IV	0.72			2.3	12.5	37.1	37.9	10.2					
	V	1.09			2.0	11.5	35.6	38.1	11.1	1.6				
	VI	1.18				12.5	39.5	39.0	8.9					
Taxus baccata	I	0.06				7.8	30.8	26.9	7.8	6.1	11.6	9.1		
	II	0.37				3.6	18.0	29.5	15.8	6.5	7.4	9.6	6.4	2.4
	III	0.52				3.1	16.8	31.2	13.8	6.5	8.2	10.6	7.1	2.7

sugar derivatives are key intermediates in the biosynthesis of bacterial sugar heteropolymers and glycoproteins [7, 34]. There is a large body of information on the occurrence of esters of dolichols with fatty acids and it has been suggested [35] that they serve as intracellular carriers of fatty acids. In the studies on plants apart from free polyprenols, their esters with acetic acid and with fatty acids were found. There are no data on the occurrence of larger amounts of phosphate esters of polyprenols and their sugar derivatives in plants, probably because this group of compounds escaped the attention of plant biochemists due to technical difficulties in isolation of very hydrophobic anionic compounds. In the case of a few plant species thoroughly examined in our laboratory (*Gingko biloba, Nephelium litchii*; W. Jankowski, unpublished) no polyprenol characteristic of the given species was found to be esterified with phosphate group. The phosphate esters of polyisoprenoid alcohols were considered mainly as coenzymes occurring only in small quantities. However, it turned out recently, that they can be accumulated in some pathological conditions, e.g. in brain tissue in dogs [36, 37]. These phenomena might be not exceptional, and checking some of the plants for the presence of polyprenyl phosphates is required.

In general, the occurrence of carboxylic acid esters of polyprenols is restricted rather to polyisoprenoid alcohols composed of more than 15 isoprene units, i.e. the plants containing families of polyprenols of this chain length and longer ones accumulate them in the form of acetic acid esters or as fatty acid esters. In most of the gymnosperms, the carboxylic acid component of the ester is acetic acid; in some of them fatty acid esters are present, e.g. in *J. communis* [23], where about 40% of carboxylic acid equivalents were 14:0, 16:0, 18:3 and 20:3. The carboxylic acid components of the polyprenyl esters were characterized in detail in our studies on *Potentilla aurea* [28]. This plant shows the tendency to acylate longer polyprenols with more hydrophobic fatty acids (Fig. 9).

In our studies performed on other species belonging to the family *Rosaceae*, and representing trees and shrubs, mainly acetic acid esters were found in the group of polyisoprenoid esters representing prenol-19, -20 and longer ones. In plants in which both free polyprenols and polyprenyl esters were present, in both groups the same prenologues were de-

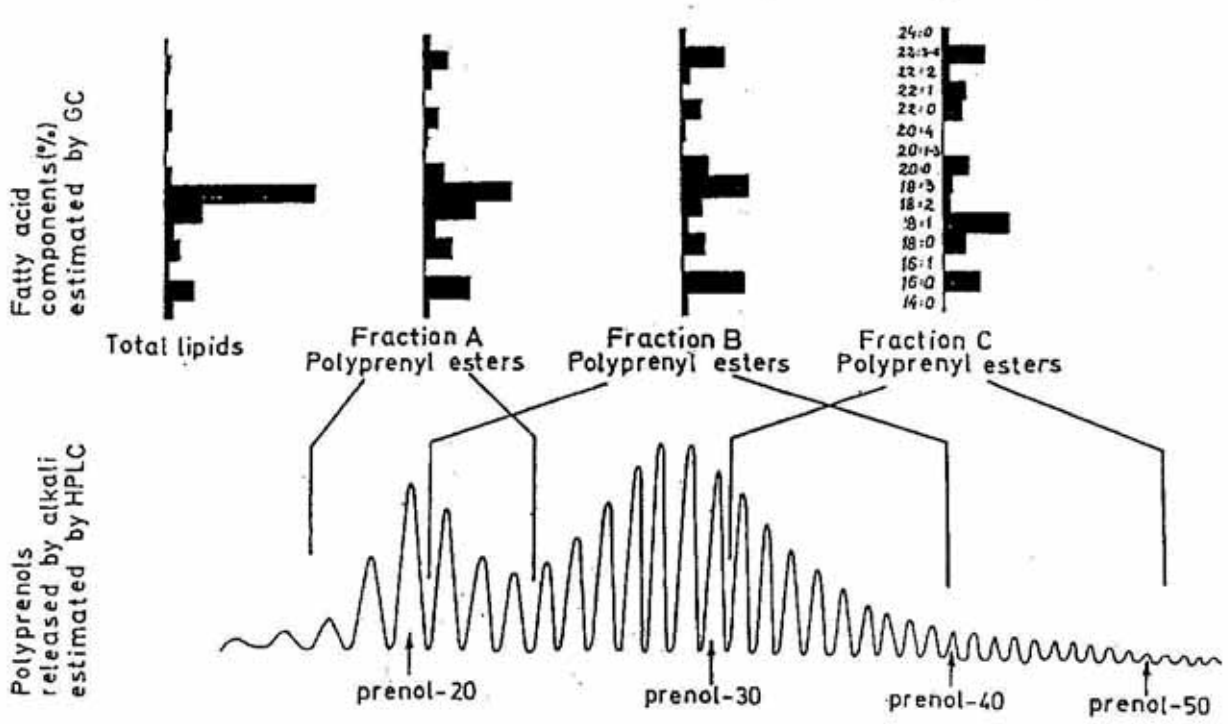


Fig. 9. Schematic illustration of fatty acid composition of polyprenyl fatty acid esters isolated from leaves of Potentilla aurea.

A, B, C, Fractions of polyprenol esters obtained by hydrophobic liquid chromatography on Lipidex-5000 of total lipids (native polyprenyl esters). Reproduced from [29].

tected [23, 24, 32]. It seems that the total pool of polyprenols undergoes at random the acylation mechanism, though there is some selectivity with respect to fatty acids taking part in the acylation. Discrete changes in the type of fatty acid acylating different polyprenols could be observed as shown in Fig. 9, and in similar studies on polyprenyl esters in *Rosa virginiana* (E. Świeżewska, unpublished).

In plants containing shorter chain polyprenols, prenol-10, -11 or -12 as dominating prenologues, the proportion of esterified polyisoprenoid alcohols varied between 5–20% (cf. Table 1 and 2). Here also the same prenologues were present in the same proportions as free alcohols and as carboxylic acid esters.

Dolichol dolichoates in which the long-chain α-dihydropolyprenols are esterified with dolichoic acid — α-dihydropolyprenolic acid represent a special type of long-chain polyisoprenoid esters [38]. These compounds have been detected in animal tissues, but they do not occur commonly, especially there were no reports on their occurrence in plants.

The possibility of habitat-dependent changes in the occurrence of polyprenols in plants

In the course of the search for plant sources rich in polyprenols not only various plants throughout the botanical systematics (as shown in most of the Tables), but various specific habitats were also examined. In the latter approach three groups of plants were examined; the Mediterranean, mainly ever-green plants (Table 15), mountain plants (Table 16) and aquatic plants (Table 17). One should add that the groups of plants characteristic of tropical and subtropical regions (Tables 1, 2, etc.) may represent tropical and subtropical habitats, but the fact that the plants were artificially cultivated in special botanical gardens does not allow to consider them as true representatives of those habitats.

The search in the group of Mediterranean plants (Table 15) demonstrated the presence of several types of polyprenol mixtures already described above on discussing the taxonomic groups of plants. We could not find a common feature of Mediterranean plants with respect to polyprenol spectrum, however the "taxon-specific" polyprenol spectra were evident; additionally some angiosperms accumulated polyprenols very efficiently and a number of studied plants were poor sources of polyprenols. The two richest sources of polyprenols were *Aristiolodin sempervirens* (*Aristolodinaceae*) and *Laurus nobilis* (*Lauraceae*). In both plants the polyprenol mixture (mainly free alcohols) contained prenol-9, -10, -11 and -12 as dominating prenologues. It seems that, in the Mediterranean flora, the polyprenol-rich or moderately rich sources are rather frequent. A few species of plants exhibited also the presence of uncommon polyprenols composed of 15, 16 and more isoprene units, e.g. *Rhamnus alaternus*. Polyprenol mixtures of unique composition were found in *Pistacia lentiscus*, *Quercus coccifera* and *Quercus ilex*. In those species two groups of polyprenols were present, one with dominating prenol-11 and -12, and another with dominating prenol-16 and -17. In the group of mountain plants the approx. 50 species studied represented herbaceous plants belonging to several systematic families and characteristic of the high mountain valleys and meadows in the Tatra mountains (Table 16). Only a few species exhibited the presence of larger amounts of long-chain polyprenols; these were: two representatives of the genus *Potentilla* (their polyprenol pattern was described above), one specie of the *Rosaceae* family, *Geum montanum*, and one specie of the family *Ericaceae*, *Vaccinia vitis-idaei*. The pattern of polyprenols in the latter plant (dominating polyprenols built up from about 17 and more isoprene units) was found recently also in other *Ericaceae* species (W. Jankowski, preliminary unpublished results).

In the group of aquatic plants consisting of 47 species only in leaves of one of them, *Myriophyllum aquaticum*, traces of prenol-11 were detected (Table 17). It should be noted that in another *Myriophyllum* species, *M. verticillatum*, the presence of polyprenols was described [39]. In other aquatic species polyprenols were not detected.

THE SHORTER CHAIN POLYPRENOLS IN THE WOOD OF *BETULA* sp.

While all polyprenols described above were isolated from green leaves, prenol-7 and similar short-chain di-*trans* poly-*cis* prenols were found in the wood. The discovery of the presence of these polyprenols in early 1960-ies by Lindgren came from the observation on the

Table 15

Long-chain polyprenols in leaves of hard-leaf ever-green Mediterranean flora.

Samples of leaves (at least two years old) were collected in May and June 1988 by Prof. Kazimierz Browicz.

Plants	Dominating prenologues
A. Rich sources (over 0.5% of dry weight)	
Aristolodinaceae	
Aristolodin sempervirens (NNW Peloponez)	prenol-9, -10, -11, -12*
Lauraceae	
Laurus nobilis (N Peloponez)	prenol-9, -10, -11, -12*
B. Fairly rich sources (0.05–0.5% of dry weight)	
Anacardiaceae	
Pistacia lentiscus (Zakinthos Island)	prenol-9, -10, -11, -12, -16, -17, -18**
Ericaceae	
Arbutus andr. (Central Greece)	prenol-17, -18, -20**
Fagaceae	
Quercus coccifera (NW Peloponez)	prenol-10, -11, -12, -15, -16, -17**
Quercus ilex (NW Peloponez)	prenol-10, -11, -12, -15, -16, -17**
Leguminosae-Caesalp.	
Ceratonia siligua (Zakinthos Island)	prenol-16, -17, -18, -19, -20**
Oleaceae	
Olea europea var. sylv. (Zakinthos Island)	prenol-15, -16, -17, -18, -19**
Rhamnaceae	
Rhamnus alaternus (NW Peloponez)	prenol-22, -23, -24, -25, -26**
Rosaceae	
Rosa sempervirens (NW Peloponez)	prenol-28, -29, -30, -31, -32**
C. Poor sources (less than 0.05% of dry weight)***	
Arbutus unedo, Buplenrum fruticosum, Cistus incanus, Cistus monspeliensis, Cistus salifolius, Erica manipuliflora, Globularia alypum, Ilex aqiufolius, Lonicera implexa Art., *Myrtus communis, Ruscus aculenta, Smilax aspera*	

*Free polyprenols; **free and esterified polyprenols; ***the following species are listed in alphabetical order irrespectively of systematic group. The type of polyprenol is not given.

difficulties in bleaching the silver birch wood in the course of cellulose production [40].

The polyprenols in birch wood occur in the form of fatty acid esters and their content may vary between 0.5 and 1.0%. The natural mixture of "betulaprenols" contains prenol-6, -7 and -8 in variable proportions. Sometimes prenol-9 is visible. Prenol-5 was never present in the betulaprenol mixture (T. Chojnacki, unpublished). It should be mentioned that betulaprenols composed of 6–8 isoprene residues occur only in wood tissue; leaves of *B. verrucosa* did not contain them. Prenol-11 was found to be the typical polyprenol in leaves of silver birch.

Betulaprenols were present in all parts of the silver birch trunk, though their content varied and the proportions of individual components of the polyprenol mixture differed slightly in various parts of the trunk. The highest amounts were found in the outer parts and in the central part of the trunk (Fig. 10). The occurrence of relatively short-chain betulaprenols seems to bear no relation to any physiological function, except that these lipids were considered to provide the trunk with mechanical resistance against frost-caused breaks affecting the trees in the cold climate (B. Lindgren, personal communication).

Table 16

List of herbaceous mountain plants tested for the presence of long-chain polyprenols.
The semiquantitative assay of polyprenol content was performed by TLC. In the polyprenol-negative plants the content of polyprenols was < 0.005%.

A. Polyprenol-positive		Approximate content (% of wet weight)
1. *Geum montanum*	prenol-17, -18, -19, -20	0.05
2. *Potentilla aurea*	prenol-19, -20, -21, -25, -26, -27, -28, -29	≥ 0.05
3. *Potentilla crantzii*	prenol-19, -20, -21, -25, -26, -27, -28, -29	0.05
4. *Vaccinia vitis-idaea*	prenol-17, -18, -19	0.05

B. Polyprenol-negative	
1. *Aconiton colibotrion*	23. *Lunaria rediviva*
2. *Anemone narcissiflora*	24. *Mulgenium alpinum*
3. *Anthyllus alpestris*	25. *Mutellina purpurea*
4. *Astrantia maior*	26. *Oxytropis campestris*
5. *Angelica archangelica*	27. *Phyteuma orbiculare*
6. *Avenastrum planicum*	28. *Polemonum caeruleum*
7. *Betonica officinalis*	29. *Polygonum verticullatum*
8. *Campanula glomerata*	30. *Prunella grandiflora*
9. *Campanula persicifolia*	31. *Pulsatilla slavica*
10. *Carlina acaulis*	32. *Salix hastata*
11. *Centaurea aplestris*	33. *Salvia glutinosa*
12. *Cincifuga europea*	34. *Sassurea alpina*
13. *Cirsium eriophorum*	35. *Sedum maximum*
14. *Cirsium eristhales*	36. *Seldanella carpatica*
15. *Delphinium kotulae*	37. *Senecio subalpinus*
16. *Dianthus praecox*	38. *Silene inflata*
17. *Digitalis grandiflora*	39. *Succisa pratensis*
18. *Heliantheum numm. grandifl.*	40. *Trollius europeus*
19. *Hieracium aurantiacum*	41. *Valeriana sambucifolia*
20. *Hieracium tetrense*	42. *Verbascum nigrum*
21. *Hieracium villosum*	43. *Veronica reucrium*
22. *Listeria ovata*	

SPECULATIONS ON THE APPEARANCE OF POLYPRENOLS IN THE COURSE OF EVOLUTION OF PLANTS

In gymnosperm plants the accumulation of long-chain polyprenols in green leaves and needles was observed in almost all species studied [25]. We could not find accumulation (even the presence) of long-chain polyprenols in various *Pteridophytina* (see above), nor in any of the 30 species belonging to *Hepaticopsida* (from the collection of Professor J. Szwejkowski in Poznań, Poland; E. Świeżewska, unpublished). The evolutionary oldest plant species that were found to be polyprenol-positive belonged to *Cycadopsida*. This systematic group, together with other gymnosperm plants which, as a rule, are rich polyprenol sources, may mark the appearance of polyprenols at the devonian

Table 17

Aquatic plants checked for the presence of long polyprenols in green leaves.

The plants were from the collection of the Wrocław Botanical Garden. In all studied samples except No. 38 the amount of polyprenols was undetectable (less than 0.005%). In *Myriophyllum aquaticum* trace amounts of prenol-11 were found.

1. *Acorus graminensis*	25. *Echinodorus loniscapus*
2. *Alternantera reimecki*	26. *Echinodorus uruguaiensis*
3. *Apongenton capuroni*	27. *Egeria densa*
4. *Apongenton ulvaceus*	28. *Heteranthera dubia*
5. *Apongenton undulans*	29. *Hydrocleis nymphoides*
6. *Anubias barteri* var. *glabra*	30. *Hygrophyla angustifolia*
7. *Anubias barteri* var. *nana*	31. *Hygrophyla diformis*
8. *Anubias barteri* var. *barteri*	32. *Hygrophyla guyanensis*
9. *Bacopa amplexicantis*	33. *Hygrophyla polisperma*
10. *Bacopa monieri*	34. *Lobelia cardinalis*
11. *Crinum (natans)* sp. *torta*	35. *Lymnophyla aquatica*
12. *Cryptocoryne affinis*	36. *Lymnophyla indica*
13. *Cryptocoryne aponogetifolia*	37. *Ludvigia natans*
14. *Cryptocoryne balansae*	38. *Myriophylum aquaticum*
15. *Cryptocoryne Grabovsky*	39. *Nuohar sagitifolius*
16. *Cryptocoryne pontederifolia*	40. *Nymphea species*
17. *Cryptocoryne vendtii*	41. *Pistia stratiotes*
18. *Echinodorus amazonicus*	42. *Potamogeton goughii*
19. *Echinodorus ascherzovianus*	43. *Potamogeton octangia*
20. *Echinodorus anrieuxii*	44. *Rotala rotundifolia*
21. *Echinodorus blecherii*	45. *Samolus veterandii*
22. *Echinodorus cordifolia*	46. *Trichocoronis (schimerzia) rivularis*
23. *Echinodorus grandiflorus*	47. *Valisneria neotropicalis*
24. *Echinodorus horemanii*	

period (270–320 million years ago). The few species of the *Cycadopsida* class that were studied in our laboratory constitute a high proportion of the total number of species in this group estimated as less than 100. The *Cycadopsida* are considered to constitute a not numerous group of the remaining, not yet extinct species.

The group of gymnosperm plants contains species in which there is present either one family of polyprenols representing a wide range of prenologues differing in size by one isoprene unit, or two polyprenol families differing in size by 5–8 isoprene units. The occurrence of two polyprenol families is very common in gymnosperm plants.

In one family of angiosperm plants, i.e. in *Rosaceae*, we found a striking similarity of their polyprenol pattern to that of gymnosperm plants. We observed similarities with respect to the size of polyprenols and in the character of the polyprenol mixture (cf. Fig. 4 C, 4 D and 4 E). These similarities may indicate that of the evolutionary young *Rosaceae* originated from gymnosperms. This conclusion is in accord with the current views in botany on the origin of *Rosaceae*.

It should be stressed that most of the angiosperm plants studied contained prenol-11 as the dominating prenologue. One should notice that the presence of prenol-11 or a similar pre-

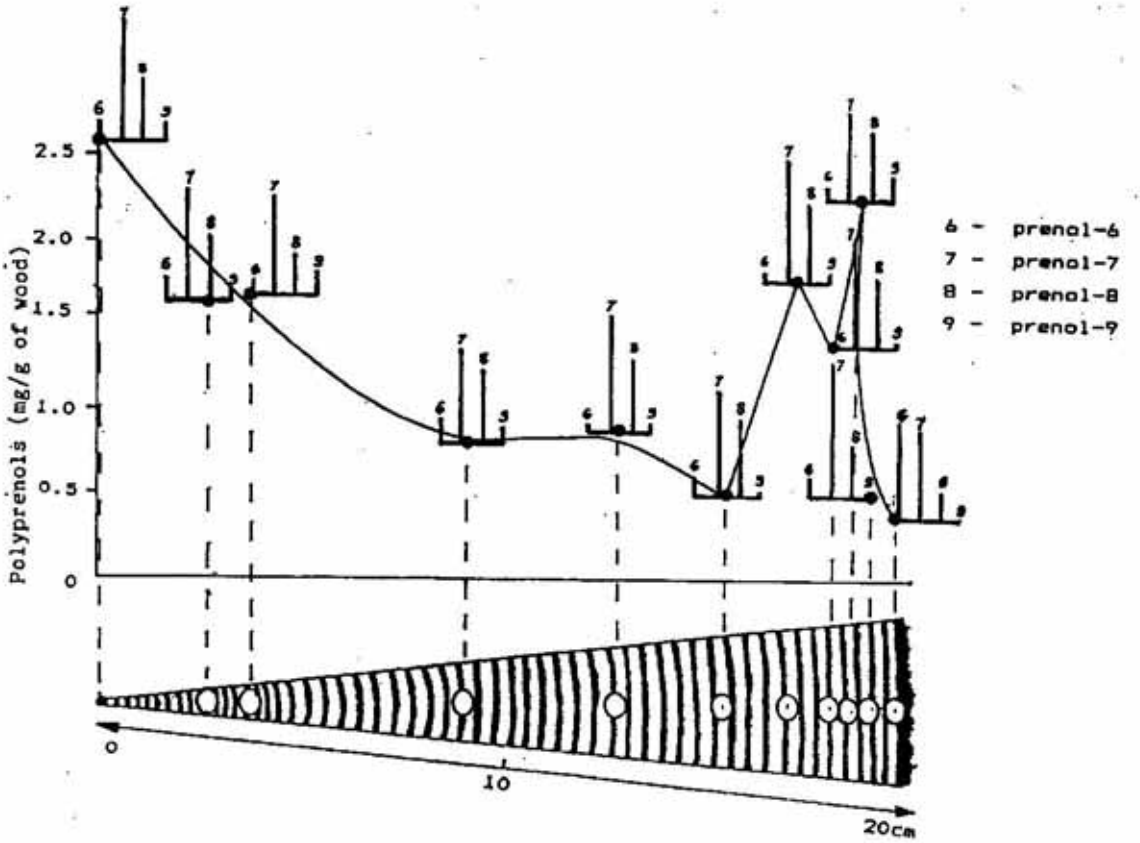


Fig. 10. Betulaprenols in the trunk of an old Betula dalearnica tree.
The content of total polyprenol fraction and proportions of individual polyprenols in various parts of the trunk are shown. The tree lived in the period of 1930–1986 close to Arrhenius Laboratory in Stockholm, Sweden. The analysis was done together with Elisabeth Peterson and Orjan Tollbom of the Department of Biochemistry of the University of Stockholm when the tree was cut down.

nol (prenol-10 or -12) is found in most plant species. The domination of prenol-11 is characteristic mainly of tropical plants. Also one of the evolutionary oldest families *Magnoliaceae*, contains prenol-11 as the dominating prenologue (Table 2). On extending the search for polyprenols to other angiosperm, apart from the very long-chain polyprenols in *Rosaceae* family built up from appox. 20 and more isoprene units, we found in the plant kingdom the accumulation of prenols of any chain-length like prenol-13, -14, -15, etc. (e.g. in families *Rhamnaceae, Sapindaceae, Capparidaceae).* It should therefore be admitted that the view on the domination of prenols composed of 11 isoprene residues and of polyprenols composed of approx. 20 isoprene units which was suggested by the former studies might be incorrect, and that there is no special preference in plant tissues for constructing linear poly-*cis* prenols of these two sizes.

On looking at the development of the research in the field of long-chain polyisoprenoid alcohols one can see that there is a striking time-dependent relation in the reports on chain length of polyprenols (Fig. 11). One can see that starting from the 1960-ies, each decade brings the discoveries of still longer polyisoprenoid alcohols in plants. Thus, the first poly-*cis*-prenol, reported in 1965 was betulaprenol composed of 6, 7 and 8 isoprene residues [40]. The occurrence of ficaprenol and castaprenol (11 and 12 isoprene residues) was described in 1967 [12, 13]. The still longer chain polyprenols composed of 16 and more isoprene units were identified in coniferous trees in early seventies [21, 22]. Later, the complexity of polyprenol spectrum (multiple families) in some conifers was demonstrated [23, 24]. In early eighties the polyprenols from leaves of *Rosaceae* trees, built from 20 and more isoprene units, and then still longer and longer polyprenol molecules, were described [31]. Those most recently described were polyprenols from leaves of species belonging to the genus *Potentilla, Rosa* and *Lum-*

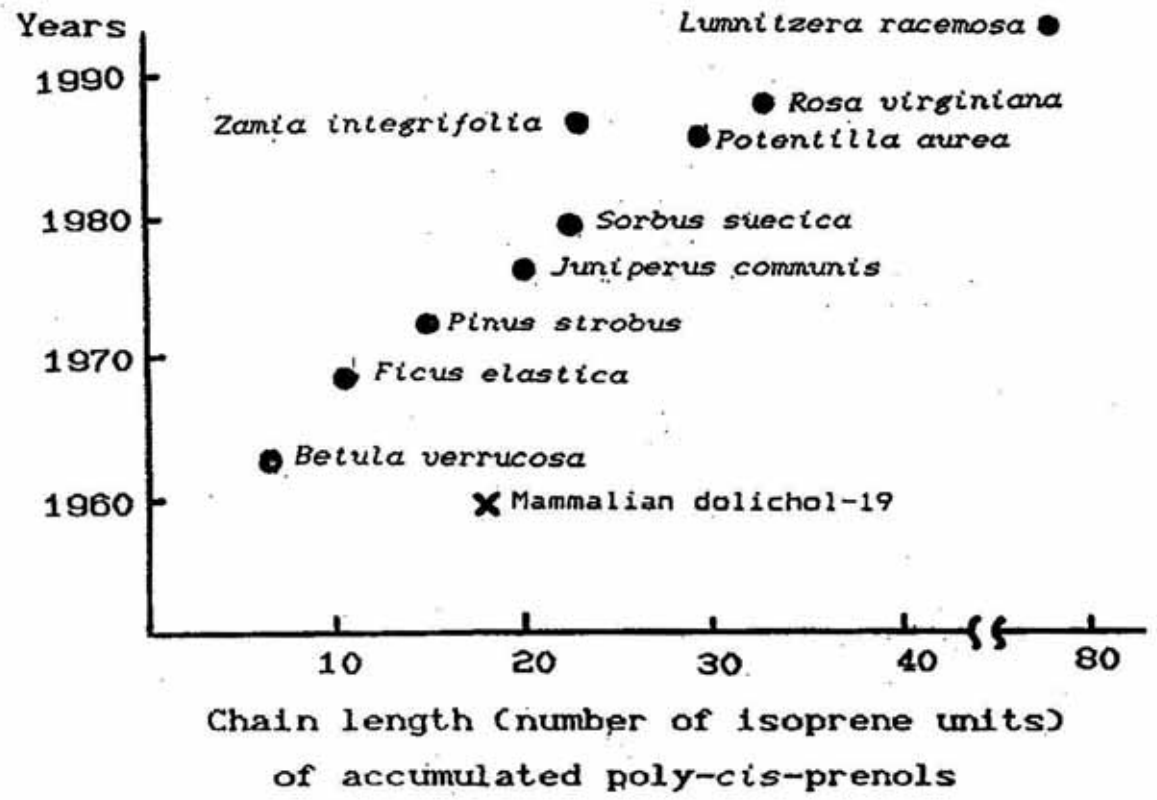


Fig. 11. Approximate time of isolation of the most characteristic polyprenols from plants.

nitzera racemosa (Skoczylas *et al.*, in preparation). The latter plant species draws the attention of phytochemists to a group of plants that has not been explored so far, and in which the occurrence of the largest size molecules that are lipids and rubber-like substances at the same time, could be a characteristic feature. It should be recalled that the polyprenols isolated recently in our laboratory from *L. racemosa* are poly-*cis*-prenols composed of up to 100 isoprene units. According to the data on the molecular size of rubber molecules, the size of about 400 isoprene units is characteristic of the natural rubber polymer [41].

SPECULATIONS ON THE ROLE AND POSITION OF POLYPRENOLS

During approx. 25 years of our search for plant polyprenols in various plant materials there occurred a spectacular development of new methods that enabled to perform the studies on isoprenoid lipids of increasingly large size. This is why now we can approach the problem of long-chain isoprenoid lipids and prove the occurrence of the "rubber-like" isoprenoid lipids which form an intermediate class of substances "filling up" the gap between low molecular isoprenoids and high molecular natural rubber. It seems that the group of substances characterized as "rubber-like" lipids might exhibit interesting physical and chemical properties. However, at first they were considered as undesired substances in the paper mill technology (betulaprenols). The further coincidence of plant polyprenols with the problem of lipid-dependent transglycosylation showed them to be very specific and efficient tools in biochemical processes and as membrane modifying factors [42].

The speculations on the possibility of using polyprenols as chemotaxonomic markers in botany have been mentioned earlier in this review. The authors are convinced that the "polyprenol pattern" of a plant is deeply encoded in the genotype, and its specificity as chemotaxonomic marker seems to be unquestionable. Though the size and the amount of polyprenol molecules are the results of action of *cis*-prenyltransferase, one should envisage

that also other factors, like the presence of accompanying lipids and the properties of membranaceous subcellular elements could be of crucial importance in formation of polyisoprenoid alcohols.

One can not predict now whether there will be a demand for poly-*cis* prenols from new technologies, like it was in the case of natural rubber with the advent of car industry, medicine, etc. The appearance of such a demand is still possible in view of a big challenge in the civilisation and perhaps the results of our search for long-chain plant polyprenols will find then full justification.

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