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Review

Chromatography of long chain alcohols (polyprenols) from animal and plant sources

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Abstract

This paper provides a comprehensive overview of existing chromatographic methods for the analysis of long chain alcohols (polyprenols) from animal and plant sources. After a brief introductory discussion on the biological aspects of the polyprenols, the review focuses on various techniques for the isolation, purification, chromatographic separation and detection of polyprenols. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Polyprenols; Long-chain alcohols; Dolichols; Bactoprenols; Ficaprenols

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1. Introduction

The polyprenols are polyisoprenoid alcohols that contain multiprenyl (polyprenyl) chains build-up from 5 to 25 and more prenyl units with a hydroxyl group placed at the end (Fig. 1).

The stereospecific head-to-tail assembly of the prenyl units creates polymers that differ as to the chain length and/or geometrical configuration. The other important structural modification of polyprenols is related to saturation of one or more residues, while other branches incorporate an exo methylene group [1]. The significant role of polyprenols as a lipid carrier in the biosynthesis of microbial cell-surface polymers has frequently been mentioned in literature. The most important polyprenols in this paper are dolichols, ficaprenols and betulaprenols, including related compounds. These substances can be found in plants, animals and other eukaryotic

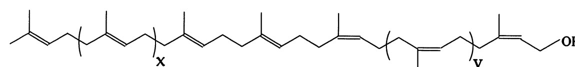

Fig. 1. Structure of polyprenol.

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organisms. The other important group of polyprenols is bactoprenols and their derivatives. We also find them in prokaryotes.

2. Results

As shown in Table 1, the α residue in dolichols is saturated. A (ω-1) and (ω-2) residues appear as *trans* (*E*) configured, the remaining internal residues have *cis* (*Z*) configuration. In several fungi, one or two residues at the ω end may be also saturated. There are bacterial polyprenols of di-*trans* and poly-*cis* type. Usually, they have a shorter chain length (about C_{55}) when compared with C_{75}–C_{120} for dolichols. These bacterial polyprenols are completely unsaturated. The chemical structure of few bacterial polyprenols was characterized in details. They show big difference in the geometry and chain length as compared with bactoprenol. For example, *Bacillus acidocaldarius* contains few polyprenol derivatives including C_{50} isoprenologues, all-*trans* isomers, and tertiary alcohols [2]. On the other hand, ficaprenol was found as a constituent of polyprenol pool in *Streptococcus mutants* [3].

The appearance of prenol derivatives in the plant kingdom is the most explored area. Polyprenols which abound in plants are characterized by large differences in chain length and contents. Shortest prenols (C_{30-40}) were found in birch wood [4]. Medium size prenols are typical constituents of angiosperm (C_{50-60}) and gymnosperm (C_{70-80}). The longest chain prenols (C_{130} and more) are present e.g. in *Araucaria* [5]. There are plant polyprenols containing two-*trans*-poly-*cis* chain (betulaprenols) or three-*trans*-poly-*cis* residues (ficaprenols) [6]. Usually, the structure of animal prenols [7] (dolichols) is linear and limited to the chain length from C_{85} to C_{105}. There are exceptions to this rule but the spectrum of polyprenol derivatives of animal origin is not so diverse as in plants. Generally, dolichols are di-*trans*-poly-*cis*-prenols with α-isoprene-saturated unit. The presence of plant dolichols was detected in seeds of monocotyledons [8] for the first time in 1984. The common incidence of betulaprenols and ficaprenols in the same organism is very rare, it was detected only once [9].

Table 1
Polyprenols and their structures

Trivial name	Shorthand structural designation
Dolichol	$WT_2C_{n-4}S$–OH ($n=15$–25 or more)
Bactoprenol	WT_2C_7C–OH
Ficaprenol	$WT_3C_{n-5}C$–OH ($n=9$–13 or more)

Isoprenoid (C_5) units are coded as follows: W, terminal (ω) unit; T, *trans* unit; C, *cis* unit; S, saturated (isopentane) unit.

Over the last 30 years, the role of polyprenol-like substances in the biosynthesis of glycoconjugates has been studied extensively. The aim of this paper is to present and evaluate the data on dolichols and polyprenols with respect to the large diversity in their chain length and type of prenol. We wish to describe an arsenal of modern chemicophysical methods such as RP-HPLC, IR, ^{1}H-NMR, ^{13}C-NMR, EI-MS and soft ionization techniques such as FD-MS, FAB-MS, etc., which are used for the elucidation of the molecular architecture of polyprenols.

Prenols, due to the universal features of their biological function, occur in the major parts of living organisms. Outdoors, we can find prenol-like compounds either as an ester of acetic, phosphoric and higher fatty acid or in a free form. With few exceptions, prenols are detected in a free form. However, in the earliest stage of the research, the TLC method was used to determine the esters of prenols and higher fatty acids.

Table 2 summarizes the most important methods and examples of chromatographic analyses of prenols in natural materials. The table gives the number of basic trends in the performance of analyses. Table 2 shows that HPLC was more successful than GLC. Usually, the main disadvantage of GLC is the concurrent degradation of analyzed compounds by thermal degradation and dehydratation. Commonly, TLC uses silica gel impregnated by paraffin or fixed by other non-polar as a reversed phase. In general, a water–acetone mixture saturated by paraffin was applied as a mobile phase. As shown from Fig. 2 and Table 2, this version of TLC was successful in many cases.

The isolation of polyprenols from a mixture of homological compounds requires a complicated preparation procedure. An exception is the isolation of polyprenols from latex of *Hevea* sp., see Fig. 3. In the first step of the isolation procedure are separated the total lipids. Secondly, a fraction of low polar

Table 2
Chromatographic conditions for separation of prenols

Source	Column	Mobile phase	Detector	Forms of compounds	Homologues	Literature
Birch	1% SE-30 on Celite	Ar, 230°C, 1 atm.	FID or MS	Free or Ac and both hydrogenated	6–9	[27]
Tobacco	5% Dexsil 300, 45×0.3	He 50 ml/min, 210–330 temp. gradient	FID	TMSi,	Solanesol	[26]
Birch, ficus, yeast	1% SE-30, 122×0.3	Ar 60 ml/min, 300 or 340°C	FID	Ac and hydrogenated Ac	6–9 and 14–16	[25]
Prenols from different company	RP-8, 5 μm, 25×0.3	MeOH–W, 100:0 or 92:8	205	Free	4+9	[58]
Solanesol, phytol	TLC-silica impregnated Ag	Hex–AcOEt–diisopropylether, 2:1:2, etc.	Rhodamine	Free	4+9	[22]
Solanesol, pig liver, ficus, pinus	RP-18, 25 cm, 5 μm	MeOH–iPrOH, 1:1, 1.5 ml/min	210	Free	11–20	[24]
Pig liver, birch, ficus	30×0.7 Lipidex 5000	Acetone–water, 80–93:20–7 preparative mode 50 mg per one chromatographic run	Collected fraction and identified by TLC	Free	7–22	[28]
Leaves infected by fungus up to 0.25‰ of fresh leaves	RP 30×0.4	*n*-Propanol	254	*p*-Nitrobenzoyl esters	10, 11, 12	[29]
Leaves infected by fungus	RP-TLC	Acetone–water, 9:1	Fluorescein	Free	10, 11, 12	[29]
Leaves of Ginko 1.97 of dry leaves	RP C_{18} 30×1	Acetone–methanol, 9:1, 3 ml/min	RI	Acetates	14–23	[30]
Leaves of plants 0.5–1.0% of wet wt.	RP C_{18} 25×0.4; 5 μm	MeOH–iPrOH–W, 60:40:2, iPrOH–Hex, 40:60, from 0 to 40%; 30 min, 1 ml/min	210	Free	10–25	[35]
	RP18 TLC	Acetone	Iodine			[35]
Magnolia leaves	CC on Lipidex 5000	Acetone–water, 30 ml/h 100×1				[33]
	RP-TLC cellulose impregnated by paraffin	W–acetone, 10:90, saturated by paraffin	Iodine	Free	9–13	[33]
Juniperus needles	CC on Lipidex 5000	1×120, 7– 2% W in acetone		Free	15–21	[34]
	RP-TLC cellulose impregnated by paraffin	W–acetone, 10:90 saturated by paraffin				[34]
	C_{18} 25×0.4	4% W in acetone, 1 ml/min	RI			[34]
Needles of pinus 1% of dry weight	RP C_{18} 120×0.3	Acetone–W 7:1	RI	Free	10–19	[31]
	HPLC normal-phase 60×1	Petrol ether ether, 199:1	RI			[31]
Needles of conifers 0.2–2.3% of dry needles	RP C_{18} 30×1	Acetone–methanol 9:1, 3 ml/min	RI	Free or acetates	12–28	[32]
Gymnosperms 0.01–3.04% of fresh weight	RP C_{18} 12×0.4; 5 μm	MeOH–iPrOH–W, 60:40:5 iPrOH–Hex, 30:70 from 0 to 50%; 45 min, 1.5 ml/min	210	Free	13–27	[41]
Gymnosperms	TLC RP-18	Acetone–hex, 95:5	Iodine	Free	13–27	[41]
Potentilla up 0.48% fresh wt.	RP C_{18} 12×0.4; 5 μm	MeOH–iPrOH–W 60:40:5 –iPrOH–Hex, 30:70 from 0 to 50%; 45 min, 1.5 ml/min	210	Free	15–35	[42]
Potentilla up 0.48% fresh wt.	TLC	AcOEt–benzen, 5:95	Iodine	Free	15–35	[42]
	RP-18 TLC	Acetone–hex, 95:5	Iodine	Free		

Table 2. Continued

Source	Column	Mobile phase	Detector	Forms of compounds	Homologues	Literature
Lower plants (mosses, algae, lichens, etc.)	C_{18}, 7 μm, 25×0.8	iPrOH–W–Hex, 41:4:5–3:0:7, 40 min	210	Free	8–26	[11]
Potentilla aurea up 0–3% of fresh leaves	RP18; 3 μm,	iPrOH–MeOH–W, 8:21:1, to 70% Hex–iPrOH, 7:3, 1 ml/min, 45 min	210	Free	18–40	[40]
Hevea leaves	RP18, 60×1	iPrOH–MeOH–W, 8:12:1 to Hex–iPrOH, 7:3, 0.5 ml/min	210	Free	10–70	[10]
Combretaceae up to 4.0% dry wt.	RP18; 3 μm	iPrOH–MeOH–W, 8:12:1 to Hex–iPrOH, 7:3, from 0 to 50 or 80%, 1.5 ml/min	210	Free	20–60	[39]
Roseceae up to 5.0% dry wt.	RP18; 3 μm	iPrOH–MeOH–W, 8:12:1 to Hex–iPrOH, 7:3, from 0 to 50 or 80%, 1.5 ml/min	210	Free	19–23 and 35–45	[37]
Pinus up to 4.8% dry wt.	RP18; 6×0.39	iPrOH–MeOH–W, 8:12:1 to Hex–iPrOH, 7:3, from 0 to 70 or 80%, 1.0 ml/min	210	Free	16–18	[38]
Plants (analytical mode)	RP18, 3 μm, 6×0.46	iPrOH–MeOH–W, 40:60:5 to Hex–iPrOH, 7:3, from 0 to 60 or 80%, 1.5 ml/min, 25 min	210	Free	15–30	[58]
Plants (preparative mode)	RP18; 5 μm, 10×1	iPrOH–MeOH–W, 40:60:5 to Hex–iPrOH, 7:3, complicated gradient, 6.0 ml/min, 20 mg per one injection	215	Free	15–30	[58]

lipids such as hydrocarbons and carotenoides is separated. Usually, after separation of high polar compounds fraction, which includes the whole spectrum of lipids, is applied the adsorption chromatography to isolate prenols and their esters [10].

A similar, but simpler, variant of the above described method uses in a second step of separation the saponification of crude lipid fraction to remove the undesired lipids. Subsequently, the unsaponifiable fraction is separated by adsorption chromatography. Both, columns and plates are suitable to isolate individuals compounds from the mixture of prenol homologues [11].

Physicochemical methods, such as NMR [12] or MS are used for the direct identification of chemical structure. The use of spectral analysis methods is beyond the scope of the paper but we will estimate the high potential of this group of methods in the future. The application of electron impact (EI) ionization can serve as an example. In years 1960–1970, the EI method, which is not suitable for the analysis of high-molecular-mass compounds, represented a sole efficient analytical technique. However, in 1990s, the EI method was replaced by soft ionization techniques such as FAB-MS or FD-MS.

For the characterization of ficaprenols (C_{50-60}) from leaves of *Ficus elastica* [13], undecaprenol from bacteria (*Lactobacillus plantaum*) [14], polyprenols from fungi *Aspergillus niger* [15] and *A. fumigatus* [16], dolichols from fungi *Phytophtora cactorum* [17] and other natural sources [18] such as leaves of horse-chestnut (*Aesculus hippocastanum*) the TLC method was successfully used. Silica gel was used as the reversed-phase impregnated by paraffin oil. A water–acetone mixture saturated by paraffin was applied as mobile phase.

Castaprenols (C_{55-65}) were identified in leaves of horse chestnut (*Aesculus hippocastanum*) [19] and other tropical and subtropical plants [20], predominantly represented by families of *Moraceae* and *Euphorbiaceae*. The major homologues were C_{45-60} prenols.

A very interesting preview paper [21] summarizes the occurrence and seasonal distribution of higher

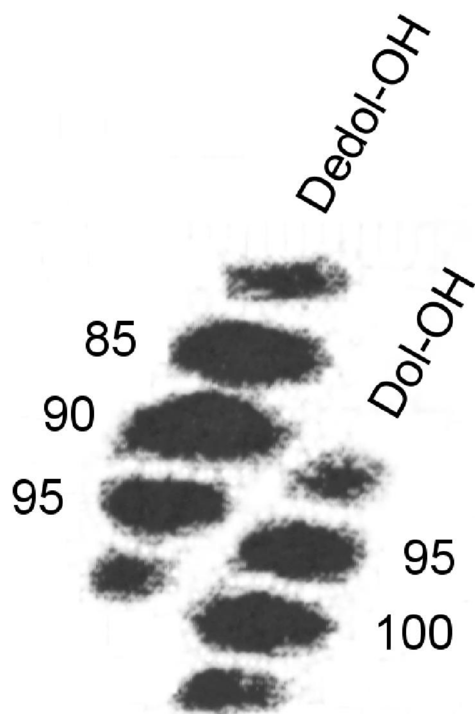

Fig. 2. Separation of polyprenols into dehydrodolichol and dolichol by two-plate TLC, dehydrodolichol of *Ginkgo biloba* and dolichol of pig liver. Reproduced from Ref. [53] with permission.

isoprenoid alcohols in the plant kingdom. The paper describes the presence of prenols in higher, but not in lower, plants. The exception to the rule is the alga *Chlorella pyrenoidosa*. The value of 0.1 μg prenols/1 mg of chlorophyll is commonly accepted as the detection limit, whence it follows that the value of the detection limit ranges about 0.1 mg prenols/1 kg of wet weight.

The chemical structure of bactoprenols from *Lactobacillus casei* was identified using MS and NMR methods. As described above, the TLC method was used for bactoprenol preparation [22].

Prenylquinones, prenylvitamines and prenols were separated by means of TLC. In addition to, the reversed-phase was impregnated with silver nitrate [23]. The paper describes the separation of tocoferols, phylloquinones, plastoquinone, ubiquinone, phytol, solanesol, etc. using different solvent mixtures e.g. hexane–ethyl acetate–diisopropyl ether, petrol ether–$CHCl_3$–acetone.

A supercritical fluid chromatographic (SFC) procedure for the determination of three major polyprenols present in the leaves of *Ginkgo biloba* was developed using dodecaprenol as internal standard. The relative standard deviation of the method was 5.8% for the determination of C_{85}, C_{90} and C_{95}

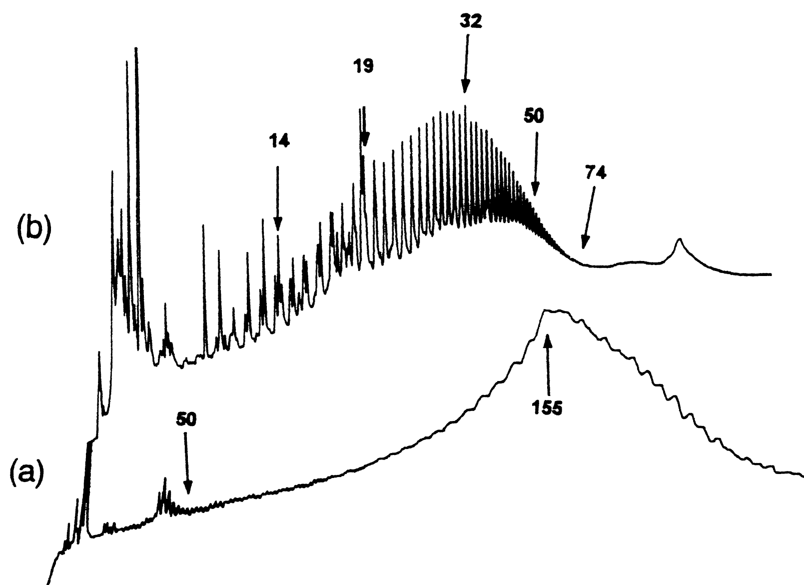

Fig. 3. HPLC profiles of fractions of rubber isolated from nascent leaves on young *Hevea* shoots. (a) Fraction with M_w up to 2×10^4, (b) fraction with M_w up to 5×10^3. Reproduced from Ref. [10] with permission.

polyprenols. The SFC assay results showed that the content of polyprenols in ginkgo leaves was higher than previously published values. The chromatogram of the highly concentrated leaf extract revealed the presence of an isoprenolog (C_{120}) not previously detected by HPLC methods [24].

The application of gas–liquid chromatography (GLC) for the separation of polyprenols can be dated back to the 1960s. Years later, the HPLC technique replaced GLC in spite of its further improvement. There are a few reasons for the rejection of GLC. The peaks were broadened when compared with those of saturated chain and retention times varied a little from one analysis to the next [25].

While it is possible to analyze a mixture of fully saturated alcohols (prepared by a foregone hydrogenation) by GLC, however, during the procedure we lose information concerning of chemical structure (e.g. *cis* and *trans* isomers). Generally, GLC techniques do not analyze higher prenols ($>C_{100}$). Retention times are extremely high when an isothermal regime is applied to the analysis of higher homologues. On the contrary, the retention time can be decreased when a temperature gradient regime is applied. Such a solution is restricted by upper limit of temperature at 340°C and makes troublesome the evaluation of results due to the fact that in a non-isothermal regime the logarithm of the retention time is not equal to the chain length.

Solanesol, the prenol included in different types of tobacco was identified by GLC [26]. In this case, GLC showed its shortcomings. When the direct separation on Dexsil 300 was used, the free solanesol and/or its derivatives such as solanesol trifluoracetate were split into many unidentifiable products of pyrolysis. When GLC analyzed pure TMS (trimethylsilyl) ether standard the results were better. The procedure is not feasible when the solanesol is mixed with other natural compounds.

Betulaprenols, obtainable from birch wood [27] are lower prenols (C_{30-45}). The natural or fully hydrogenated betulaprenols were identified using GLC. Lipidex 5000 [28] (hydroxyalkoxypropyl dextran) was used for the preparation of few milligrams of pure prenols. The procedure is feasible, but it is complicated by a very long elution time. The observed elution time of betulaprenol C_{45} was about 100 h. The preparation of dolichol C_{95} took longer. Even if the elution time is very long, the purity of obtained prenols is very high, see the purity of commercial dolichols (Sigma catalog). Prenols C_{45-60} with usual isoprenoid unsaturation were isolated from *Quercus ilex* leaves infected by fungus *Microsphaera alphitoides* [29]; both TLC and RP-HPLC methods were used for their identification.

Long-chain betulaprenols were isolated from leaves of *Ginkgo biloba* [30]. As shown in Table 2, fully unsaturated homologues C_{70-115} such as ω-terminal 13–15-*cis* α-terminal prenols were identified by RP-HPLC. For the first time, the verification of chemical structure was performed using ^{1}H and ^{13}C-NMR and FD-MS methods.

Polyisoprenols (pinoprenols) were isolated from needles of *Pinus sylvestris* [31]. The total amount of prenols in needles was about 1% of dry weight. RP-HPLC technique was used for the isolation of prenols with chain length in the range C_{50-95} (Table 2). GLC (column 30 cm×3 mm, 1% SE-30, temperature gradient 10°C/min in the range 300–375°C) was applied and MS was used for identification.

Polyprenols (C_{60-130}) obtained from conifers either in free form (alcohols) or bound as acetate esters [32] were identified using ^{1}H and ^{13}C NMR and FD-MS techniques.

Polyprenols from leaves of *Magnolia campbellii* occur as a mixture of alcohols composed of 9–13 isoprene units [33]. The geometric isomers were partially separated after triple development of the TLC plate. As proven by ^{1}H-NMR spectral analysis of enriched fraction, the major part of three *trans* polyprenol C_{55} was followed by a minor fraction (5–10%) consisting of a geometrical isomer. The isomer has 1 or 2 *trans* bonds and the *cis* configuration of the rest of both isomer molecules was the same.

Prenols with chain lengths C_{55-105} were isolated successfully from the needles of *Juniperus communis* [34] (Table 2) using RP-HPLC. The yield of prenols was about 1% of the fresh weight of needles. Free prenols together with prenol esters with long chain fatty acids in the ratio of about 1:1 were found. The main fraction of esters included 14:0, 13:0, 18:3 and 20:3 acids. Traces of other fatty acids were identified. Unfortunately, MS spectra of prenol esters were not published [34] ‘‘because the compounds were not volatile in the conditions of analysis’’. As

mentioned above [18,57], the application of RP-HPLC method permitted identification of the other compounds such as phytol, geranylgeraniol or solanesol.

The occurrence and seasonal distribution of C_{50-60} polyprenols together with C_{100} and similar long-chain polyprenols in leaves of plants was investigated [35]. In higher plants, a number of *cis* configured long chain prenols was identified using, for the first time, RP-HPLC (Table 2) with a complex gradient regime. Fig. 4 shows its successful application for the separation of prenols.

Polyisoprenoid alcohols from leaves (*Prunus*, *Sorbus*, *Juglans*, *Magnolia*, etc.) were studied by ^1H NMR spectroscopy and HPLC. Data on dynamics of relative monthly (July–October) content of each isoprenolog of polyprenols were published [36]. The maximum accumulation was observed in late September and in October.

In the leaves of *Potentilla aurea* [37], the appearance of prenols with two comparable maxima in the chain length was described for the first time (Fig. 5). The polyprenols comprised up to 0.3% of the fresh weight of the leaves. The main fraction contained long chain *cis*-polyprenols in the form of fatty acid esters. The polyprenol mixture exhibited a complex distribution pattern with two maxims in which prenols-18 or 19 and prenol-35 are the dominant prenologues (Fig. 6).

In green needles of *Pinus mugo* [38] the most abundant polyprenols occur as a mixture of prenologues. The dominant alcohol is built of 16 isoprene units. The typical spectrum of polyprenols (prenol-15, -16 and -17) was the same irrespective of the location of plants. The distinct morphological differences were observed in the various selected forms of this species. The constant pattern of the polyprenols spectrum was preserved throughout the 2-year life span of needles, although the level of polyprenols increased by 2–3-fold. The polyprenol pattern in the *Pinaceae* family differs from species to species, thus it may serve as a chemotaxonomic

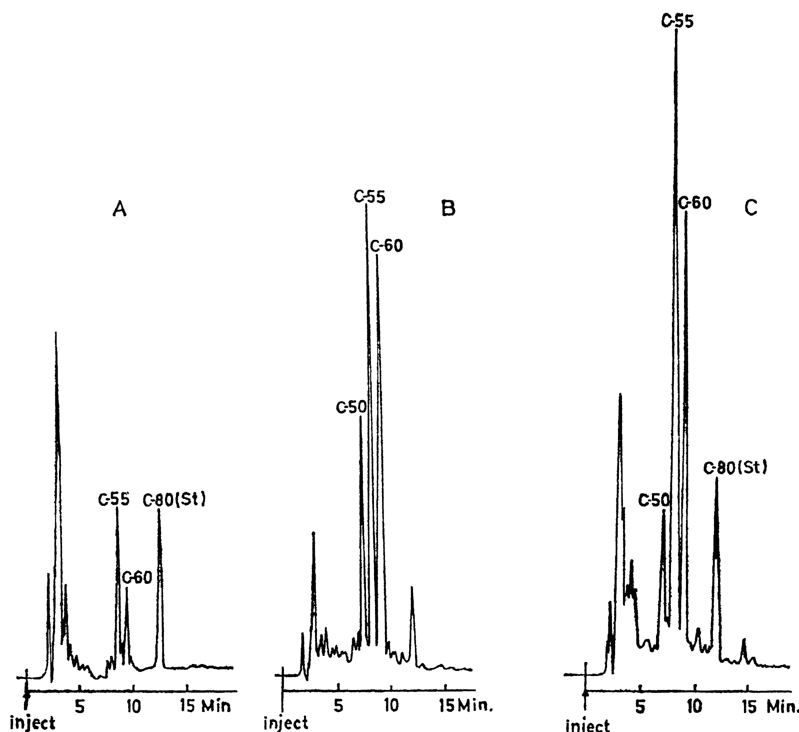

Fig. 4. HPLC of polyprenols from leaves of *Carya cordiformis* at various ages. (A) 5 weeks, (B) 15 weeks and (C) 27 weeks. Reproduced from Ref. [35] with permission.

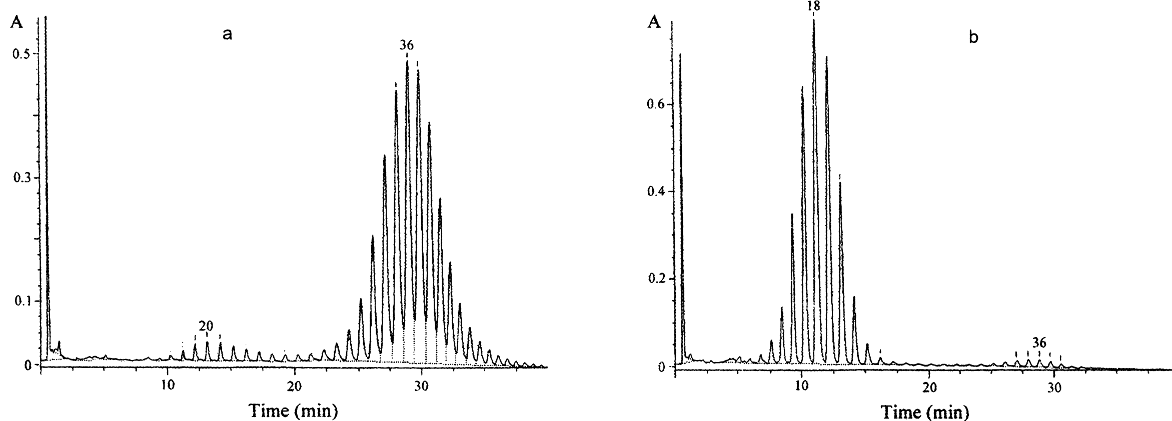

Fig. 5. HPLC of longer (a) and shorter (b) chain polyprenols from *Prunus incisa*. Reproduced from Ref. [37] with permission.

criterion within this group. Repeatedly, esters of fatty acids were identified by RP-TLC technique. The presence of natural free prenols was not detected. The esters of fatty acids were found at a level that was 5–10 times lower than the major portion of polyprenyl acetates (Fig. 7).

The presence of poly-*cis*-prenols with chain lengths of 20–60 isoprene units or up to 70 units in leaves of plants belonging to *Combretaceae* family [39] was a common attribute of this group of plants. The polyprenols of this group were found in half of the 20 species studied. Polyprenols mostly occurred in the form of fatty acid esters. Only in one species — *Combretum molle*, were the polyprenols found in

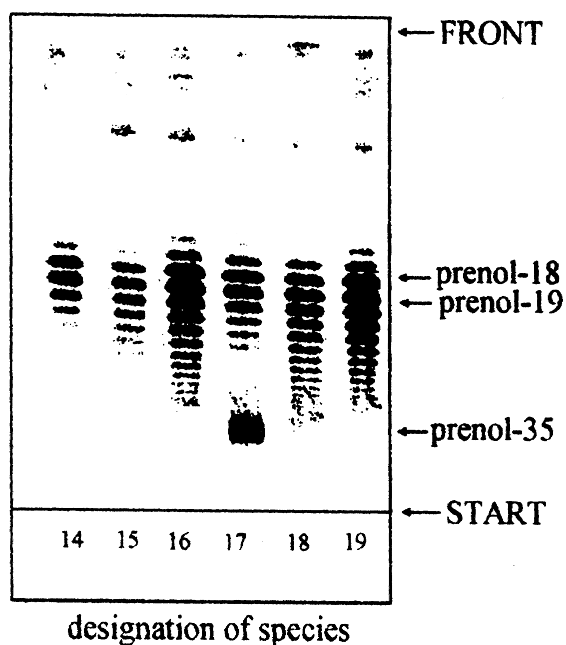

Fig. 6. Total lipid extracts of the species of genus *Prunus*; TLC on RP-18. Reproduced from Ref. [37] with permission.

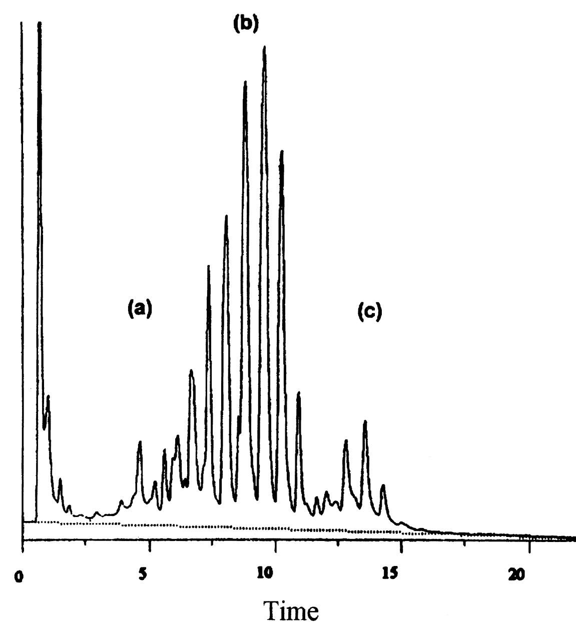

Fig. 7. Typical chromatographical patterns of *P. mugo* polyprenyl family: free polyprenols (a), polyprenyl acetates (b) and polyprenyl esters with fatty acids (c). Reproduced from Ref. [38] with permission.

the form of free alcohols. The amount of long-chain polyprenols varied with plant species; the richest source was *C. molle* (about 4% of dry mass of leaves). Typical polyprenol groups that characterize the other systematic families of plants were not found in the *Combretaceae* sp.

Leaves of *Potentilla aurea* [40] contain mainly very long chain *cis*-polyprenols in the form of fatty acid esters. The polyprenol mixture exhibited a complex distribution pattern with two maxims in which prenol-20 and prenol-28 are the dominant prenologues. The polyprenols comprised up to 0.3% of the fresh weight of the leaves. The paper [40] is unique in that it summarized the fatty acid content in a tabular form and described the isolation of di-*trans*-poly-*cis* prenols.

Over a hundred species of gymnosperm plants were examined for the presence of long chain polyprenols [41]. In green needles of more than 60 species, the poly-*cis* long chain prenols were found in the form of acetic acid esters. The main constituent of the natural polyprenol mixture in *Cycadopsida* was prenol-18 or prenol-20.

Like in all species of *Pinaceae*, in *Coniferopsida* sp. a single polyprenol family consisted of two families differing in the size of polyprenol molecules was presented. The first family can be characterized by prevailing prenol-17 content, the second one by prenol-23. Such a complex mixture of polyprenols was also found in *Araucariaceae*, *Cupressaceae*, *Taxiodiaceae* and in *Taxopsida* sp. Seasonal variation of the polyprenol content was observed in the green leaves.

The observed concentration of polyprenols in the leaves of *Potentilla anserina* was greater than 0.3% of fresh weight [42]. The actual polyprenols fraction comprised a mixture of poly-*cis* fully unsaturated analogues up to 29 isoprene units long. Generally, in *Potentilla sp.,* the polyprenol mixture is composed of two families. The first group includes the medium chain-length polyprenols (built up of about 20 isoprene units) and the second one contains the very long prenologues of ~24–28 isoprene units. The paper cited above is the first report of the occurrence of polyprenyl alcohols of such chain length in plant material, and the first report of the presence of multiple polyprenol mixture in angiosperms; a useful modification of polyprenols preparation technique from plant material is described. The separation method is based on solid-phase extraction with hydrophobic gel Lipidex-5000.

Contemporary improvements and development in instrumental techniques confirmed that the chemical architecture of prenols can contain two chain lengths; prenols can be build-up by the customary 15–19 isoprene units together with other longer chains of about 25–30 units.

Efficient HPLC methods for the determination of dolichols in tissues of rats and human plasma have been developed [43]. For the most part, tissue concentration of dolichols was determined by HPLC with UV detection. Usually, the plasma level of dolichols was detected fluorometrically after derivatization with anthracene-9-carboxylic acid. This method was sensitive enough to determine the concentration of dolichols in human plasma. The chemical structure of the separated compounds was identified by means of FD-MS or NMR techniques. Using this procedure, dolichols with chain lengths of C_{85-100} were recognized. Table 2 summarizes the chromatographic conditions. Two procedures for quantitative determination of dolichol by HPLC were studied and applied to analyze its distribution in tissue and subcellular structures [44]. To determine dolichyl phosphates, a lipid extract was treated by acid and alkaline hydrolysis. Subsequently, after hydrolysis with acid phosphatase, the content of dolichols was determined by HPLC. Rat spleen exhibited the highest dolichol content (114 pg/g) followed by lower values in liver and brain. The distribution pattern was similar in all organs, with 18 and 19 isoprene residues as the dominating components. Human organs contained considerably higher concentrations of dolichol, with the 19 and 20 isoprene residues as the main components. In rat liver, outer mitochondrial and Golgi membranes, lysosomes and plasma membranes contained considerable amounts of dolichol. A drastic increase in the dolichol content was observed in rat liver hyperplastic nodules while human liver cirrhosis and hepatocarcinoma showed a marked decrease in dolichol content. In the latter case, the distribution pattern of dolichol concentrations was also changed. It was found that dolichols are presents in large amount of about 10% in human testis, 18% in rat liver and in phosphorylated form in human liver (2%). Rat liver

mitochondria and microsomes contained the activated form of polyprenols at 4 and 31%, respectively. The identification of dolichols was performed by means NMR and RP-TLC techniques, in all examples cited above. Table 2 summarizes the chromatographic conditions used.

A lipid fraction enriched in polyisoprenoid alcohols was prepared from the seeds of a number of crop plants, using chromatography on reversed-phase Florisil [45]. Analysis by HPLC of a lipid fraction isolated soybeans showed a series of peaks corresponding to α-saturated polyprenol homologues (dolichols) with the chain lengths from 15 to 22 isoprene units. Similar results were obtained with seeds of other dicotyledonous species (rapeseed, peanuts, mung beans, navy beans and peas). In contrast, the analysis of monocotyledonous seeds such as wheat, rye, barley, rice and corn by HPLC technique provided split peaks. Such chromatograms indicated the presence of two approximately equal distinct groups of homologous compounds. The analysis of polyisoprenoid material that was isolated from wheat germ indicated the presence of dolichols and α-unsaturated homologues (polyprenols). When the raw material was treated with manganese dioxide, the polyprenols were selectively oxidized to the corresponding aldehydes. Afterward, the aldehydes were separated from dolichol fraction by means of TLC. The identification of the chemical structure of the separated compounds was performed by infrared and nuclear magnetic resonance spectroscopy and confirmed by comparison with authentic standards on HPLC. It was found that the concentration of polyisoprenoid alcohols in seeds varied from 1 to 16 mg/100 g. Using this sophisticated procedure, the separation of very similar compounds (M_w 1108 or 1106) was performed. The difference in molecular mass between the compounds was about two units.

Careful inspection of the TLC pattern of the neutral lipid fraction of bovine thyroid reveals, in addition to cholesteryl esters and dolichyl fatty acid esters, the presence of a not yet identified compound in the most apolar lipid region [46]. This unknown compound was purified on a preparative scale by silica acid column chromatography, Lipidex-5000 gel-filtration chromatography and preparative TLC. By chemical (hydrolysis and reduction) and spectroscopic (UV, IR, NMR and MS) methods this lipid was identified as dolichyl dolichoate. The homologue pattern was analyzed by RP-HPLC and FD-MS.

Leaves of twelve species of the genus *Capparis* were examined for the presence of long chain polyprenols [47]. In a number of species the accumulation of polyprenols was up to about 0.3% of dry weight of tissue. In all studied species polyprenols composed of C_{60-75} formed the main polyprenol family. In the majority of the plants studied lower quantities of an additional polyprenol family were present, in which prenologues composed of 19, 20 or 21 isoprene units were dominating. In one species, *Capparis coriacea*, the presence of dolichols with a hydrogenated α-unit followed by partial splitting of observed peaks was documented.

In the review paper [48] dolichols, polyprenols and their phosphates were investigated by fast atom bombardment MS (Fig. 8). With the view of decreasing molar response with the molecular mass, suitable matrices and doping conditions were developed. The sensitivity of the arrangement was much higher for the phosphates, which are already in an ionized form, than for the parent alcohols. All derivatives gave rise to pseudomolecular ions. The polyprenols also gave rise to $[M-18+H]^+$ ions and the phosphates produced fragment ions by elimination of $[(C_5H_8)_{n+2}]$. The stationary phase prepared from *m*-nitrobenzyl alcohol and sodium acetate, where the relative intensities of pseudomolecular ions $[M+Na]^+$ was about 100%, was found to be optimal. It is remarkable that the negative FAB-MS spectrum did not show any ion $[M-H]^-$. Similarly, as shown above an ion $[M+Na]^+$ at 2651 that exhibited a weaker interaction was found in the polyprenol C_{185} spectrum.

Free dolichols, dolichol fatty acid esters and dolichyl phosphates in human serum were analyzed [49]. In order to determine the level of each dolichol class, the samples were pretreated using three different methods prior to fluorescent derivatization. To estimate the concentrations of free dolichol, samples were treated by alkaline methanol and kept at room temperature for 1 h. In the case of dolichyl fatty acid ester, samples were saponified at 100°C for 1 h. To estimate dolichyl phosphate, saponified lipid extracts were treated with acid phosphatase. Each sample of pretreated dolichol preparation was treated with anthracene-9-carboxylic acid. Afterward, the content

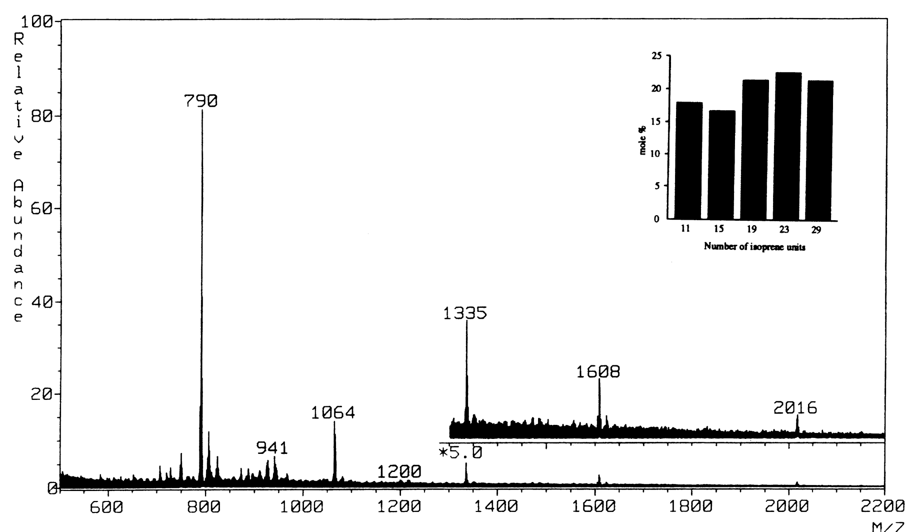

Fig. 8. Positive FAB mass spectrum of mixture of polyprenols -11, -19, -23 and -29 and dolichol-15 in a matrix of NBA with sodium acetate. Reproduced from Ref. [48] with permission.

of 9-anthroyl derivatives was determined fluorometrically. The three types of dolichols can be determined using HPLC. The other advantage of this simple method consists in the use of UV adsorption derivatives that increases the sensitivity of the method at least 40 times.

Leaves of *Lumnitzera racemosa* contain ~1–2% (dry weight) of long-chain poly-*cis*-prenols composed of 15–100 isoprene units [50]. In a natural mixture of these polyisoprenoid alcohols two families can be distinguished: one with dominating prenologue composed of 17 isoprene units. The second one consists of ~80 molecular species of polyprenols built up to 100 isoprene units. In this second family the dominating prenologues are composed of 25–30 isoprene units and prenologues composed of 50–100 isoprene units form a high proportion, thus making this lipid fraction similar to natural rubber. The long-chain polyprenols of *L. racemosa* are of di-*trans* poly-*cis* type. They occur as esters of fatty acids e.g. 16:0, 18:2 and 18:3. The long-chain polyprenols of *L. racemosa* can be considered as a group of intermediate compounds between prenyl lipids and polyisoprene rubber polymer.

A procedure for the rapid identification and determination of non-polar isoprenoid lipids from animal tissues was developed [51]. The complete determination can be carried out by RP-HPLC of just two samples. The first, extracted from unaltered tissues and suitably processed by column chromatography, provides information about free cholesterol, cholesteryl esters, coenzymes Q, free dolichols and dolichyl esters. The second, obtained from saponified tissues, can be used to detect both total cholesterol and total dolichols. Specific calibration graphs were constructed for the determination of the different constituents. This is the first case when the simultaneous analysis of fatty acid esters with dolichols and free dolichols fractions were performed.

Polyprenols of different chain lengths composed of five to approximately fifty isoprene units are frequently encountered in the unsaponifiable lipid fraction from leaves of *Spermatophyta* [52]. An extensive search for these lipids has confirmed the common occurrence and frequent accumulation of di- or tri-*trans*-poly-*cis* fully unsaturated prenols. Accumulation of α-dihydropolyprenols in leaves seems to be very scarce. Available data obtained in our and other laboratories allow drawing an overview — describing occurrence of these prenyllipids in some main taxons of *Spermatophyta*.

A novel TLC procedure [53] was devised to separate dolichol and dehydrodolichol, with the

concomitant separation of each family with respect to the carbon chain length. This method involves development of the polyprenols successively on two different plates, a silica-gel plate and a reversed-phase plate.

Negative-ion desorption chemical ionization (DCI) tandem MS was applied to the analysis of nanomole quantities of semisynthetic polyisoprenyl phosphates [54], the chain length of which ranged from 7 to 20 isoprene units. The DCI spectrum of all the compounds tested shows the presence of independently generated ions $[M-HPO_3-H]^-$, $[M-H_3PO_2-H]^-$ and $[M-H_3PO_4-H]^-$ resulting from the loss of a part of or the entire phosphate group of a polyisoprenyl-P. In tandem MS, the $[M-H_3PO_4-H]^-$ fragment produces series of ions 68 mass units apart, indicative of the polyisoprenoid nature of a compound. Studies with deuterated and α-saturated polyisoprenyl phosphates demonstrated that fragmentations of the $[M-H_3PO_4-H]^-$ ion proceed from both ends (α and ω) of a polyisoprenoid chain and may occur at either allylic (A) or vinylic (V) sites. Fragments of masses equal to $[n\times 68-1]$ and $[n\times 68-13]$ (where n is the number of isoprene units and $3\leq n$ is less than the total number of isoprene residues within a polyisoprenoid chain) comprise the αA and ωV series, respectively, and represent the most abundant ions in tandem mass spectra of the $[M-H_3PO_4-H]^-$fragment of polyprenyl phosphates. α-Saturated dolichyl phosphates can be distinguished easily from corresponding polyprenyl phosphates not only on the basis of a 2-amu shift of the $[M-H_3PO_4-H]^-$ ion and the α series of fragments, but also because of the presence of an additional (A+14) series of ions 14 amu heavier than fragments resulting from the allylic cleavages of an α-saturated polyisoprenoid chain.

A family of monoglycosyl polyprenylphosphates was isolated from *Mycobacterium smegmatis* [55], containing arabinose, ribose, and mannose. The isoprenoid nature of the lipid components was established by ^{1}H-NMR, and fast atom bombardment mass spectroscopy (FAB-MS) demonstrated the presence of C_{50} decaprenyl-P derivatives and smaller amounts of the C_{35} octahydroheptaprenyl-P products. The configuration of the mycobacterial decaprenol was established as mono-*trans* octa-*cis*. Combined GC–MS, FAB-MS–MS and ^{1}H-NMR allowed characterization of one of the primary components as β-D-arabinofuranosyl-1-monophosphodecaprenol.

The polyprenol pattern in leaves of fruit trees belonging to the *Rosaceae* (*Prunus*, *Malus)* and *Cornaceae* (*Cornus)* families are presented [56]. The content of polyprenyl acetates varied within plant species between 10 and 50 mg per gram of dry weight. In genus *Prunus, Cornus* and in representatives of species *Malus domestica*, a mixture of polyprenols composed of 18, 19, 20, 21 isoprene units was found. In six species of genus *Prunus* (sour-cherry): *P. serrulata-spontanea, P. yedoensis, P. fruticosa, P. kurilensis, P. subhirtella* and *P. incisa* the presence of a second polyprenol family, i.e. the group of prenologues consisting of prenol -35, -36, -37, etc. up to -42 was detected.

In lower plants, i.e. mosses, liverwort, algae and lichens [11] several classes polyisoprenoid alcohols in the wide range C_{40-130} were found (Table 3). They were isolated by means of RP-HPLC and identified by EI-MS, FD-MS, IR and ^{1}H- and ^{13}C-NMR.

Mosses predominantly contain betulaprenols [11] (polyprenols with two *trans* and other *cis* isoprene residues) with average chain length C_{60}. Liverwort has two maxima of chain lengths, first at C_{70} and second at C_{115}. It contains predominantly ficaprenols (three *trans* and other *cis* isoprenes) with minor (up to 5%) of some betulaprenols. Algae have similar composition to mosses; only the average chain length is moved to higher values (~C_{70}). Lichens are different in the average chain length and in the composition of long chain alcohols. They contain ficaprenols with two maxims, first at C_{80} and second at $C_{105-110}$ and dolichols (two *trans*, many *cis* and one saturated isoprene residues).

The method of separation of single polyprenols (or dolichols) from a mixture of isoprenoid alcohols is described [58]. Application of an RP-HPLC apparatus equipped with a semipreparative ODS column resulted in preparation of long-chain (dihydro) polyprenols of high purity (>95%). This approach substantially decreases the time scale of the conventional chromatographical preparative procedure. The method can be widely used in chemical and biochemical projects, where single polyprenols or dolichols are required.

The advance in instrumental technique that

Table 3
Composition of prenols from lower plants [11]

	Content of prenologues (%)																		
No. of units	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
M_w [a]	562	630	698	766	834	902	970	1038	1106	1174	1242	1310	1378	1446	1514	1582	1650	1718	1786
Mosses																			
D.s.	0.0	1.0	5.9	12.6	29.6	25.9	14.7	6.7	3.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T.t.	0.9	1.8	17.1	31.3	22.7	13.6	7.2	3.6	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P.c.	1.0	5.1	16.3	39.8	18.5	10.2	6.1	2.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A.v.	0.0	0.7	6.6	23.4	37.2	19.7	8.8	2.9	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P.sp.	0.9	2.6	10.4	54.8	18.3	5.2	4.3	2.6	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M.sp.	0.8	3.2	11.2	19.2	33.6	18.4	10.4	2.4	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B.sp.	0.0	0.7	11.6	19.2	23.4	20.5	13.7	6.8	3.4	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A.a.	0.0	0.6	10.2	14.8	26.1	23.9	11.9	6.8	3.4	1.7	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Liverwort																			
M.p.	0.0	0.0	0.0	0.7	3.9	13.7	24.6	7.2	2.6	0.7	0.7	2.0	3.9	5.2	7.8	12.6	9.7	4.1	0.6
Algae																			
C.f.	0.0	0.0	0.0	0.8	4.6	13.7	38.9	20.5	11.5	6.9	2.3	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A.sp.	0.0	0.0	0.6	3.1	7.4	15.3	23.9	19.6	14.1	8.0	4.9	2.5	0.6	0.0	0.0	0.0	0.0	0.0	0.0
N.o.	0.0	0.6	2.5	5.0	10.0	17.5	26.3	18.1	11.9	5.6	1.9	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lichens																			
A.c.	0.0	0.0	0.0	0.5	2.5	5.4	9.4	12.8	22.2	13.3	5.4	2.5	3.9	8.9	5.9	3.8	2.0	1.0	0.5
A.t.	0.0	0.0	0.4	1.3	3.9	9.9	13.8	17.7	22.8	7.3	2.6	0.9	1.3	3.9	6.9	3.4	2.2	1.3	0.4
E.m.	0.0	0.0	0.8	1.6	3.3	3.7	6.1	10.2	19.2	13.5	8.6	3.3	3.7	9.5	7.8	4.7	2.4	1.2	0.4
H.p.	0.0	0.0	0.0	0.0	0.6	1.7	5.0	12.6	19.5	10.7	2.8	1.7	4.4	8.0	12.6	10.6	5.8	3.5	0.5
C.sp.	0.0	0.0	0.4	1.7	3.4	8.0	11.0	12.2	17.3	10.5	6.8	3.4	1.3	2.5	8.0	6.3	5.1	1.7	0.4

D.s., *Dicranum scoparium*; T.t., *Thuidium tamarisunum*; P.c., *Polytrichum commune*; A.v., *Anomodon veticulosus*; P.sp., *Plagiomnium sp.*; M.sp., *Mnium sp.*; M.p., *Marchantia polymorpha*; B.sp., *Brachythecium sp.*; A.a., *Atrichum angustatum*; C.f., *Chara fragilis*; A.sp., *Acrosiphonia sp.*; N.o., *Nitellopsis obtusa*; A.c., *Anaptychia ciliaria*; A.t., *Aspicilia transbaicalica*; E.m., *Evernia mesomorpha*; H.p., *Hypogomnia physodes*; C.sp., *Cladonia sp.*

[a] Molecular weight by FD-MS.

brought about the qualitative change over TLC to HPLC is apparent from Fig. 9. The rapid development of HPLC method is followed by findings of new and larger polyprenols and this trend is well documented in Fig. 9. Thirty years ago C_{35} polyprenols represented the larger limit of detection; current techniques allow identifying polyprenols up to C_{500}.

The dolichol and dolichyl monophosphate content of exponentially growing revertants of RER2 deleted yeast (*Saccharomyces cerevisiae*) cells (Delta rer2) and of cells overexpressing SRT1 have been determined by HPLC analysis. Dolichols and dolichyl monophosphate with 19–22 isoprene units, unusually long for yeast, were found, and shown to be utilized for the biosynthesis of lipid intermediates involved in protein N-glycosylation [59].

The prenyltransferase undecaprenyl pyrophosphate synthetase (di-*trans*,poly-*cis*-decaprenylcistransferase EC 2.5.1.31) was purified from the soluble fraction of *Escherichia coli* by TSK-DEAE, ceramic hydroxyapatite, TSK-ether, Superdex 200 and heparin–Actigel chromatography [60].

The *Lactarius volemus* rubber showed a unimodal molecular distribution, centered around a M_w of 2400, with a shoulder in the high-molecular-mass region and two small peaks in the low-molecular-mass region. Each fraction, except for the highest one, contained a dimethylallyl-*trans*-*trans* terminal, and poly-*cis* isoprene units with a hydroxyl or fatty acid ester terminal group. The lowest m.w. fraction showed a corresponding to homologues of polyprenols with peaks from 17-mer to higher than 90-mer, having two centres around 21- and 32-mer by HPLC analysis. The distribution of chain-length between

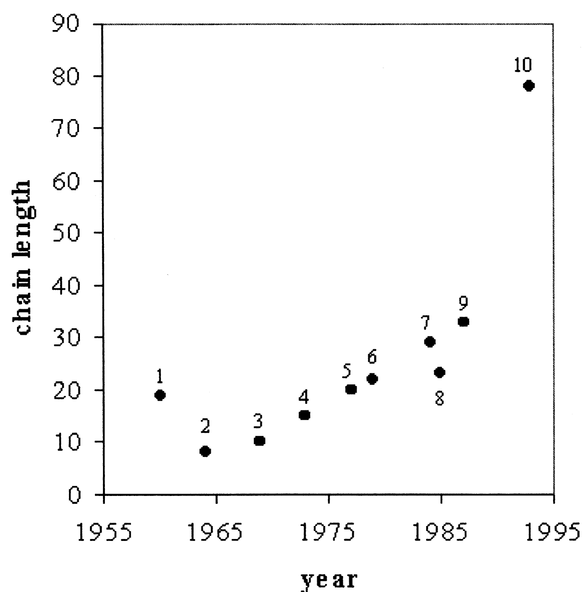

Fig. 9. Approximate time of isolation of the most characteristic polyprenols from plants. 1 = mammalian dolichol, 2 = *Betula verrucosa,* 3 = *Ficus elastica,* 4 = *Pinus strobus,* 5 = *Juniperus communis,* 6 = *Sorbus suecica,* 7 = *Zamia integrifolia,* 8 = *Potentilla aurea,* 9 = *Rosa virginiana* and 10 = *Lumnitzera racemosa.* Reproduced from Ref. [36] with permission.

35- and 90-mer in the next lowest molecular fraction, showed a 5 or 6 periodical repeating isoprene unit [61].

Solanesol (a C_{45} polyprenol) is a major component of tobaccos generally ranging in quantity from 0.4 to 4%. While this polyprenol was first isolated from tobacco, it is now considered to be a ubiquitous leaf component. The progression of solanesol changes during growth and air curing for middle stalk burley tobacco is shown in paper [62].

The paper shows by ESI-MS that the T-cell antigen receptor and the CD1c protein mediate recognition of an evolutionarily conserved family of isoprenoid glycolipids from *Mycobacterium tuberculosis* whose members include essential components of protein glycosylation and cell wall synthesis pathways. A CD1c-restricted, mycobacteria-specific T-cell line recognized two previously unknown mycobacterial hexosyl-1-phosphoisoprenoids and structurally related mannosyl-b1-phosphodolichols. These results define a new class of broadly distributed lipid antigens presented by the CD1 system during infection in vivo and suggest an immune mechanism for recognition of senescent or transformed cells that are known to have altered dolichol lipids [63].

To identity the prenol from *Schizosaccharomyces pombe* cells, they were incubated with a metabolic precursor of prenol, tritiated mevalonolactone. The cells incorporated only a modest amount of label, about 1000 dpm per million cells, into base-stable lipid and only 1% of that radioactivity was incorporated into prenol. They found by normal-phase silica HPLC and more directly by the lack of reactivity with MnO_2 that the labelled lipid was predominantly dolichol, not polyprenol, RP-HPLC demonstrated that in *S. pombe*, dolichol ranged between 14 and 18 isoprene units with dolichol-16, 17 being the most abundant prenol. This dolichol is of an intermediate length, between the dolichol of *S. cerevisiae* and that of mammalian cells [64].

3. Conclusions and future prospects

The modern techniques for prenols separation and determination including TLC, GLC and HPLC methods were reviewed. In biological matrices, prior to assay of prenols, a preparation treatment of the sample is often required to remove interfering endogenous components and to preconcentrate the analytes and fractionate them into separated groups. Based on the use of extraction, a significant progress was achieved in the methodology of sample pretreatment for chromatography with classical adsorbents (Lipidex gels, lipophilic ion-exchange gels), silica or chemically modified silica gels (e.g. octadecyl-bonded silica). Among the techniques suitable for prenols separation, we can select either TLC or HPLC. In fact, despite its rapidity and simplicity, TLC exhibits insufficient resolving power for the complex composition of biological extracts. HPLC should be preferred for the evaluation of prenol profiles since it has fewer disadvantages than TLC. These inherent disadvantages may be passed by fast development in LC–MS and LC–MS–MS techniques, especially if their cost and analytical ruggedness will become comparable to those of GC–MS. To conclude, for the analysis of very complex prenol mixtures, especially for ester of prenols and fatty

acids, multimode separation and detection systems would provide more accurate data than any single method.

4. Note added in proof

A high-resolution analysis of polyprenol mixtures was achieved by SFC. The separation of polyprenols was examined on an octadecylsilane-packed column with liquid carbon dioxide as the mobile phase and ethanol as modifier. Using this chromatography system, the resolution of separation between octadecaprenol and nonadrecaprenol was two times higher than that using conventional RP-HPLC [65].

The culture of *Coluria geoides* was analysed by HPLC and polyprenols were accumulated in roots. The mixture of several prenologues with the dominating prenol composed of 16 isoprenoid units was analyzed. The content of polyprenols in tissue was approx. 300 μg/g of dry weight [66].

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