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PLANT POLYPRENOLS AND THEIR BIOLOGICAL ACTIVITY

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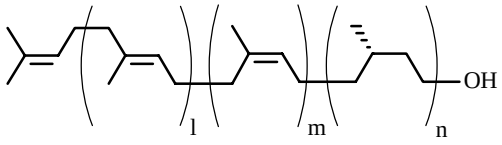
Research on the isolation, identification, and biological activity of plant polyprenols is reviewed.

Key words: polyprenols, distribution, isolation, chemical transformations, isoprene unit, biological activity.

Many vitamins, hormones, and coenzymes are based on isoprene chains. Thus, polyprenols (PP) including dolichols, which were not previously recognized as low-molecular-weight bioregulators, are interesting. They act as coenzymes of menbrane-active transport systems for polysaccharides, peptidoglycans, and carbohydrate-containing biopolymers, which are found in pro- and eukaryotes [1, 2]. PP are also interesting because of their important role as lipophilic sugar transporters in the biosynthesis of bacterial polysaccharides and glycoproteins. The physiological function of endogenous PP of plants is not yet fully elucidated although it is known that they open Ca^{2+} channels of bilayer membranes [3] and enhance the biosynthesis of nuclear proteins during preliminary soaking of cotton seeds [4]. Their use as chemotaxonomic criteria has been proposed [5].

PP (**1**) are unsaturated acyclic branched alcohols with a primary hydroxyl at the isoprene terminus. The quantity and geometric configuration of the units varies depending on the plant family.



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l = 2 - 3, m ≥ 3, n = 0

Gymnosperms and bacteria typically have PP with m = 2 (betulaprenols) whereas compounds with m = 3 (ficaprenols) and m + n varying from 4 to 25 are more typical of angiosperms.

The lowest PP include C_{10}-prenols [geraniol (**2**) and nerol (**3**)], C_{15}-farnesol (**4**), C_{20}-geranylgeraniol (**5**) and its hexahydro derivative phytol (**6**), and C_{25}-geranylfarnesol (**7**); the highest, solanesol (**8**), C_{55-65} castoprenols (**9**), etc. They are widely distributed among plants [6-9] and are found in both the free and bound states as esters of acetic and higher fatty acids [8, 9].

PP are represented by abbreviated formulas where ω is the terminal isoprene unit, t is a *trans*-isoprene unit, c is a *cis*-isoprene unit, and s is a saturated nonterminal unit.

ω-t-OH (**2**)	ω-c-OH (**3**)	ω-t-t-OH (**4**)
ω-t-t-t-OH (**5**)	s-s-s-t-OH (**6**)	ω-t-t-t-OH (**7**)

It was found in 1965 that lipophilic compounds containing monosaccharides are formed during the biosynthesis of carbohydrate biopolymers in the cell wall of gram-positive bacteria from nucleosidediphosphate sugars. These themselves donate glycosyl units of polymeric chains [9-13]. Research carried out in 1967 showed that this lipophilic component is C_{55}-undecaprenol. Further experiments demonstrated that these compounds are widely distributed in nature [14-17].

Free plant PP include C_{45}-C_{65}-compounds. Microbes contain polyprenolphosphate sugars; animals, compounds as

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dolichols (**10**) containing from 14 to 24 isoprene units with the terminal saturated unit. Dolichols were detected in both plants and microbes [18, 19].

ω-t-t-c_{11-21}-s-OH (**10**)

DISTRIBUTION

PP are recognized as a class of natural polyterpenes. They have been found in the green parts of many plants and possess a wide spectrum of biological activity.

New PP are usually named for the plant from which they are isolated, for example, betulaprenols from birch leaves (*Betula verrucosa*, Betulaceae), malloprenols from mallotus plants (*Mallotus japonicus*, Euphorbiaceae), pinoprenols from pine (*Pinus*, Pinaceae), castoprenols from horse chestnut (*Aesculus hyppocastanum*, Hippocastanaceae), etc. A number signifying the number of isoprene units is added to the name of a pure compound. For example, malloprenol-10, castoprenol-11, pinoprenol-14, etc.

If the stereochemistry is known and the structure is accurately established, then PP are often named by E-(*trans*) and Z-(*cis*) types, i.e., E- or Z- is added to the number designating the C atom. For example, malloprenol-10 is called (2Z,6Z,10Z,14Z,18Z,22Z,26E,30E,34E,38Z)-3,7,11,15,19,23,27,31,35,39-decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaen-1-ol [20]; glycinoprenol-10, (2Z,6Z,10Z,14Z,18Z,22Z,26E)-3,7,11,15,19,23,27,31,35,39-decamethyl-2,6,10,14,18,22,26-tetracontaheptaen-1-ol [21].

In most instances plant PP contain more Z- than E-isoprene units. Therefore, they are classified into three groups: betulaprenols (m = 2) with farnesyl; ficaprenols (m = 3) with geranylgeranyl, and those with one or more saturated prenyl unit at the *ω*-terminus [21].

The first representative of plant PP was solanesol (C_{45}), which was isolated first from tobacco leaves (*Nicotianum*, Solanaceae) [16, 22, 23]. A detailed study of its structure found that it is the *trans*-type. Compounds of this type were observed later in other plants [24].

ω-t-t-t-t-t-t-t-t-OH (**8**)

A prenol of lower molecular weight is hexahydro-C_{25}-prenol (P), which was isolated from potato leaves (*Solanum tuberosum*, Solanaceae) [25, 26]. The following formula was proposed for it:

(s+1)-s-s-t-c-OH (**11**)

Birch wood (*Betula verrucosa*) contains a mixture of betulaprenols C_{30}-C_{45} (**12**) bound to fatty acids. Among these, the C_{35}-C_{45} prenols dominate. Free C_{50}-C_{65} prenols are also found in trace amounts [9, 27, 28]. Like bacterial PP, these compounds contain two E-type isoprene units.

In contrast with this, prenols with a longer isoprene chain C_{50}-C_{65}-dominate in birch leaves (*Betula verrucosa*). Lower betulaprenols with n = 6-9 are found in trace amounts [29, 30].

The isolation of malloprenols and their esters with linolenic acid from *Mallotus japonicus* (Euphorbiaceae) leaves has been reported [20, 31]. The principal components in the PP fractions are C_{45}-C_{55} prenols with 9-11 isoprene units, 3 of which are the E-type.

The dynamics of malloprenols accumulation have been studied. It was found that the malloprenols content increases from April to September from 0.002 to 0.2%, i.e., by 100 times.

Leaves of *Cleome spinosa* (Capparidaceae) afforded claeomiprenols, a mixture of nona-, deca-, undeca-, and dodecaprenols in the ratio 7:43:42:8. The configuration of these is analogous to that of malloprenols [32, 33].

The isolation of C_{40-60} prenols from mulberry leaves (*Morus bombysis*, Moraceae) has been reported. It was shown that mulberry PP consist mainly of a mixture of undeca- and dodecaprenols of E-Z-mixed types [34, 35]. The mulberry PP have the configuration of ficaprenols.

Fig leaves (*Ficus elastica*, Moraceae) of the decorative fibrous plant yielded ficaprenols, which contain 10-12 isoprene

units. Among these, ficaprenol-11 (undecaprenol) dominates. Chromatography demonstrated the presence of trace amounts of ficaprenol-9 and -13 [36].

Leaves of horse chestnut (*Aesculus hippocastanum*, H.) afforded PP called castoprenols. These contain 11-13 isoprene units in which, in contrast with ficaprenols, castoprenol-12 dominates. Castoprenol-10 was observed by chromatography in trace amounts [37].

ω-t-t-t-c-c-c-c-c-c-c-c_{1-3}-OH (**9**)

PP of the pine family are especially well studied [8, 38-45]. Research found that prenols in various pine species are found in the free and bound states.

White pine wood (*Pinus strobus*, Pinaceae) yielded a mixture of polymeric homologs C_{90}-PP with 18 isoprene units [38]. PP of white-pine needles were found to occur as acetates of polymeric homologs with n = 14-21 with C_{85} PP of 17 isoprene units dominating [38].

Wood and needles of Scotch pine (*P. sylvestris*, Pinaceae) were extracted with acetone and petroleum ether. Separation over a column isolated PP acetates, alkaline hydrolysis of which in aqueous alcoholic KOH produced free prenols with 10-19 isoprene units [8, 39].

Their principal components are pinoprenols with 15 and 16 isoprene units. Pinoprenols with 13, 14, and 17 isoprene units are comparatively rare. Other prenols with 10, 11, 12, 18, and 19 isoprene units are found in trace amounts [8, 39, 40].

A comparison of data for PP isolated from wood and needles of Scotch pine, with solonesol and dolichol, led to the conclusion that PP from this plant contain poly Z-,2E-isoprene units [8, 41] and belong to the betulaprenol type [42]. Leaves of Scotch pine afforded PP in which prenols with n = 15 dominate and have the ficaprenol configuration [43].

A study of the composition of the PP fraction from Crimean pine needles (*P. krimica*, Pinacae) [44] found that the PP in them, like in *P. sylvestris*, occur as acetates and consist of polymeric homologs with 11-19 isoprene units. The main components are prenols with 15-17 isoprene units.

Needles of two species, *P. densiflora* and *P. thunbergii*, in contrast with *P. strobus* and *P. sylvestris*, have PP in the free and bound states with n = 11-22. Their principal components are PP with n = 16-17 at 28.3:30.0 and 32.3:32.3%, respectively [41].

Green needles of *P. mugo* contain a mixture of PP with 15-17 isoprene units as acetates. The principal component is a C_{80} alcohol. A study of the PP accumulation dynamics found that the level of PP in the second year of growth increases by 2-3 times [45].

It should be noted that, depending on the pine species, the dominant PP vary in amount of isoprene units. For example, PP with n = 17-18 dominate for *P. strobus*; n = 16-17, *P. sylvestris*, *P. densiflora*, and *P. thunbergii*; n = 15-17, *P. krimica*; and n = 16, *P. mugo*. All these species have characteristically PP with the betulaprenol configuration, i.e., they contain 2 E-isoprene units.

It was noted [17] that needles of European fir (*Picea abies*, Pinaceae) contain PP. The green wood of this species is the main raw material for chemical processing and is used to produce food products and medicinal-prophylactic preparations [46-48].

PP concentrates with molecules ranging from 14 to 16 isoprene units long were isolated from green wood of conifer species of *Pinus*, *Picea*, and *Abies* growing in the Komi Republic [49].

A study of the thermal stability of PP from green needles found that 3.5-4.5% of the mass is lost in the range 0-280°C. This is due to evaporation of water and volatile impurities and not PP destruction. According to the authors, the process is not accompanied by thermal effects [50].

PP were also isolated from products of processing green wood of European fir [*Picea abies* (L.) Karst]. Extraction with benzene gives a provitamin concentrate that contains PP, chlorophylls, needle wax, essential oils, and balsamic paste [51].

The composition of a concentrate of higher fatty acids from green conifer wood was studied. According to chromatographic analysis, the concentrate contains hydrocarbons (3-4% of the total fraction), PP (2-4%), total organic acids (61-65%), sesqui- and diterpene alcohols (7-8%), and sterols (10-12%) [52].

Comparison of the compositions of extracted substances from needles and shoots of European fir found that the majority of the substances are extracted by an isopropyl alcohol. Next, fractional extraction by diethylether and petroleum ether was performed. The petroleum-ether extract of needles consists mainly of C_{60-90} PP alcohols (83%) acetates whereas the shoot

extract contains esters of higher fatty acids and sterols (87%) [51].

PP fractions with 12-18 isoprene units were isolated from leaves of this plant. Among them, piceoprenol-15 dominates [53].

A study of the PP content in needles of European fir of various age found that the PP in them occur as acetates. PP fractions in which the PP content is ~1% of the absolute needle mass were isolated. The maximal PP content in needles in the first year of growth occurs in January-February. Furthermore, a big difference is noted in the PP content of needles in the first and second year of growth. For example, the amount of PP in September in two-year needles is 1.25% whereas it is only 0.46% of the absolute mass in the first year, i.e., about 2.5 times greater [54]. The principal components are prenols with 14-16 isoprene units.

The biosynthesis of PP in fir needles is analogous to that for plant PP [55], i.e., it occurs via sequential *cis*-condensation of isopentylpyrophosphate with *trans*-geranylgeraniol pyrophosphate [56].

A report on the chemical composition of bark from Siberian fir (*Abies sibirica*, Pinaceae) noted that the PP in it occur as acetates [56]. A study of the chemical composition of the inner bark layer found that 1.1% of the neutral part of the extract contains esters of polymeric PP homologs according to HPLC. Among these, homologs with chain lengths from 11 to 18 isoprene units are found. The principal components are prenols with n = 16-17. The positions of the E- and Z-double bonds assign them to the betulaprenol class. It should be noted that the PP fractions of fir bark contain a small quantity of C_{45-85} dolichols (3%), the stereochemistry of which is unknown.

Research on still another Pinaceae species (*Cedrus deodora*) found that the PP of this species, like for other representatives, occur as acetates of polymeric homologs with n = 13-23. The dominant component is a C_{90} prenol [41].

A review of the literature on Pinaceae PP leads to the conclusion that PP of this family are characteristically found mainly as the acetates. Their configuration belongs to the betulaprenol class in which Z-isoprene units dominate. Furthermore, it was found that PP of pines (Pinaceae) differ according to species and can be used as a chemotaxonomic criterion [5].

Needles of *Juniperus communis* (Cupressaceae) afforded free and bound PP with 14-21 isoprene units. Research found that the dominant prenols have n = 16-17 (24.6:24.6 free and 25.2:22.5 bound). In contrast with this, needles of *Abies alba* and *Picea abies* have only bound PP. PP with n = 13-15 dominate (23.9:32.7:27.6) for *P. abies* (n = 12-18) whereas those with n = 15-17 isoprene units dominate (20.2:38.5:22.6) in *A. alba* (n = 11-19) [57].

Japanese researchers studied PP from leaves of *Cryptomeria japonica* and *Metasequoia glyptostroboides* (Taxodiaceae), *Sciadopitys verticillata* (Sciadopitydaceae), *Chamaecyparis obtusa*, *Juniperus chinensis* and *J. rigida* (Cupressaceae), *Araucaria brasilina* (Araucariceae), *Podocarpus macrophylla* and *P. nagi* (Podocarpaceae), *Cephalotaxus harringtonia* (Cephalotaxaceae), and *Taxus cuspida* and *Torreya nicifera* (Taxaceae) [58]. The compositions of the PP fractions of *J. chinensis* and *J. rigida* differ little from that of *J. communis* C., i.e., they consist of polymeric homologs with n = 14-23. The principal components are C_{85-90} prenols in the ratio 20.9:16.8 and 26.8:18.9 free and 22.8:18.4 and 26.5:19.8 bound.

Needles of *A. brasilina* (Araucariceae) yielded free PP with n = 12-26. Prenols with 22-23 isoprene units dominate. Free PP isolated from *P. macrophylla* and *P. nagi* (Podocarpaceae) have isoprenols with n = 12-28. Prenols with n = 24 dominate in the former; with n = 23, in the latter.

PP of *C. harringtonia* (Cephalotaxaceae) occur as acetates of polymeric homologs with n = 15-23. The main component is a C_{90} prenol. Acetates of PP of various composition are typical of *T. cuspida* and *T. nicifera* (Taxaceae) with n = 14-26 for the former and n = 14-20 isoprene units for the latter with the C_{90} prenol dominant.

For *C. japonica* (Taxodiaceae), C_{85-90} prenols dominate among the polymeric homologs with n = 14-25. For *M. glyptostroboides* of this same family, PP with n = 16-24 are typical with $C_{105-110}$ prenols as the principal components. It should be noted that PP of this family occur as the acetates. For *S. verticillata* (Sciadopitydaceae), PP with n = 12-25 and predominance of the C_{85} prenol are typical [58].

Research found that the PP content in needles of conifers is higher than in broad-leafed species [29, 30, 53].

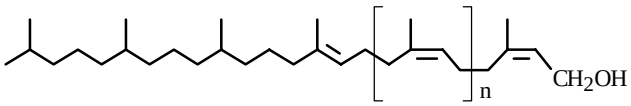
The seasonal accumulation dynamics of PP in both broad-leafed (*Aesculus hippocastanum*, Hippocastanaceae) and evergreen (*Hevea brasiliensis*, Euphorbiaceae) species have been reported. The PP content in leaves of evergreens increases from April to October by 80-600 times [29].

Leaves of various woody plants contain a large number of PP with Z-double bonds.

PP acetates with n = 14-23 were isolated from leaves of *Gingko biloba* (Gingkoceae) by extraction with hexane:acetone (4:1), treatment with methanol and hexane, and subsequent separation over a silica-gel column. The principal components of the PP fraction are prenols with n = 17-18 (25.6 and 39.2%) [59]. The PP of *G. biloba* have the betulaprenol configuration.

Research on the composition of seabuckthorn (*Hippophae rhamnoides*, Ebagnaceae) leaves found that the PP in them occur in the free state and bound to higher fatty acids [60, 61]. The dominant components are prenols with n = 13-14. A study of the chemical composition of the leaves using TLC detected a significant quantity of PP [60]. According to the literature, the contents of free and bound PP are 0.043 and 0.045%, respectively, of the mass of air-dried leaves [61]. The fractions were analyzed qualitatively and quantitatively using HPLC. The free PP of seabuckthorn consist of C_{45-65} prenols, mainly C_{55} (63.2%) and C_{50} (22.5%). The bound PP consist of C_{45-95} prenologs and C_{70-100} dolichols. It was found that the PP and dolichols of seabuckthorn are bound to 12:0-24:0, 16:1, 16:2, 16:3, 18:1, and 18:3 fatty acids.

Leaves of soy (*Glycine max* Merrill, Fabaceae) afforded two PP fractions. The first PP fraction contains a phytyl substance called glycinoprenols-9, -10, and -11. Spectral analysis assigned to them the following formula:



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n = 4 - 6

The second fraction contained ficaprenols-10, -11, and -12 [21].

A comparison of PP from plants of the Magnoliaceae, Moraceae, and Cucurbitaceae families found that prenol-11 dominates the polymeric homologs in the first family whereas prenols with 20-30 isoprene units dominate in plants of the last family [5, 62].

Leaves of *Potentilla aurea* (Rosaceae) contain long-chain PP bound to C_{14-24} fatty acids. The number of isoprene units is from 18 to 42 with prenols with n = 20 and 28 dominant. Furthermore, PP with n = 25-42 and a saturated terminal hydroxyl are detected as minor components [63].

The PP of leaves of certain Rosaceae species such as *Crataegus crusgalli*, *Cotonoaster lucida*, *Prinus serotina*, and *Sorbus suecica* (intermedia) were studied. The PP of this family occur in the free and bound states. They contain 17-30 isoprene units with C_{95-100} prenols acetates dominating. The contents of the PP fractions vary from 0.5-1% of the moist plant mass [64].

Free C_{50-60} prenols, the content of which is >0.5% of the moist plant mass, were isolated from leaves of *Magnolia liliflora* Pesrouss (Magnoliaceae), *Carya cordiformis* Koch. and *Juglans regia* L. (Juglangaceae), and *Rhus typhina* L. (Anacardiaceae) [65]. A study of the accumulation dynamics of PP in these plants found that their content increases as the leaves age. The content is maximal in 18-24-week leaves.

A comparison of the PP in the aforementioned species shows that their compositions differ quantitatively. For example, C_{95-105} PP dominate in *Crataegus crus-galli* and *S. suecica* (intermedia) (20.8, 23.1, 16.7 and 16.3, 42.6, 14.9%, respectively); C_{90-100}, in *Cotonoaster lucida* and *Prinus serotina* (15.5, 27.9, 20.7 and 23.1, 35.6, 18.6%, respectively).

Research on the PP composition of leaves of *Magnolia liliflora* Pesrouss (Magnoliaceae) (I), *Carya cordiformis* Koch (Juglangaceae (II), *Juglans regia* L. (Juglangaceae) (III), and *Rhus typhina* L. (Anacardiaceae) (IV) determined that the C_{50-60} content in 18-24 weeks after the start of budding is 5.5, 6.1, 31.4 (I); 12.0, 54.7, 33.3 (II); 25.7, 64.4, 9.9 (III); and 0, 55.9, 44.1 (IV).

It should be noted that C_{55} prenol dominate in plants of the Juglangaceae family; C_{60}, Magnoliaceae; and C_{55}, Anacardiaceae. The C_{50} PP are absent.

Leaves of fruiting plants of the Rosaceae (*Prinus* and *Malus*) and Cornaceae families yielded acetates of PP with 18-21 isoprene units, the content of which is 1-5%/g of dry mass. Other polymeric homologs, i.e., PP alcohols with 35-42 isoprene units, were observed in leaves of other plum species (*Prunus* Sour Cherry, *P. serrulata-spontanea*, *P. yedoensis*, *P. fruticosa*, *P. kurilensis*, *P. subhirtella*, and *P. incisa*) [65]. In all instances prenols with 18-19 isoprene units dominate. Certain varieties, for example, representatives of the Prinus family, contain prenols with 35-40 isoprene units in addition to the prenols noted above. The PP content in leaves of analyzed fruit trees is from 1 to 5% of the dry mass. The maximal content occurs at the end of the vegetative period, i.e., autumn.

Analytical results indicate that prenols with a lower isoprene number dominate in certain plant species whereas prenols with a longer chain occur in trace amounts or vice versa. For example, prenols with a longer chain length dominate in *P. incisa* or *P. subhirtella*. They occur in trace amounts in other Rosaceae species. The PP in fruiting plants occur mainly as acetates.

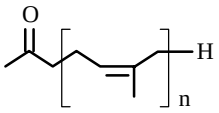
However, they also contain esters with fatty acids and with longer chains [66].

PP of certain tropical and subtropical plants such as *Plumeria rubra* (Apocynaceae), *Anaxogorea brevipens* and *Annona reticulata* (Annonaceae), *Begonia hederaefolia* (Begoniaceae), *Eucommia ulmoides* (Eucommiaceae), *Hura crepitans*, *Mallotus barbatus*, *Exoecaria bussei*, *Codiaeum variegatum*, *Euphorbia tirucalli*, *Euphorbia speldens*, *Putranjiva roxburghii*, and *Hevea brasiliensis* (Euphorbiaceae), *Mammea americana* (Guttiferae), *Ficus subrepanda*, *F. craterostoma*, *F. triangularis*, *F. elastica*, *F. retusa*, *F. altissima*, *F. bengalensis*, *F. lyrata*, and *F. religiosa* (Moraceae) have been studied [67]. It was shown that these typically have a high content of free PP of the Z/E configuration with n = 8-14 and predominance of C_{50-60} prenols. The PP content depends on the family and species. For example, plants of the Moraceae family have the maximal PP content. It varies from 0.13 (*F. religiosa*) to 3.93 (*F. subrepanda*). However, the content in plants of the Euphorbiaceae family varies from 0.14 (*E. splendens*) to 3.01 (*H. crepitans*); Annonaceae, 0.59-1.48; Apocynaceae, Eucommiaceae, and Guttiferae, 0.33, 0.21, and 0.51 mmole/kg, respectively.

A systematic investigation of cotton (*Gossypium hirsutum*, Malvaceae) leaves components showed that PP occur in them in the free state [68]. Later they were observed as a mixture of 16 components [69]. Cotton PP are isoprenologs with 10-13 isoprene units. Undecaprenol (n = 11) dominates. The content of undecaprenol in the PP fraction depends on the type and lineage of the cotton and reaches 70%. Tridecaprenol occurs in trace amounts.

The accumulation dynamics of cotton PP indicate that their content increases as the plant matures. The PP content as a function of type and lineage varies from 1 to 4% of the air-dried mass [3, 4, 68, 69].

A detailed study of PP fractions from cotton leaves detected bombyprenols (**13**) with n = 8-10 [70, 71], which were previously identified in *Nicotianum* (Solanaceae) leaves [72, 73]. PP with n = 5-8 were also identified. According to spectral data, these belong to the glycinoprenols (**14**), which were found previously in soy (*Glycine max*, Fabaceae) leaves [21].



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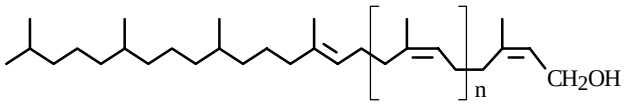
n = 8 - 10



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n = 5 - 8

Thus, the information presented above indicates that PP differ in molecular weight depending on the plant species. Broad-leaf trees contain from 6 to 13 prenols; conifers, 10-21; fruit trees, 11-42; grasses, 5-30.

ISOLATION METHODS

PP are usually isolated from the neutral part of plant extracts. However, optimal conditions are developed for each specimen as a function of plant composition. For example, extraction of *Picea abies* needles by hexane, chloroform, or isopropanol gives the greatest amount of extracted substances in the isopropanol. The isopropanol extract is mixed with petroleum ether. The substances soluble in petroleum ether are separated into acids (21.9%) and neutral substances (75.9%). The chemical composition of *P. abies* needles and shoots shows that the content of neutral substances is 2.0-2.5 times greater in the needles than in the shoots [17].

Separation of the neutral substances by column chromatography isolates from them hydrocarbons, esters of di- and triacylglycerines, aldehydes, alcohols, sterols, polyoxy compounds, carotinoids, and chlorophyll derivatives.

Other researchers isolate PP fractions by extraction with petroleum ether (40-60°) in a Soxhlet extractor. Then, the extract is washed with aqueous NaOH (2%) and chromatographed over a silica-gel column with elution by diethylether: petroleum ether (5:95) [54].

Conditions for isolation of PP from green wood of *Pinus* and *Picea* species have been optimized [49]. The plant material is extracted with ethanol. The extract is condensed, held at 10°C, and filtered to remove solids. The filtrate is saponified by aqueous KOH (20%) and treated with saturated NaCl solution. The mixture is extracted by hexane and separated over a column packed half with aluminum oxide (lower) and half with silica gel (upper).

In other instances, PP are isolated from needles of *Juniperus communis* (Cupressaceae) by extraction with acetone in

a Soxhlet extractor. The extract is diluted with an equal volume of water and re-extracted with petroleum ether. The petroleum-ether fraction is separated using column chromatography over aluminum oxide. Free (benzene—ethylacetate 95:5, R_f 0.6) and bound (petroleum ether—benzene 2:1, R_f 0.5) PP are isolated [57].

PP are isolated from seabuckthorn leaves [60] by extraction with diethylether, treatment with aqueous alkali (2%) and water, and drying. The neutral part of the extract is chromatographed over a silica-gel column with elution by petroleum ether mixed with diethylether. The pure PP are isolated preliminarily by HPLC in a Milikhrom instrument using a column (6.3×0.2 cm) packed with Silasorb-C_{18} sorbent and elution by acetone—methanol (2:3).

PP are isolated from soy (*Glycine max*, Fabaceae) leaves by extracting the plant material with methanol at room temperature for two weeks. The methanol extract is condensed, diluted with water, and extracted with hexane. The extract is separated over a silica-gel column with elution by benzene to give PP fractions. The quantitative content is determined by HPLC (Zorbax ODS column, 21.2×250 mm, hexane—methanol 1:4) [21].

PP fractions are isolated from cotton (*G. hirsutum*, Malvaceae) leaves by drying the leaves, extracting with ethanol, hydrolyzing with alkali, and re-extracting with benzene or hexane. The hexane extract is separated using column chromatography to give the PP [3, 4, 67].

PP MODIFICATION

Numerous studies of the isolation and synthesis of PP have been reported. Data on their chemical transformations are limited. The modification is usually carried out in order to establish the structure or to study PP derivatives as potential biologically active substances. Previous reports contain mainly data on the acylation of the hydroxyl or its oxidation to an aldehyde. Thus, results from modification of solanesol are described in a series of works [22, 23, 74]. Rowland et al. first isolated solanesol (**8**) and acylated it to give the acetate (**15a**) and solanesyl-3,5-dinitrobenzoate (**16**). Reaction with 3,4,5-triiodobenzoylchloride gives solanesol triiodobenzoate (**17**) [23].

$$H\text{-}(CH_2\text{-}CMe{=}CH\text{-}CH_2)_9\text{-}OCOR,\ R{=}CH_3\ (\mathbf{15a}),\quad C_6H_3(NO_2)_2\ (\mathbf{16}),\quad C_6H_2I_3\ (\mathbf{17})$$

Phosphorylation of solanesol [73-75], lower betulaprenols [75, 76], and ficaprenol [75, 77-79] by *bis*-(triethylammonium)phosphate in the presence of trichloroacetonitrile gave the corresponding phosphates:

$$H\text{-}(CH_2)\text{-}CMe{=}CH\text{-}CH_2)_n\text{-}OH+(Et_3N)_2H_3PO_4 \rightarrow H\text{-}(CH_2\text{-}CMe{=}CH\text{-}CH_2)_n\text{-}O\text{-}PO(OH)_2+$$
$$+H\text{-}(CH_2\text{-}CMe{=}CHCH_2)_n\text{-}O\text{-}PO(OH)\text{-}O\text{-}PO(OH)_2$$

Acetates of castoprenols were isolated and oxidized with MnO_2 to synthesize the corresponding aldehydes [37].

$$H\text{-}(CH_2\text{-}CMe{=}CH\text{-}CH_2)_n\text{-}OH \rightarrow H\text{-}(CH_2\text{-}CMe{=}CH\text{-}CH_2)_{n-1}\text{-}CH_2\text{-}CMe{=}CH\text{-}CHO$$
$$n = 11\text{-}13$$

Oxidation of malloprenol by CrO_3 in pyridine gave the corresponding aldehydes [31]. Malloprenols were acylated by acetic anhydride and *p*-bromobenzoylchloride in the presence of pyridine to give the acetate and *p*-bromobenzoate of malloprenol (**19**) [29].

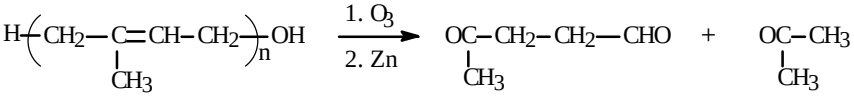
$$H\text{-}(CH_2\text{-}CMe{=}CH\text{-}CH_2)_n\text{-}OH+BrC_6H_4COCl \rightarrow H\text{-}(CH_2\text{-}CMe{=}CH\text{-}CH_2\text{-})_n\text{-}O\text{-}COC_6H_4Br\ (\mathbf{19})$$
$$n = 9\text{-}11$$

Acylation of PP from cotton leaves by acetic, propanoic, and benzoic anhydrides [80] gave the PP esters in 69, 52, and 58% yields, respectively.

$$H\text{-}(CH_2\text{-}CMe{=}CH\text{-}CH_2)_n\text{-}OH + (RCO)_2O \rightarrow H\text{-}(CH_2\text{-}CMe{=}CH\text{-}CH_2)_n\text{-}O\text{-}COR + ROOH$$

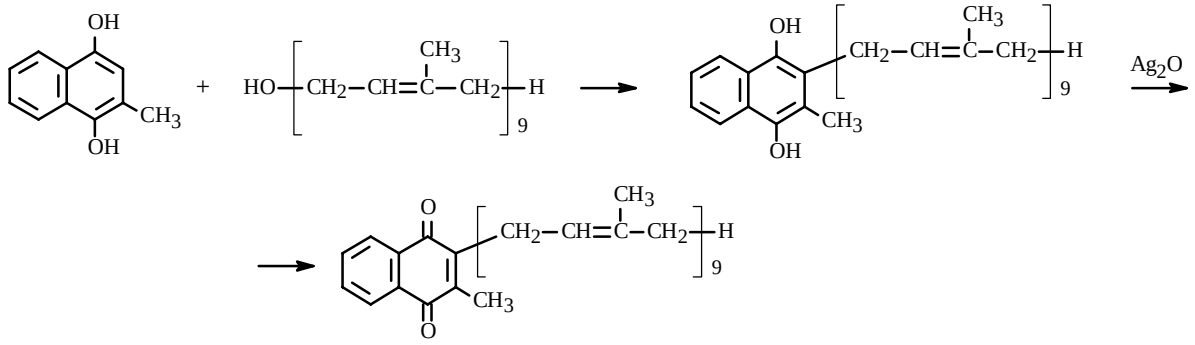
$$R = CH_3,\ CH_3CH_2,\ C_6H_5;\ n = 10\text{-}12$$

Ozonolysis of malloprenol in ethylacetate at -70°C gave the ozonide, reduction of which by powdered zinc gave levulinic aldehyde and acetone. These were identified as the 2,4-dinitrophenylhydrazones [31].



n =10 - 12

Other researchers [22] condensed solanesol and 2-methyl-1,4-naphthohydroquinone. Oxidation of these gave analogs of vitamin K_2 (**18**).



IDENTIFICATION METHODS

PP fractions are identified in most instances using TLC on Silufol UV-254 plates and comparison with authentic samples, GC, HPLC [8, 33, 79, 80] and the combination of physical methods such as IR, PMR, ^{13}C NMR spectroscopies and mass spectrometry [3, 16, 20, 36, 37, 81, 82].

The mass spectra of PP contain weak peaks for the molecular ions. Ions with *m/z* $[M - 18]^+$ and fragment ions corresponding to loss of a 3,3-dimethylallyl group, $[M - 69]^+$, are stronger and more characteristic. Then, series of ions from the loss of one, two, or more isoprene units, $[M - n68]^+$, are formed.

Ions such as $[M - Ac]^+$, $[M - Ac - 69]^+$, $[M - Ac - 69 - n68]^+$ and fragments with *m/z* 135, 121, 107, 105, 95, 93, 81, and 69 are typical of bound PP, i.e., acylated PP [3].

Biogenetic and comparative spectral data for solanesol and betulaprenols suggested that they and their acetates have the following structures:

$$\omega\text{-t-t-c-}C_{1\text{-}4}\text{-C-OH or}$$

$$(CH_3)_2C{=}CH\text{-}CH_2\text{-}[CH_2\text{-}C(CH_3){=}CH\text{-}CH_2]_{n\text{-}2}\text{-}\ CH_2\text{-}C(CH_3){=}CH\text{-}CH_2OR\ (\mathbf{12})$$

$$R{=}H,\ OCCH_3$$

Their molecules were demonstrated to contain two E-type isoprene units [27, 28].

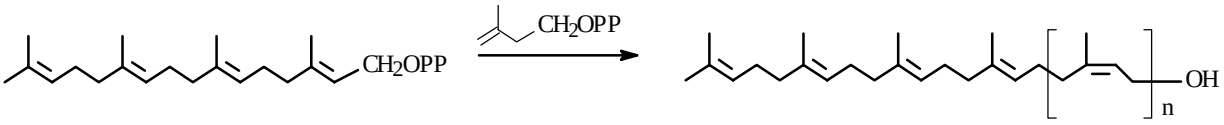
Quantitative HPLC analysis of TMS-derivatives of PP isolated from Scotch pine wood and needles found that the content of principal components with 15 and 16 isoprene units are 33;43 and 24.2;35.1% whereas the fractions of PP with 13,

14, and 17 isoprene units are 3;10;9 and 3;7.8;18.8%, respectively. The remaining prenols with 10, 11, 12, 18, and 19 isoprene units are found in trace amounts [8, 40, 41].

PMR and ^{13}C NMR spectroscopies are used to study fully the PP structures. The number of isoprene units in molecules dominating the prenol PP fraction can be calculated from the ratio of signal strengths at 1.74 ppm [3H, singlet, –C($\underline{CH_3}$)=CH–CH_2OAc], 1.57 and 1.65 (singlets of *trans*- and *cis*-methyls, respectively, of isoprene units), and 5.31 (1H, triplet, >C=CH–CH_2O) and 5.08 (broad singlet, protons on C atoms of double bonds). The position of the H_3C signals is characteristic. The number of *cis*- and *trans*-isomers and the number of unsaturated isoprene units in the PP can be determined from it [3, 8, 20, 39, 41, 54, 58, 63].

Thus, the PMR spectrum of malloprenol-11 (90 MHz, $CDCl_3$, δ, ppm, J/Hz) has signals at 1.59 (12H, s, E-C$\underline{Me}$=CH), 1.67 (21H, s, Z-C$\underline{Me}$=CH), 1.72 (3H, s, Z-CMe=C$\underline{H}CH_2$OH), 2.0-2.1 (40H, =CHC$\underline{H_2}$C$\underline{H_2}$C=), 4.06 (2H, d, J = 7, =CHC$\underline{H_2}$OH), 5.1 (10H, m, $W_{1/2}$ = 13 Hz, =C$\underline{H}$), and 5.42 (1H, t, J = 7, =C$\underline{H}CH_2$OH). This is consistent with a 7:3 ratio for the Z- and E-isoprene units [20].

Biogenesis [20] and spectral data [31] proved that malloprenol-10 from *Mallotus japonicus* (Euphorbiaceae) leaves has the structure (2Z,6Z,10Z,14Z,18Z,22Z,26E,30E,34E)-3,7,11,15,19,23,27,31,35,39-decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaen-1-ol. Biogenesis found that sequential Z-condensation of isoprene units with (2E,6E,10E)-geranylgeranylpyrophosphate leads to the formation of malloprenols.



Ficaprenols were also identified using PMR, mass, and IR spectra. Physical and biogenetic investigations found that ficaprenols contain only three *trans*- internal isoprene units that are formed from *trans*-geranylgeranylpyrophosphate [36].

The spectral properties of castoprenols were studied in detail. It was found that the prenols contain three *trans*-terminal units. The rest are *cis*-isoprene units [37], like in ficaprenols [36].

Spectral studies found that pine PP contain mainly Z-double bonds in addition to two terminal isoprene units with the *trans*-configuration [40, 41].

The ratio of methyl signal strengths showed that PP of *P. krimica* contain two E- and poly-Z-isoprene units [44].

The PMR spectra of PP from European fir needles showed that they have a high content of Z-methyls. The principal components are prenols with 14-16 isoprene units [54].

The PMR spectra of PP isolated from Siberian fir bark have 3:1 ratios of integrated intensities for methyl-proton signals on Z- and E-double bonds at 1.66 and 1.58 ppm. Therefore, they are assigned to the betulaprenols [56].

The stereochemistry of PP from leaves of *Potentilla aurea* (Rosaceae) was studied in detail using PMR and ^{13}C NMR [63]. The PMR are consistent with reported Z- and E-PP data [40, 81]. Internal Z- and E-isoprene units in the PP were determined using ^{13}C NMR spectroscopy. The ^{13}C NMR spectra contain signals at 32.01 ppm that correspond to *trans-cis*; at 32.25, *cis-cis*; at 39.72-39.74, *trans-trans*; and *trans*-terminal isoprene units. The lack of a signal at 40.0 ppm indicates that *cis-trans* bonds are absent. PP of *P. aurea* are assigned to betulaprenols according to the spectral data.

Deca- and undecaprenols isolated from seabuckthorn leaves were identified using PMR and ^{13}C NMR spectra and mass spectrometry. It was found that decaprenol has 3E- and 6Z-; undecaprenol, 3E- and 7Z-isoprene units. The presence of a signal at 131.12 ppm and the absence of a signal at 40.0 ppm in the ^{13}C NMR spectrum indicates that the E-double bonds are located at the ω-terminus and are typical of ficaprenols [60].

The PMR spectrum of acetyl-PP has distinctive signals at 2.02-2.04 (3H, s, $OCOCH_3$) and 4.55 (2H, d, =$CHCH_2$OAc) [3, 44, 54, 78].

The IR spectrum of PP exhibits absorption bands at 835 cm^{-1} that correspond to C–H deformations of trisubstituted olefins. Characteristic C–O bands of allyl primary alcohol appear at 1000 cm^{-1}. The region near 1365 cm^{-1} belongs to C–H deformations of CH_3; at 1450 cm^{-1}, to –C–H of CH_3 and CH_2 deformations. The C=C vibrations in isoprenols appear at 1660 cm^{-1}. The region near 2845 cm^{-1} is typical of –C–H vibrations of CH_2; at 2918 cm^{-1}, –C–H of CH_2 and CH_3; at 2956 cm^{-1},

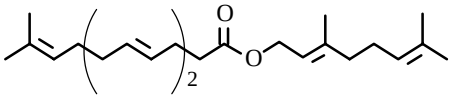
–C–H of CH_3, at 3024 cm^{-1}, –C–H vibrations of =CH. Vibrations of free hydroxyl appear at 3575 cm^{-1}; of polymeric OH chains, at 3310 cm^{-1} [36, 37].

It should be noted that the exact location of E- and Z-isoprene units is difficult to determine because plant prenols occur as mixtures of polymeric homologs. The location is often relative. However, the location of E- and Z-isoprene units can be accurately determined in pure PP [20, 21, 31].

PP are synthesized in multiple steps [83-85]. Regardless of recent success in constructing PP molecules, total synthesis of higher prenols is still problematical. Thus, synthesis of the very simple geraniol derivatives (E,E)-farnesol and (E,E,E)-geranylgeraniol [86] requires the use of organometallic reagents and low temperatures (-78°C). A mixture of PP with internal and terminal double bonds and a 5:1-31:1 Z/E ratio, i.e., optically impure isomers, is formed. Another relatively simple isoprenol alcohol (3R)(-)geranyllinalool and its (3S)(+)-isomer, which are widely distributed in nature [87] and are important as intermediates in the synthesis of pheromones [88, 89], are synthesized in seven steps [90]. Therefore, the principal starting materials for preparing their PP are natural sources. The main starting material for manufacturing highly effective medicinal and agricultural preparations comes from plants.

PHYSIOLOGICAL ACTIVITY OF PP

The physiological activity of PP was first studied nearly 40 years ago. Adami et al. first found that the geranyl ester of farnesylacetic acid, gefarnate (**20**), possesses anti-ulcer activity [91].

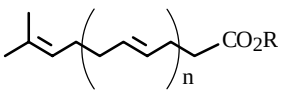


20

The wide spectrum of biological activity of PP is probably due to their membrane-active properties and their ability to bind PP fragments with biologically active groups. They play an important role in the biosynthesis of carbohydrate chains of natural macromolecules that is catalyzed by membrane-bound enzymes. PP phosphate sugars are involved in the biosynthesis of several cell-wall components of bacteria and yeast and bacterial capsule polysaccharides [11].

According to the literature [54], PP of conifers accumulate in cell membranes and fulfill two functions. They are carbohydrate acceptors in reactions that form repeating polysaccharide chains. They stabilize cell membranes during temperature drops during the year. Thus, the second function of PP for deciduous trees is not so important. This is reflected in their lower content.

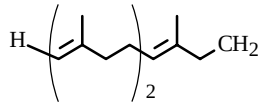
It was also found that PP isolated from 108-F cotton leaves have the unique property of opening calcium channels in membrane bilayers [3].

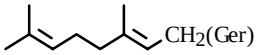


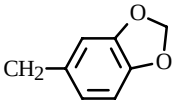
21

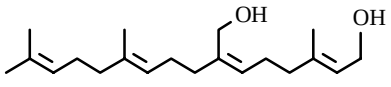
R = Me, Et, $CH_2C_6H_4Cl$–n

n = 2, 4, 6 - 11



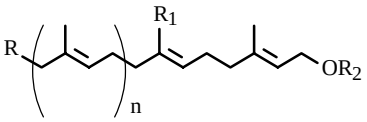






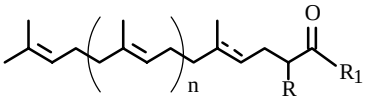
22

Research has shown that prenylacetic esters (**21**) inhibit gastrointestinal ulcers [92-95]. Further searches for natural PP sources [96, 97] based on Thai folk medicine identified a compound (**22**) from *Croton* (*C. sublyratus* Kurz and *C. columnaris*) plants that exhibits a stronger effect than gefarnate (**20**) for treating gastrointestinal ulcers [97-99]. Systematic investigation of analogs of these compounds led to preparations for prophylaxis and cure of stomach and duodenal ulcers. Long-chain prenyl alcohols (**23**), aldehydes (**24**), ketones (**25**), acids (**26**), and amines (**27**) also exhibit high anti-ulcer activity [100-102].



23: R = H, $R_1 = CH_2OR_2$, R_2 = H, Ac, Me, Bz, $COC_{11}H_{23}$-H in positions on $C_{(2Z)}$, $C_{(6E/Z)}$, $C_{(10E/Z)}$, $C_{(14E)}$

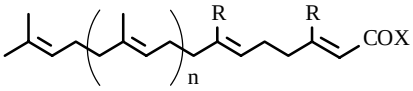
n = 7, 8



24, **25**

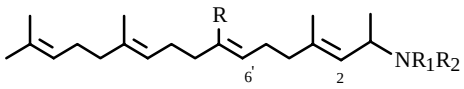
R = H, Alk, COAlk; R_1 = H, Alk

n = 1, 2



26: R = H X = OH, OAlk, NAlk

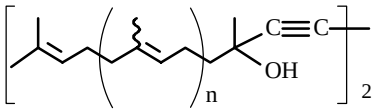
n = 4, 9, 10



27: R, R_1, R_2 = H, Alk

Biological testing of a series of compounds (**23**) showed that free alcohols with the (6Z)-configuration have higher anti-ulcer activity than other isomers. Introducing oxygen- and nitrogen-containing groups at the $C_{(7)}$ position increases the effectiveness of the compounds [97-99, 103, 104].

PP containing 7-11 isoprene units and their esters with aliphatic and aromatic acids possess hypotensive activity and exceptionally low toxicity [105-108]. High hypotensive activity of dimeric acetylenic alcohols containing the prenyl group (**28**) [107] and higher polyisoprenylcarboxylic acids (**23**) [98, 104, 105] has been reported in the patent literature.



28

n = 2, 4

A method for using PP to restore liver functioning has been patented [109, 110].

Derivatives of β,γ-dihydropolyprenyl alcohols (**29**) have been proposed as prophylactic and therapeutic agents

$$H\text{-}(CH_2\text{-}CMe{=}CH\text{-}CH_2)_n\text{-}CH_2\text{-}CHMe\text{-}CH_2\text{-}CH_2OR \ (\mathbf{29}),$$

n = 5-7, R = H, alkyl, aliphatic, or aromatic acyl group.

The compounds listed above can be used to treat diseases caused by immunological imbalances and to treat infectious diseases [111].

Preparations based on PP alcohols and their esters are used to treat hypertonia [112]

$$(CH_2{-}CMe{=}CH{-}CH_2)_n{-}CH_2{-}OR,$$

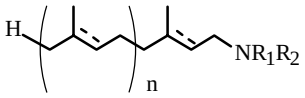
n = 7-10, R = H, Ac.

PP isolated from *Gingko biloba* leaves are used to manufacture preparations for treating nervous disorders [112].

PP can normalize immune functions and increase resistance to infections due to asthma, rheumatoid arthritis, pneumonia, meningitis, sepsis, and other diseases [114, 115].

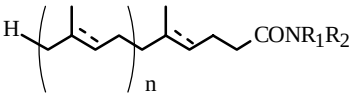
The antitumor activity of PP is an important property [112, 116].

PP amines (**30**-**32**) exhibit antithrombic activity and can be used for prophylaxis and treatment of cardiac stenosis, chronic atherosclerosis, and brain thrombosis [117].



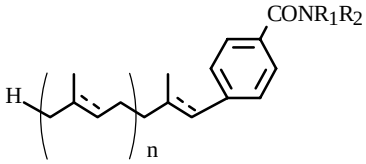
30

n = 2 -5



31

n = 2 - 5



32

n = 2 - 5

NR_1R_2 - residues of mono- , diamins, oxyamins

Recent results showed that PP derivatives such as PP acids, esters, and amides can be used to treat allergies, tumors, and skin diseases [112, 114, 115].

Prenylphenylsulfoacids and polyprenylmonophosphates are used to treat AIDS [107, 118, 119].

Russian researchers developed the semisynthetic preparation phosprenyl based on a concentrate obtained from conifer needles. Its principal component is a phosphorylated prenol with five isoprene units. The preparation is recommended as a treatment for serious viral diseases in animals [120-122].

Thus, the biological function of endogenic plant PP is not yet completely understood. However, data on their physiological activity have appeared.

Cotton PP were used to manufacture preparations with high growth-stimulating activity. The effect of undecaprenol, an intermediate in the synthesis of this preparation, on the level of protein biosynthesis by cotton sprout nuclei in vivo and in vitro was studied to determine the mechanism of action. It was shown that it doubles the level of biosynthesis of nuclear proteins after preliminary soaking of seeds in a 0.1% solution [4].

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