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REVIEW



The history and recent advances in research of polyprenol and its derivatives

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The reduction pathway leading to the formation of dolichol was clarified in 2010 with the identification of SRD5A3, which is the polyprenol reductase. The finding inspired us to reanalyze the length of the major chain of polyprenol and dolichol from several plant leaves, including mangrove plants, as well as from animal and fish livers by 2D-TLC. Polyprenol- and dolichol-derived metabolites such as polyprenylacetone and epoxydolichol were found together with rubber-like prenol. This review focuses on analyses of polyprenol and its derivatives, including recently found epoxy polyprenol and polyprenylacetone. Attention has also been paid to the chromatographic behavior of rubber-like prenol on TLC.

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Prenol; dolichol; epoxide; acetone; rubber

A naturally occurring isoprenoid alcohol with a long carbon chain length (dolichol) was found in human tissues in 1960 [1], and its function has been elucidated as being a sugar carrier lipid during the biosynthesis of glycoproteins [2]. Since then, a number of studies on its biosynthesis, distribution, and function have been carried out, the details of which were reviewed by Chojnacki and Dallner [3] in 1988. At that time, however, the reduction pathway to form dolichol from polyprenol was still unclear. Members of the polyprenol family can be separated using reversed-phase chromatography, and it is possible to analyze the differences between the polyprenol and dolichol families by reversed-phase HPLC. The difference of the retention time between each family member is really small - they are eluted as partially overlapping doublet. Careful modification of the gradient results in an acceptable separation. Polyprenols (dehydrodolichols) are more abundant in plants than in animals, and their distribution in plants summarized by Swiezewska and Chojnacki in 1994 [4]. In addition to reversed-phase HPLC, two-dimensional (normal-phase and reversed-phase) thin-layer chromatography (2D-TLC) has also been used to analyze various dolichols and polyprenols [5]. The 2D-TLC method is superior to reversed-phase HPLC in that the polyprenols are completely separated from the dolichols: however, the sensitivity is lower than that of HPLC.

The reduction pathway leading to the formation of dolichol was clarified in 2010 with the identification of SRD5A3, which is the polyprenol reductase [6–8]. The analysis of polyprenol and dolichol was done by using

reversed-phase HPLC. These findings inspired us to reanalyze the length of the major chain of polyprenol and dolichol from several plant leaves, including perilla by 2D-TLC [9]. When we used larger amounts of unsaponifiable lipids from perilla leaves to clearly visualize the separation between the polyprenol and dolichol, 2D-TLC revealed the occurrence of two families with chains longer than C₈₀ (16 isoprene units) along two oblique lines, as shown in Figure 1. This surprising finding led us to further analyze the unsaponifiable lipids of various plant leaves, including mangrove plants as well as the unsaponifiable lipids of animal and fish livers by 2D-TLC [10–13].

More recently, two kinds of polyprenol- and dolichol-derived metabolites were found by 2D-TLC. One is epoxydolichol [13], which is found in fish liver, and the other is polyprenylacetone [12], which is found in the leaves of mangrove plants. It is not clear at present if these two of metabolites, or any of the polyprenols that can be detected in plants, play important roles inside or outside of cells. The structure, biosynthesis, and function of polyisoprenoids and their cellular significance have recently been reviewed [14–16], and recent studies on isoprenoid generating systems (MVA and MEP pathways) in plants have been summarized [17–19]. Therefore, this review focuses on analyses of polyprenol and its derivatives, including epoxy polyprenol and polyprenylacetone, recently carried out using 2D-TLC. Attention has also been paid to the chromatographic behavior of rubber-like prenol on TLC. Their chemical structures are shown in Figure 2.

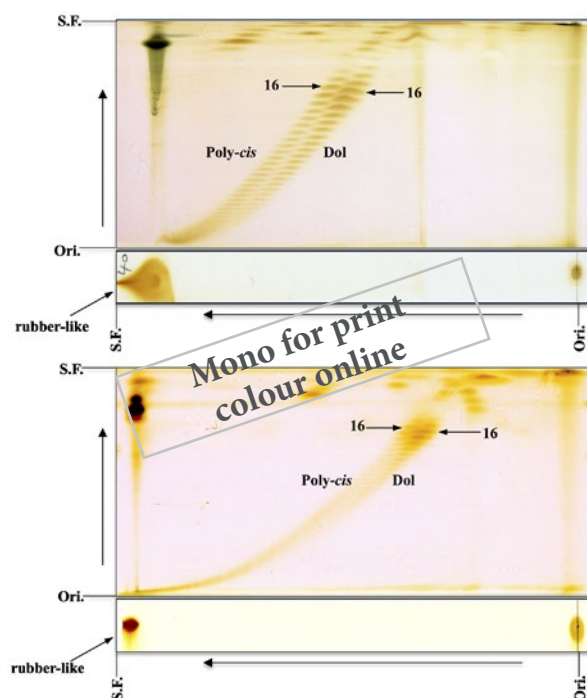


Figure 1. 2D-TLC of poly-*cis* prenol and dolichol from perilla leaves. Upper panel: the first, toluene:ethylacetate (4:1); the second, acetone. The acetone development was repeated 10 times. Lower panel: the first, toluene:ethylacetate (49:1); the second, acetone:methylethylketone (1:1). Rubber-like prenol is indicated with an arrow. Poly-*cis*, poly-*cis* prenol; Dol, dolichol. 16 = number of isoprene units. Ori., origin; S.F., solvent front (Sagami unpublished data).

Materials and methods

Terpenone (a 3:2 mixture of geranylgeranyl acetone and geranylneryl acetone, Eizai Co.) was obtained from a local drug store. Polyprenols (C_{85-95}) from *Ginkgo biloba* were provided by Kuraray Co. Polyprenols (C_{55-60}) from silkworm feces were provided by Takasago Perfumery Co. Dry leaves *Eucommia ulmoides* leaves (tochu in Japanese) were purchased in a local herbal medicine shop. Leaves of perilla (*Perilla frutescens*) and apple mint (*Mentha suaveolens*) were obtained from a local super market and a campus field at Tohoku University, respectively. Lavender (*Lavandula angustifolia*) and laurel (*Laurus nobilis*) were purchased from a garden shop. *Luminitzera racemosa* leaves were obtained in Okinawa Prefecture, Japan.

Dry leaves of basil (*Ocimum basilicum*), rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*), winter savory (*Satureja montana*), marjoram (*Origanum majorana*), thyme (*Thymus vulgaris*), peppermint (*Mentha x piperita*), and common sage (*Salvia officinalis*); roots of dandelion (*Taraxacum officinale*) and echinacea (*Echinacea purpurea*); stems (leaves) of artichoke (*Cynara scolymus*); and roots (stems) of kutki (*Picrorhiza kurroa*) were used from collections of Prof. Yoshihiro Shidoji of University of Nagasaki. Livers of Pacific bluefin tuna (*Thunnus orientalis*, kuromaguro in Japanese) were a generous gift from the Aquaculture Research Institute of Kinki University with the help of

Prof. Shigeru Shigeoka of Kinki University. Skipjack tuna liver was obtained from a local fish shop. Mass spectra of bombiprenone (potato leaves) and rubber-like compounds (apple mint leaves) were acquired at Tohoku University, at the Institute of Organic Chemistry PAS, and at the Institute of Biochemistry and Biophysics PAS.

Analysis of unsaponifiable lipids

Plant leaves were dried at 65 °C overnight and then extracted with chloroform:methanol (2:1). The extracts (less than 1.0 g) were saponified in KOH (0.45 g), water (2.0 mL), and ethanol (2.0 mL) at 65 °C 3 h ~ overnight and the saponified mixture was extracted with hexane (4.0 mL). In the case of fish livers [13], Pacific bluefin tuna and skipjack tuna livers (8.51 g and 8.57 g, respectively) were saponified in KOH (1.125 g), water (5.0 mL), ethanol (5.0 mL), and pyrogallol (0.05 g) at 65 °C overnight and the saponified mixture was extracted with hexane (10 mL). In the case of both the plant and fish extracts, the hexane extracts were analyzed by two-dimensional thin-layer chromatography (2D-TLC) [5]. A prenol sample (between 0.1 and 1 μ g) is required for detection with iodine vapor. The first silica-gel TLC was carried out with toluene:ethylacetate (4:1, 9:1, 19:1, or 49:1), and the transfer of polyprenols and dolichols to the second RP- C_{18} silica-gel plate was done with acetone or acetone:methylethylketone (1:1), followed by the second round of chromatography using acetone or acetone:methylethylketone (1:1). To reuse the reversed-phase plates, it is necessary to completely remove the iodine from the plate in the hood and develop the plate once with dichloromethane or chloroform.

Large quantities of rubber-like prenol of apple mint leaves for structural characterization were prepared using 865 g of leaves (wet weight). The dried leaves (178 g) were extracted with chloroform:methanol (2:1) (900 mL), and the extracts (7.1 g) were saponified in KOH (4.5 g), water (20 mL), and ethanol (20 mL) overnight at 65 °C and then the mixture was extracted with hexane (40 mL). The crude extracts (ca. 600 mg) was mixed with silica gel powder (ca. 6 g) and applied to a silica-gel column (4.7 \times 12 cm) equilibrated with acetone. After acetone elution (3 mL, 37 frs.), the rubber-like compounds were eluted with chloroform. Fractions (55–73) were combined and dried, followed by analysis by NMR.

Polyprenol

Polyprenol is the generic name for 1,4-polyisoprene alcohols, and structurally classified into main two groups, poly-*trans* and poly-*cis* prenols, as shown in Figure 2.

Poly-*trans* prenol

Geraniol (C_{10}), farnesol (C_{15}), and geranylgeraniol (C_{20}), which are acyclic mono, sesqui, and diterpene alcohols, respectively, are known as essential oils, and

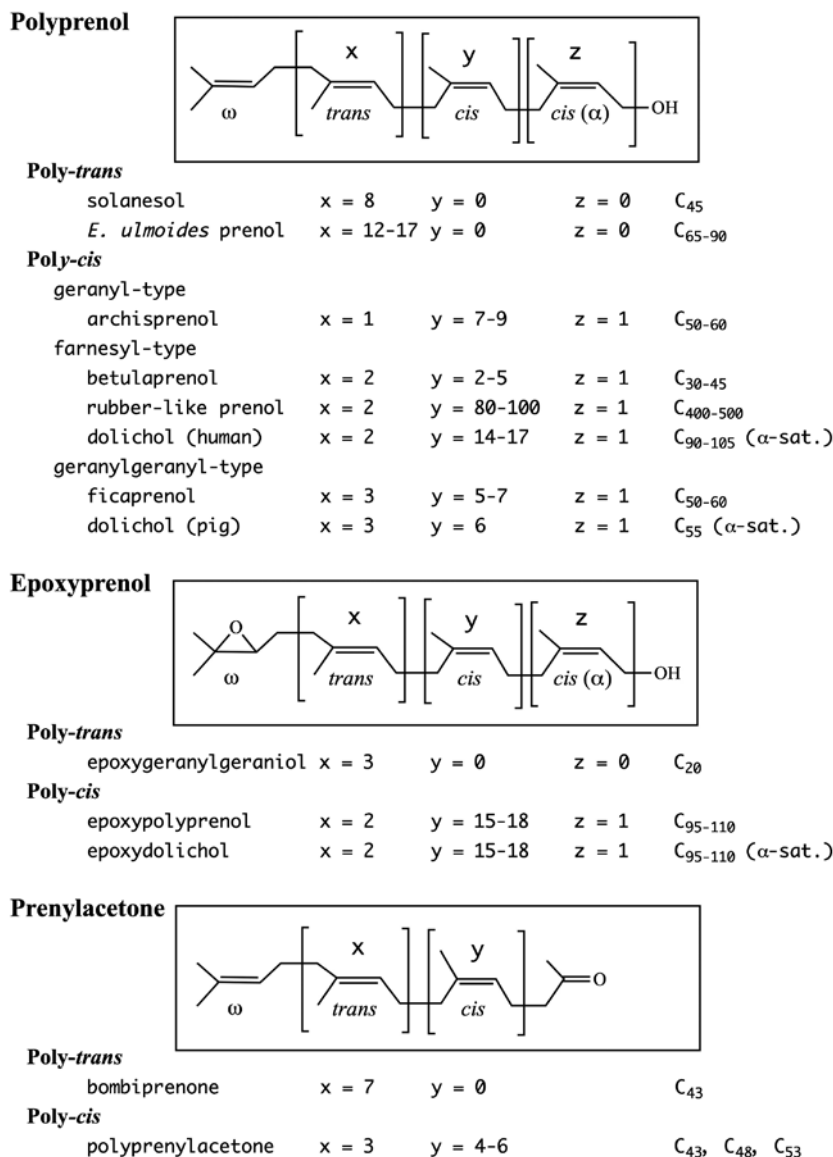


Figure 2. Chemical structure of poly-prenol, epoxy-poly-prenol, and poly-prenylacetone.

are examples of poly-*trans* prenol. Phytol (C₂₀), another *trans*-prenol, is a side chain of the chlorophyll molecule. A triene, 12,13-dehydrogeranylgeraniol was isolated from the aquatic plant *Saururus cernuus* [20]. Solanesol (C₄₅), which was isolated from flue-cured tobacco leaves (*Nicotiana tabacum*) in 1956 [21], is a typical poly-*trans* prenol, and its structure is shown in Figure 2. Recently, poly-*trans* prenol with chain lengths longer than C₆₅ has been found in tissues of *E. ulmoides* [22,23]. The *trans* isomers were found in the leaves, seed coats, and roots but not in the bark and seeds. The poly-*trans* prenol, poly-*cis* prenol, and dolichol in *E. ulmoides* were clearly separated by family using capillary column chromatography. 2D-TLC was also used to separate those polyisoprenoids into three groups along oblique lines consisting of each family as shown in Figure 3 (upper). The poly-*cis* prenol and dolichol of perilla leaves are shown in Figure 3 (lower). When the poly-*cis* prenol, dolichol, and poly-*trans* prenol of *E. ulmoides* were mixed with the poly-*cis* prenol and dolichol of perilla, the members of the three

isoprenoid families were actually separated by family as shown in Figure 3 (middle). It should be noted that compounds indicated with an arrow remained near the top (with the solvent front) on the first silica gel thin-layer plate.

Poly-*cis* prenol

The family of poly-*cis* prenol is classified into three groups based on stereochemistry as shown in Figure 2. Geranyl-type prenol (mono-*trans* poly-*cis*) include archisprenol of *Arachis hypogaea* [24]. Farnesyl-type prenol (di-*trans* poly-*cis*) include betulaprenol of *Betula verrucosa* [25,26], rubber-like poly-*cis* prenol of *L. racemosa* [10], and undecaprenol of *Lactobacillus plantarum* [27]. Geranylgeranyl-type prenol (tri-*trans* poly-*cis*) include ficaprenol from *Ficus elastica* [28], malloprenol from *Mallotus japonicus* [29], moraprenol from *Morus bombycis* K. (mulberry) [30,31], castaprenol from *Aesculus hippocastanum* (horse chestnut) [32], and cleomeprenol

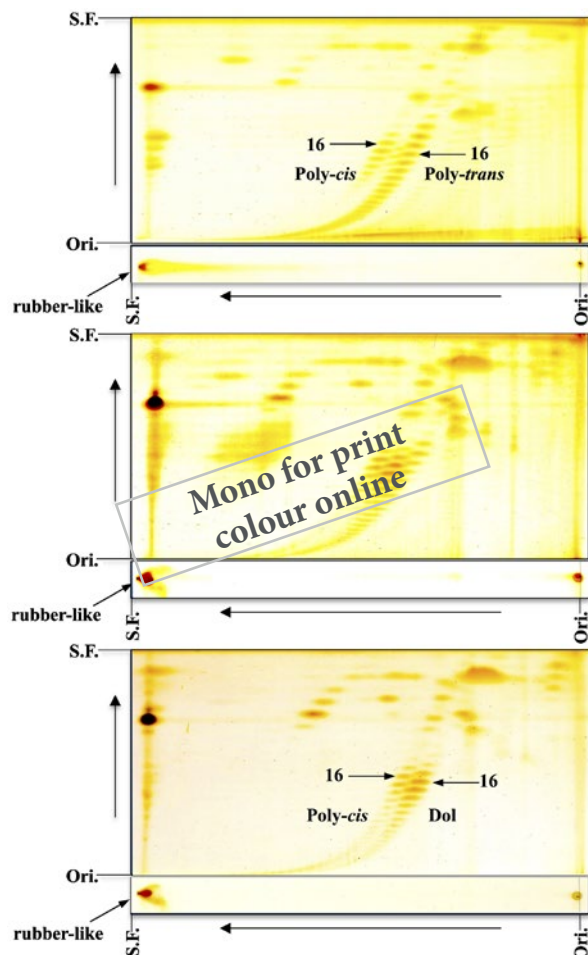


Figure 3. 2D-TLC of polyisoprenoid from the leaves of *E. ulmoides* and perilla. Upper panel, *E. ulmoides*; middle panel, a mixture of *E. ulmoides* and perilla; lower panel, perilla. The first, toluene:ethylacetate (19:1); the second, acetone (once). Poly-*cis*, poly-*cis* prenol; Poly-*trans*, poly-*trans* prenol; Dol, dolichol. 16 = number of isoprene units. Rubber-like prenol is indicated with an arrow. Ori., origin; S.F., solvent front (Sagami unpublished data).

from *Cleome spinosa* [33]. Dolichol corresponds to poly-*cis* prenol with an α -saturated isoprene. Human C_{90-105} dolichol has a farnesyl-type structure, whereas pig liver C_{55} dolichol has a geranylgeranyl-type structure.

Acyclic poly-*cis* prenol (C_{25}), with one *cis*-isoprene unit attached to phytol group, was isolated from potato (*Solanum tuberosum*) leaves in 1969 [34]. Poly-*cis* prenol (glycinoprenol C_{45} , C_{50} , and C_{55}) from the leaves of soy bean (*Glycine max* Merrill) possesses a phytol residue, 4 to 6 internal *cis*-isoprene residues, and a *cis* α -terminal residue aligned in that order [35].

So far, the polyprenols with the longest chains (up to C_{500}) have been found in the leaves of a mangrove plant (*L. racemosa*) and were as rubber-like polyisoprenoid alcohols [10]. Figure 4 (upper) shows the 2D-TLC of the unsaponifiable lipids of *L. racemosa*. Two families separated along oblique lines corresponding to the poly-*cis* prenol and dolichol were observable on the second RP- C_{18} silica gel thin-layer plate, and, as expected, a strong spot corresponding to rubber-like prenol was

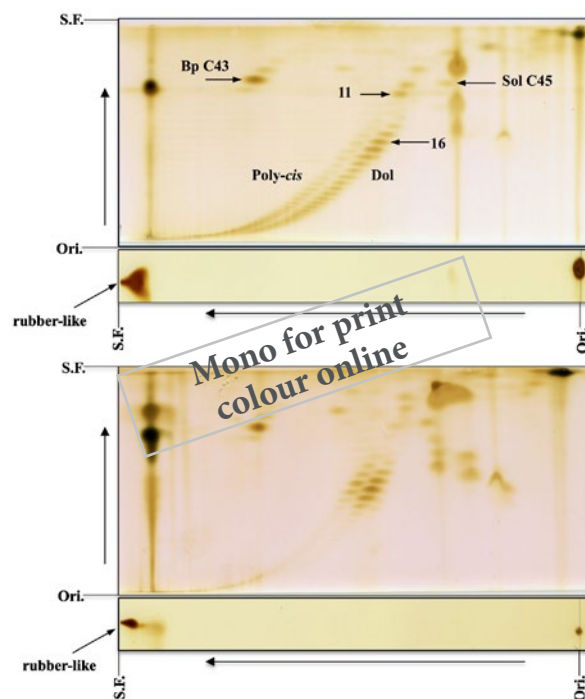


Figure 4. 2D-TLC of rubber-like prenol from the leaves of *L. racemosa* and apple-mint. Upper panel, *L. racemosa*; lower panel, apple-mint. The first, toluene:ethylacetate (4:1); the second, acetone (once). Poly-*cis*, poly-*cis* prenol; Dol, dolichol. 11, 16 = number of isoprene units. Bp C43, bombiprenone C_{43} ; Sol C45, solanesol. Rubber-like prenol is indicated with an arrow. Ori., origin; S.F., solvent front (Sagami unpublished data).

detected on the first silica gel thin-layer plate. In the 2D-TLC of the unsaponifiable lipids of perilla leaves, there were compounds that remained on the first silica gel plate, as indicated with an arrow in Figure 1, implying the presence of rubber-like prenol in perilla. This is also the case for unsaponifiable lipids of apple mint shown in Figure 4 (lower) and lavender leaves (figure not shown). The rubber-like prenol has also been detected in unsaponifiable lipids of dry leaves (basil, rosemary, oregano, winter savory, marjoram, thyme, peppermint, and common sage), roots (dandelion and echinacea), stems (artichoke), and roots (kutki) (Sagami and Shidoji unpublished data). To confirm the presence of rubber-like prenol, the lipids of apple mint leaves were purified by silica gel chromatography, and the purified compounds were analyzed by NMR spectroscopy. As expected, rubber-like signals (*cis*-isoprene) were detected although the intensities were low (Sagami and Swiezewska unpublished data).

Two *cis*-prenyltransferase genes (*RER2* and *SRT1*) have been found in yeast [36–39].

The products of these two genes are responsible for the syntheses of the carbon-backbones of the C_{70-90} and C_{95-120} dolichols, respectively. During an analysis of the endogenous lipids of a mutant yeast (#64), a shorter-chain (C_{55-60}) polyprenol was found along with a C_{70-80} dolichol by 2D-TLC [40], though the stereochemistry of the shorter polyprenol remains unclear. Preliminary

characterization of a C_{55} polyprenol detected at the stationary phase of growth of some yeast strains indicated the presence of internal two *trans*-isoprene residues [41]. Pig liver also contains a family of shorter-chain polyprenols dominated by a C_{55} -dolichol and a long chain (C_{95-100}) dolichol [42]. The number of internal *trans* isoprene residues of the former compounds was consistent with a geranylgeranyl-type dolichol. It might be that three polyprenol families (one shorter and two longer) occur in yeast and two polyprenol families (one shorter and one longer) occur in pig.

Epoxyrenol

The general chemical structure of epoxyrenol is shown in Figure 2. The epoxide is located on the ω -isoprene residue in poly-*cis* and poly-*trans* prenols.

Epoxy poly-*trans* prenol

All-*trans* 14,15-epoxygeranylgeraniol was identified as an active substance in the oil of the fruits from *Pterodon pubescens* Benth [43]. It shows prophylactic activity against infections from cercariae of *Schistosoma mansoni*; however, all-*trans* geranylgeraniol is inactive. Later, the natural product was established to have the *S* configuration [44].

Epoxy poly-*cis* prenol

Epoxydolichol with minor epoxydopolyprenol was found by chance during an analysis of unsaponifiable lipids in fish livers. In the case of skipjack tuna liver a new family of compounds was detected on the second RP- C_{18} plate [13]. The new family of compounds migrated faster than dolichoic acid (C_{95-100}) and slower than endogenous dolichol in the normal-phase direction, implying that they are more polar than the alcohol. The NMR and mass analyses strongly supported the presence of epoxy functionality on the ω -isoprene residue. The epoxydolichol family was similarly detected in Pacific bluefin tuna (kuromaguro in Japanese) liver, as shown in Figure 5 (upper), though the content was lower than that of skipjack tuna liver in Figure 5 (lower).

Ishinaga and Ueda reported the occurrence of novel dolichyl derivatives in rat spleen [45]. The compounds were not dolichyl dolichoate [46], which is present in bovine thyroid. They also described that these compounds were not found in young rats (4 months of age) but were found in old rats (approximately 12 months of age). The compounds might correspond to epoxydolichyl derivatives. Ward et al. recently reported the occurrence of a series of natural dolichol (Dol) and oxidized isoprenoid lipids in neuromelanins in various regions of the human brain by LC/MS analysis [47]. The oxidized isoprenoid lipids include Dol (+16), Dol (+32), Dol (+48), Dol (+64), and dolichoic acid (+16),

reflecting addition of oxygen (s) along the isoprenoid chain. Dol (+16) might be epoxydolichol.

Epoxydolichol has a farnesyl residue on the ω -isoprene group; this is also the case for epoxy squalene, which is a precursor for the formation of lanosterol. In this respect, it may be important to note whether the chirality at the third carbon from the ω terminal is the same as that of epoxy squalene (which is *S*) [48]. In comparison, the chirality of epoxy poly-*trans* C_{20} prenol is *S* and that of the juvenile hormone (epoxy poly-*trans* C_{15} prenoic acid) was determined to be *R* [40,49].

Hevea rubber has been reported to contain an epoxide group, and the concentration is estimated to be one epoxide group per approximately 190–320 isoprene units in *Hevea* rubber from fresh latex or a variety of its clones [50,51]. It is uncertain if the epoxide is located on an internal isoprene unit or on the ω -isoprene unit. The discovery of the epoxydolichol (epoxydopolyprenol) in fish liver suggests epoxydopolyprenol (epoxy dolichol) and epoxy-rubber-like prenol may also be present in plants, especially on the ω -isoprene group. Since Tanaka presumed that the biosynthesis of natural rubber starts with an unidentified initiating species containing two *trans*-isoprene units and a peptide groupv [52], it is possible to assume that one intermediate in the biosynthetic pathway of rubber might be an epoxy compound of a rubber-like polyprenol.

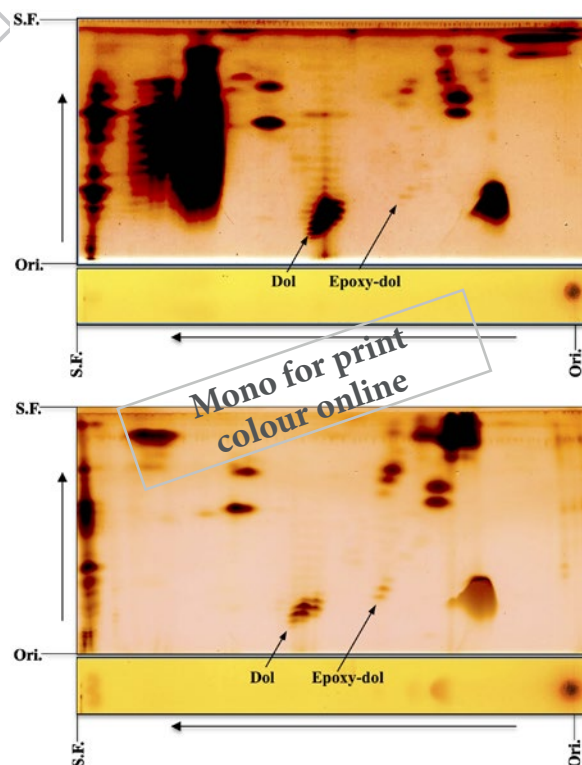


Figure 5. 2D-TLC of epoxydopolyprenol from livers of Pacific bluefin tuna (upper) and skipjack tuna (lower). The first, toluene:ethylacetate (9:1); the second, acetone (once). Dol, dolichol; Epoxy-dol, epoxydolichol. Ori., origin; S.F., solvent front (Sagami and Shidoji unpublished data).

Prenylacetone

The general chemical structure of prenylacetone is shown in Figure 2. Polyprenyl derivatives are also found as poly-*trans* and poly-*cis* compounds.

Poly-*trans* prenylacetone

Shorter carbon-chain prenylacetone such as geranyl acetone (C₁₃) or farnesyl acetone (C₁₈) was found as a volatile compound in fruits such as tomatoes and watermelons [53]. A 43-carbon ketone isolated from the unsaponifiable matter of silkworm (*Bombyx mori* L.) feces was identified as a polyisoprenoid compound (bombiprenone, C₄₃) with 8 isoprene units with each unit having one *trans* double bond to which an acetyl residue was attached at the terminal unit [54]. The ketone was also found in the leaves of mulberry trees (*M. bombycis* K.) [55], which comprises the entire diet of silkworm.

Apart from their natural occurrence, poly-*trans* prenylacetones (C₁₈, C₂₃, C₂₈, C₃₃, C₄₃, and C₄₈) have been chemically synthesized, and the antiulcer effects of those acyclic acetones on cold-restraint stress induced gastric ulcers in rats were reported [56]. Geranylgeranyl acetone mixed with geranylneryl acetone showed a particularly potent antiulcer effect. A 3:2 mixture of geranylgeranyl and geranylneryl acetones (general name, teprenone) is, at present, known as an antiulcer pharmaceutical drug.

Through feeding experiments using protoplast of *Botryococcus braunii* B race strain, Inoue et al. identified farnesal and 3-hydroxy-2,3-dihydrofarnesal as metabolites from farnesol [57]. The authors described the possibility that 3-hydroxy-2,3-dihydrofarnesal is readily decomposed into geranyl acetone and acetaldehyde. By analogy, solanesol (C₄₅) (see the section of poly-*trans* prenol) may be converted to solanesal and then 3-hydroxy-2,3-dihydrosolanesal, which might be decomposed into bombiprenone (C₄₃) and acetaldehyde (C₂).

Poly-*cis* prenylacetone

Although bombiprenone (C₄₃) is often detectable by 2D-TLC in the leaves of many plants, an isoprenoid family is sometimes found near a bombiprenone family. Figure 6 (upper) shows the case of unsaponifiable lipids of laurel leaves. Figure 6 (lower) shows the case of the laurel sample mixed with teprenone (a mixture of geranylgeranyl acetone and geranylneryl acetone). As expected, geranylneryl acetone ran faster than geranylgeranyl acetone. The right oblique spots directly below the geranylgeranyl acetone spot correspond to poly-*trans* prenylacetone (C₂₈, C₃₃, C₃₈, C₄₃ and C₄₈) with the main spot being bombiprenone (C₄₃). It is believed that the left oblique spots directly below the geranylneryl acetone spot correspond to poly-*cis* prenylacetone (C₂₈, C₃₃, C₃₈, C₄₃, C₄₈, C₅₃, and C₅₈) with the main being C₄₈ and C₅₃.

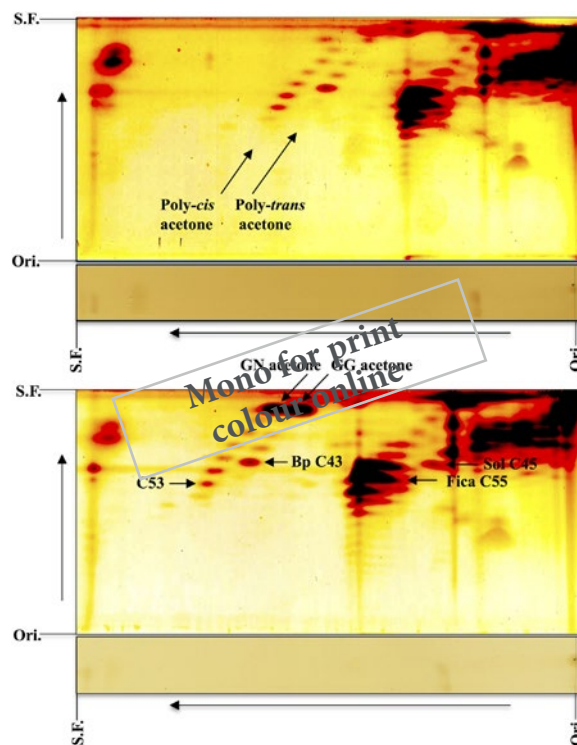


Figure 6. 2D-TLC of poly-*cis* and poly-*trans* acetones from laurel leaves. Upper panel, unsaponifiable lipids; lower panel, unsaponifiable lipids plus teprenone. The first, toluene:ethylacetate (9:1); the second, acetone (once). GN acetone, geranylneryl acetone; GG acetone, geranylgeranyl acetone. Sol C45, solanesol; Fica C55, ficaprenol C₅₅; Bp C43, bombiprenone C₄₃. Ori., origin; S.F., solvent front (Sagami unpublished data).

Since strong spots corresponding to ficaprenol (C₅₀ and C₅₅) are observed on the RP-C₁₈ plate, their derivatives might be prenylacetone (C₄₈ and C₅₃). These poly-*cis* prenylacetones as well as poly-*trans* prenylacetone (bombiprenone) were detected in *Allium tuberosum* (nira in Japanese), *Boehmeria nivea* var. *nipponnivea* (karamushi in Japanese), *Laurus nobilis* (gekkeiju in Japanese), and *Euphorbia supina* (konishikisou in Japanese) (Sagami unpublished data). More recently, Basyuni et al. analyzed the unsaponifiable lipids of North Sumatran mangrove leaves by 2D-TLC and found poly-*cis* prenylacetones ranging from C₂₃ to C₈₈ in *Sonneratia caseolaris* and *Xylocarpus granatum* and ranging from C₂₃ to C₁₀₈ in *Aegiceras corniculatum* [12].

Polyisoprene [58,59], both the *cis*-isomer [poly (*cis*-1,4-isoprene)] and the *trans*-isomer [poly (*trans*-1,4-isoprene)], are synthesized by more than 2500 plants, such as *E. ulmoides*, and some fungi. Although their biosynthetic pathways have not yet been established, their bacterial degradation pathways have recently been clarified. In the initial O₂-dependent oxidation process shortened rubber oligomers with aldehyde and keto groups at their termini are generated after the oxidative cleavage of the double bonds of poly (*cis*-1,4-isoprene) and poly (*trans*-1,4-isoprene), and the aldehyde groups that are unstable toward oxidation should be oxidized

to the corresponding acids outside of the bacterial cells. Since the keto moieties correspond to acetone groups, the initial microbial oxidative process may give us a clue toward understanding the formation of polyprenyl acetone from polyprenol in plants.

Perspectives

Analysis of polyprenyl compounds by 2D-TLC has allowed the identification of several new metabolites leading to the following new insights.

- (1) Rubber-like prenol may occur widely in the plant kingdom. It is possible to easily detect rubber-like prenol on a thin-layer silica gel plate (5 × 20 cm). The plate is firstly developed (up to 15 cm) with toluene:ethylacetate (9:1) and then developed (up to 2 cm) with acetone toward the second dimensional direction, followed by exposure of the plate to iodine vapor. Compounds that migrate up to the solvent front with toluene:ethylacetate (9:1) and that remain on the solvent front after development with acetone are expected to be rubber-like prenol.
- (2) The finding of epoxydolichol in fish livers suggests dolichyl proteins, which are presumably different from dolichyl-phospho proteins and dolichylthio proteins [60,61], may also be present because the epoxy structure is easily associated with an amino group of peptides or proteins to form dolichyl- and polyprenyl proteins. Regarding polyprenyl acetones, more information is necessary to learn whether they are intermediates in a catabolic pathway and whether they are also found in both the animal kingdom and the plant kingdom.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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