View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

POLYPRENOLS IN *JUNIPERUS COMMUNIS* NEEDLES

W. SASAK, T. MAŃKOWSKI and T. CHOJNACKI
Institute of Biochemistry and Biophysics, Polish Academy of Sciences, ul. Rakowiecka 36, 02-532 Warszawa, Poland

and

W. M. DANIEWSKI
Institute of Organic Chemistry, Polish Academy of Sciences, ul. Kasprzaka 44/52, 00-961 Warszawa, Poland

Received 24 February 1976

1. Introduction

The search for long chain polyprenols in plant material stems from the role of prenyl phosphates in the synthesis of complex polysaccharides and glycoproteins [1]. In the biosynthesis of bacterial sugar polymers fully unsaturated C_{55}-polyprenol in its phosphorylated form is involved and it also occurs in several tropical plants [2,3]. In the formation of mammalian glycoproteins the lipid–sugar intermediates contain dolichol – a C_{95}-polyprenol in which the OH-terminal isoprene residue is saturated [1]. Hannus and Pensar [4] have found C_{60}–C_{85}-polyprenols in *Pinus sylvestris.* According to Zinkel and Evans [5] similar polyprenols are present in *Pinus strobus.* The present study was aimed to find a plant species among *Conifera* containing larger amounts of C_{95}-polyprenol, i.e. the polyprenol of the same chain length as mammalian dolichol. Such compound might be useful for biochemical studies on the biosynthesis of glycoproteins as a dolichol substitute.

2. Materials and methods

2.1. *Isolation of polyprenol esters and free polyprenols*

Green needles of *Juniperus communis, Abies alba* and *Picea abies* were collected from 2–3-year old branches of plants growing in a wild state in early spring. Dry needles were extracted in a Soxlett apparatus with acetone. Acetone extract (1 vol.) was triturated with 1 volume of water and 2 volumes of petroleum ether (40–50°C b.p.), and organic layer was collected. After evaporating the solvent on a rotary evaporator, the oily residue was chromatographed on alumina column (acid-washed, Brockman grade III) using increasing concentrations of ethyl ether in petroleum ether [6]. The prenol ester fraction was eluted with 5% ethyl ether and the fraction of free polyprenols with 30% ethyl ether. Preparative thin layer chromatography on 1 mm layer of Silica gel G (Merck, Darmstadt, Germany) was employed for purifying prenol esters (petroleum ether–benzene, 2:1, v/v, R_f= 0.5) and free polyprenols (benzene–ethyl acetate, 95:5, v/v, R_f = 0.6). Detection of spots on the edge of the plate was performed with anisaldehyde [7]. Prenyl esters were hydrolyzed with ethanolic KOH [6] and free polyprenols obtained from the hydrolysate were purified by preparative t.l.c. (see above).

2.2. *Isolation of C_{95}-polyprenol from* Juniperus communis

Free polyprenols (1 g) were applied on the top of column (1 × 120 cm) of hydroxy (C_{15}) alkoxypropyl Sephadex (Lipidex-5000, Packard-Becker, B.V. Groningen, Netherlands) equilibrated with 7% water in acetone. The column was eluted with acetone containing decreasing concentrations of water

(7% to 2%) using 3 litre of effluent similarly as described previously [8]. The course of elution of individual prenologues was checked by reversed phase t.l.c. [2] using 4% water in acetone saturated with paraffin as solvent. Fractions of single prenologues eluted from the column were analysed by mass spectrometry. Fractions containing pure C_{95}-polyprenol were collected and analysed by n.m.r. spectrometry.

2.3. *Composition of natural polyprenol mixtures*

Natural mixtures of free polyprenols, up to 0.2 mg, were analysed by high pressure liquid chromatography on a column (0.4 × 25 cm) of μ-Bondapak C_{18} (Waters Associates) using 4% water in acetone as eluent at the flow rate 1 ml/min. Home made apparatus equipped with refractive index detector was used. Quantitative estimation was done by planimetry of peaks, assuming the refractive indices of different polyprenols were the same. C_{95}-polyprenol served as a guide for identifying the peak of this polyprenol and of other prenologues in studied mixtures.

2.4. *Fatty acid composition of polyprenol esters from* Juniperus communis

For the assay of long chain fatty acids, two 80 mg portions of polyprenol esters were subjected to base catalysed methanolysis [9]. To one of them a known amount of methyl oleate was added as internal standard. The resulting fatty acid methyl esters were assayed by gas liquid chromatography on a 5 ft. column of 10% polyethylene glycol adipate on Diatomite CQ at 180°C. The content of fatty acid methyl esters was estimated according to Carroll [10]. For the assay of short chain fatty acids two 70 mg portions of polyprenol esters were hydrolysed with KOH (as above) and the hydrolysates treated with excess of H_3PO_4. To one of them known amounts of sodium acetate and sodium butyrate were added as internal standards. The analysis of free fatty acids was performed by gas liquid chromatography on a 5 ft. column of 10% polyethylene glycol adipate containing 2% of H_3PO_4 on Diatomite CQ at 100°C. For both types of analysis argon (40 ml/min) was a carrier gas and Pye 104 gas chromatograph equipped with flame ionization detector was used.

2.5. *Mass spectra*

Mass spectra were recorded at 290°C with the direct inlet probe with 70 eV ionizing voltage on the AEI-MS-902S mass spectrometer.

2.6. *N.m.r. spectra*

^{1}H-n.m.r. spectra were recorded in CCl_4 with a Jeol-JNM-100 instrument.

3. Results and discussion

The polyprenols comprised about 1% of the dry weight of green needles in *Juniperus communis.* Free polyprenols and polyprenol esters were found in equal amounts. As shown in table 1 the same family

Table 1
The content of free and esterified polyprenols in needles of coniferous trees estimated by high pressure liquid chromatography on μ-Bondapak C_{18}. Figures represent the % content of polyprenol-11, -12 etc. in natural mixtures

	Number of isoprene residues										
Origin of polyprenols	11	12	13	14	15	16	17	18	19	20	21
Juniperus communis (free)				1.4	5.6	24.6	24.6	11.6	10.6	13.6	8.1
Juniperus communis (esterified)				3.1	10.8	25.2	22.5	11.4	11.4	10.4	5.2
Picea abies (esterified)		0.9	23.9	32.7	27.6	10.1	3.3	1.5			
Abies alba (esterified)	0.6	0.9	1.3	4.6	20.2	38.5	22.6	8.4	2.9		

of polyprenols (C_{70} – C_{105}-juniperoprenols) were found in these two fractions. In *Picea abies* and *Abies alba* only esterified polyprenols were found. They comprise also about 1% of the dry weight of green needles. In contrast to the finding of Hannus and Pensar [4] that prenyl acetates are present in *Pinus sylvestris* mass spectrometric analysis of natural polyprenol esters from *Juniperus communis* has shown no signal resulting from the presence of polyprenol acetate. Gas chromatography of juniperoprenol esters hydrolysate demonstrated the absence of acetate and other short chain fatty acid. Instead, long chain fatty acids methyl esters were found upon base catalysed methanolysis of this material. Four fatty acids : 14:0, 16:0, 18:3 and 20:3 were dominating, and they accounted for 42% of total acid equivalents. A number of other fatty acids were present, though they occurred in lower amounts. It can be assumed therefore that polyprenols in *Juniperus communis* are esterified with long chain fatty acids. The molecular ions of these esters were not visible in mass spectrometry, because the compounds were not volatile in the conditions of analysis.

Chromatography of larger amounts of natural mixture of free polyprenols from *Juniperus communis* on Lipidex-5000 (fig.1), in contrast to a clear-cut separation obtained by high pressure liquid chromatography (fig.2) gave some overlapping of fractions of individual prenologues. The fractions containing pure individual prenologues were collected and the identification of

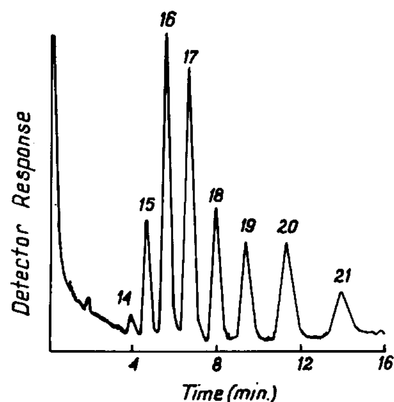

Fig.2. High pressure liquid chromatography of polyprenol mixture from *Juniperus communis* needles on μ-Bondapak C_{18}. Numbers indicate the number of isoprene residues in a separated prenologue.

polyprenols was performed by mass spectrometry. The pattern of fragmentation was typical for fully unsaturated polysoprenoid alcohols with weak molecular ion (M^+) and a stronger one (M^+–18) corresponding to molecular ion less water [11]. The n.m.r. spectrum of C_{95}-juniperoprenol (juniperoprenol-19), fig.3, confirmed the results of mass spectrometric

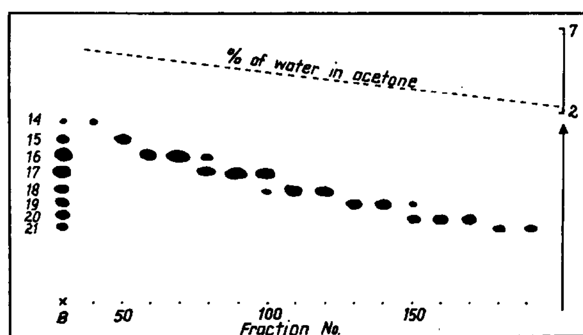

Fig.1. Column chromatography of juniperoprenols on Lipidex-5000. The distribution of polyprenols in eluate is illustrated by tracing of reversed phase thin-layer chromatogram. Lane B represents original mixture of juniperoprenols with indicated number of isoprene residues. Numbers along the bottom of the tracing are 12 ml fractions.

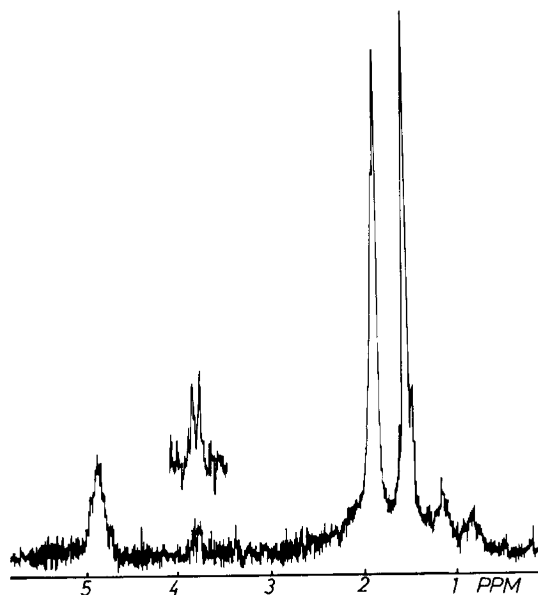

Fig.3. N.m.r. spectrum of C_{95}-juniperoprenol at 100 MHz in CCl_4.

analysis, showing characteristic signals common for fully unsaturated plant polyprenols with the OH-terminal isoprene residue in *cis* configuration [12]. The number of internal *trans*-isoprene residues calculated from n.m.r. spectrum according to Feeney and Hemming [12] was 3.

The results presented in this paper extend the knowledge of natural occurrence of long-chain *cis/trans* polyprenols in several species of *Conifera*. Especially the needles of *Juniperus communis* were found to be rich in fully unsaturated C_{70-105}-polyprenols. With respect to the chain length and *cis/trans* configuration they resemble the dolichols of eucaryotic cells.

Acknowledgements

We thank Dr G. Dallner for the gift in Lipidex-5000 and Dr P. Franke, Mr S. Maciaszek and Mrs J. Hertel for the help in performing analyses. This investigation was carried out as project 09.3.1. of the Polish Academy of Sciences and profited from the partial support of the Agricultural Research Service, US Department of Agriculture.

References

[1] Hemming, F. W. (1974) in: MTP International Review in Science, Biochemistry of Lipids (Goodwin, T. W. ed.), Vol. IV, pp. 39–98, Butterworth, London.
[2] Wellburn, A. R. and Hemming, F. W. (1966) Phytochem. 5, 969–975.
[3] Sasak, W. and Chojnacki, T. (1973) Acta Biochem. Polon. 20, 343–350.
[4] Hannus, K. and Pensar, G. (1974) Phytochem. 13, 2563–2566.
[5] Zinkel, D. F. and Evans, B. B. (1972) Phytochem. 11, 3387–3389.
[6] Stone, K. J., Wellburn, A. R., Hemming, F. W. and Pennock, J. F. (1967) Biochem. J. 102, 325–330.
[7] Dunphy, P. J., Kerr, J. D., Pennock, J. F. and Whittle, K. J. (1966) Chem. Ind. (London) 1549–1550.
[8] Chojnacki, T., Jankowski, W., Mańkowski, T. and Sasak, W. (1975) Anal. Biochem. 69, 114–119.
[9] Christie, W. W. (1973) in: Lipid analysis, pp. 89–90, Pergamon Press.
[10] Carroll, K. K. (1961) Nature 191, 377–378.
[11] Wellburn, A. R., Stevenson, J., Hemming, F. W. and Morton, R. A. (1967) Biochem. J. 102, 313–324.
[12] Feeney, J. and Hemming, F. W. (1967) Anal. Biochem. 20, 1–15.