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POLYISOPRENOLS IN *PINUS SYLVESTRIS* NEEDLES

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Abstract—A polyisoprenoid fraction was isolated from the needles of *Pinus sylvestris* and comprised about 1% of the dry weight. MS, IR, GLC and HPLC analyses showed that the fraction consisted of polyisoprenylacetates, with 10–19 isoprene units predominantly in the *cis* configuration.

INTRODUCTION

DURING recent years polyisoprenoid compounds[1,2] have received much attention. Polyisoprenoid side-chains are of considerable importance as components of numerous biochemically important compounds, such as different quinones, tocopherols, etc. Furthermore, polyisoprenols (primary allylic polyisoprenoid alcohols) in the form of phosphates act as membrane-bound lipid intermediates in the transfer of glycosyl fragments from sugar nucleotides to polymeric acceptors in bacterial walls.[3] It has also been suggested that polyisoprenyl phosphates function in the synthesis of complex polysaccharides or glycoproteins in plants and animals.[4]

Besides being found in micro-organisms and animal tissues polyisoprenols have been reported in numerous plants, principally in the green parts.[1,2] The polyisoprenols that have been found in plants consist of 10–13 isoprene units, usually predominantly in the *cis* configuration. Polyprenols with 6–9 isoprene units have been found in the wood and, to a lesser extent, in the leaves of *Betula verrucosa*,[5,6] and a polyprenol with 9 isoprene units and all-*trans* configuration, solanesol, has been found in tobacco leaves.[7] Recently the occurrence of a C_{90} polyisoprenol, consisting of 18 isoprene units (or a homologous series of polyisoprenols averaging 18 units) was reported in *Pinus strobus* needles.[8] Since it has not yet been possible to ascertain the position of *cis* and *trans* residues in the molecules it cannot safely be asserted that the polyprenols from different species are identical; they

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have therefore been called castaprenols,[9] ficaprenols,[10] betulaprenols[5] and heveaprenols,[11] etc., indicating the species from which they are isolated. This paper reports on the composition of the polyprenol fraction in extracts from *Pinus sylvestris* needles.

RESULTS AND DISCUSSION

The polyisoprenols isolated from the foliage of *Pinus sylvestris* were found to be esterified with acetic acid. These esters comprised about 5 6% of the petrol soluble extract and about 1% of the dry weight of the foliage. The polyisoprenol esters were isolated from the petrol soluble extract by means of preparative HPLC. The polyisoprenol esters were then purified by repeated TLC-treatment. After alkaline hydrolysis the free polyisoprenols were also subjected to similar purification.

Pinoprenol esters

MS of the unhydrolyzed isoprenol ester fraction showed that it consisted of a homologous series of acetates, which we named "pinoprenols". Their identity was checked by acetylation of the hydrolyzed pinoprenols; the acetylated pinoprenols showed MS identical with those of the original pinoprenol esters. In the MS of the pinoprenol ester fraction M^+ of *m/e* 740, 808, 876, 944, 1012, 1080, 1148, 1216, 1284 and 1352 corresponding to homologues with 10–19 isoprene units were detected. The pinoprenol ester homologues showed a small M^+ and a much more prominent ion corresponding to loss of HOAc ($M^+ - 60$). Fragmentation proceeds with a loss of the ω-terminal isoprene unit ($M^+ - 60 - 69$), followed by a loss of isoprene units one at a time from the ω-end until an abundant fragment at *m/e* 135 is left, resulting in the characteristic and abundant fragments ($M^+ - 60 - 69 - n.68$). The most abundant fragments in the spectra are represented by ions below *m/e* 135, namely at *m/e* 69, 81, 95, 93, 121, 107, 105. This fragmentation pattern is that expected for polyisoprenoid acetates.

The IR spectrum of pinopenol ester fraction differed from that of the pinoprenol fraction obtained after hydrolysis in those respects expected for an acetylated primary allylic polyisoprenoid alcohol. The absence of the O–H absorption at about 3330 cm^{-1} and the shift of the band at 1000 cm^{-1} characteristic of primary allylic alcohols to 1020 cm^{-1}, the C=O stretching at 1740 cm^{-1} typical for esters and the strong band near 1235 cm^{-1} due to C–O stretching of the acetate group.[9–13]

Pinoprenols

MS of the pinoprenol fraction showed that it consisted of an homologous series of polyisoprenols. By recording MS at different phases of the vaporization curve it was established that the major homologues were those with 15 and 16 isoprene units, together with smaller amounts of pinoprenols with 13, 14 and 17 isoprene units and trace amounts of homologues with 10, 11, 12, 18 and 19 isoprene units. The M^+ *m/e* 698 (pinoprenol-10), 766 (pinoprenol-11), 834 (pinoprenol-12), 902 (pinoprenol-13), 970 (pinoprenol-14), 1038 (pinoprenol-15), 1106 (pinoprenol-16), 1174 (pinoprenol-17), 1242 (pinoprenol-18) and 1310 (pino-

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prenol-19) were all detected in the spectra for pinoprenol fraction. The different pinoprenols shows a small M^+ and a more abundant ion corresponding to loss of H_2O (M^+-18). Subsequent fragmentation proceeds as described for pinoprenyl acetates.

The pinoprenol fraction was analyzed by reverse phase HPLC and the individual homologues of the fraction were easily separated and collected. MS of the eluted fractions confirmed that the homologues with 15 and 16 isoprene units were the major components of the pinoprenol fraction.

TMS-derivatives of the pinoprenols, as well as the pinoprenol ester fraction itself, were also analyzed by GLC on a short column. The relative composition of the prenols, determined by GLC and HPLC, is given in Table 1. The major components pinoprenol-15 and pinoprenol-16 represent together nearly 80% of the fraction, while pinoprenol-14 and pinoprenol-17 each represent about 10% of the fraction. Small amounts of pinoprenol-13 and pinoprenol-12 were also found, 3 and 1% respectively, while only trace amounts of other pinoprenol homologues were detected.

TABLE 1. COMPOSITION OF THE HYDROLYZED PINOPRENOL FRACTION IN EXTRACTS FROM NEEDLES OF *Pinus sylvestris*

Polyprenols*	% Of petrol soluble fraction	% Of prenol fraction
Pinoprenol-10	tr	tr
Pinoprenol-11	tr	tr
Pinoprenol-12	0·1	1
Pinoprenol-13	0·2	3
Pinoprenol-14	0·6	10
Pinoprenol-15	1·8	33
Pinoprenol-16	2·4	43
Pinoprenol-17	0·5	9
Pinoprenol-18	tr	tr
Pinoprenol-19	tr	tr

* The figures indicate the number of isoprene units in the molecule.

The IR-spectrum of the pinoprenol fraction was very similar to those published for castaprenols.[9] The characteristic absorption bands for polyisoprenoid structure (C–H deformation of a trisubstituted olefin at 835 cm^{-1}, C=C stretching at 1660 cm^{-1}, C–H stretching of =CH at about 3030 cm^{-1}, C–H deformation of $-CH_2$ and –Me at 1450 cm^{-1} and C–H deformation of –Me at 1370 cm^{-1}) and for primary allylic alcohol groups (C–O stretching of primary allylic alcohols at 1000 cm^{-1} and the O–H stretching at about 3330 cm^{-1}) are well registered in the IR spectrum. By comparing the spectrum with published spectra[13] of solanesol (all-*trans*) and dolichol (mainly *cis*) it was established that the pinoprenols contained more *cis* than *trans* isoprene units.

In an investigation[14] simultaneously performed on silvichemicals in technical foliage of domestic spruce (*Picea abies*) we have isolated a prenol fraction containing polyprenols ("piceaprenols") with 12–18 isoprene units and piceaprenol-15 predominates. These piceaprenols were found in about the same amounts as pinoprenols and were also esterified with acetic acid. The occurrence of these polyprenol esters seems to be strictly limited to

[14] HANNUS, K., LAX, B. and PENSAR, G. Unpublished work.

the needles. In wood, even from very slender branches we were not able to detect any polyprenol esters.

It is interesting to note that most other investigations report polyisoprenols in an unesterified form, whereas our investigation shows that the polyisoprenols isolated from needles of indigenous pine and spruce are in the acetate form. These polyisoprenyl acetates furthermore represent a major non-polar lipid group in the needles of these softwoods. Whether or not the accumulation of these acetates only represents a storage pool of isoprenols, inactivated through esterification, for the biochemically active phosphate derivatives or whether they also have some other role, e.g. structural, is as yet unknown.

EXPERIMENTAL

Isolation of the pinoprenols. Milled wood and defiberized needles from branches with a max dia of 6–7 mm of *Pinus sylvestris* from S.W. Finland were extracted in a Soxhlet apparatus first with Me_2CO and then with petrol (30°–40°). The extract was treated with petrol (60°–80°) and the soluble portion (16% of the total extract) was analyzed for polyprenol esters. The prenol ester fraction was separated from the petrol soluble extract on a Porasil C column using HPLC. The pinoprenol esters were hydrolyzed with ethanolic KOH,[15] then cooled, neutralized and extracted with Et_2O. The hydrolyzed material was purified by silica gel TLC to give the pinoprenol fraction. *MS* of pinoprenol esters, free pinoprenols, acetylated pinoprenols and of single homologues in the pinoprenol fraction, were recorded by direct inlet at (150°–200°) at 70 eV. *HPLC* analyses were performed with a Water Associates Inc., model ALC-201, equipped with a refractive index detector and a 70 kg/cm² pumping system. The polyprenol esters were eluted on a 60 × 1 cm column packed with Porasil C using petrol (30°–40°)–Et_2O (199:1). The hydrolyzed prenol ester fraction was analyzed using a reverse phase on a 120 cm × 3 mm column packed with Corasil/C_{18} and elution with Me_2CO–H_2O (7:1). The pinoprenol homologues were completely resolved, collected and identified by MS. *GLC* was performed with a FID instrument using a short column (30 cm × 3 mm) packed with 1% SE-30 on Gas Chrom Q (100/120 mesh). The column temp. was programmed from 300° to 375° at 10°/min. *TLC* was used mainly to purify fractions and commercially available silica gel plates with preparative (2 mm) as well as analytical (0·25 mm) layers were used. The plates were developed with petrol (30°–40°)–Et_2O (95:5) for prenol esters (R_f 0·30) and petrol (30°–40°)–Et_2O (1:1) for free prenols (R_f 0·42). *IR* spectra were determined as thin films between two KBr discs.

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