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LONG CHAIN POLYISOPRENOID ALCOHOLS IN LEAVES OF CAPRARIS SPECIES*

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Leaves of twelve species of the genus Capparis were examined for the presence of long chain polyisoprenoid alcohols. In a number of species the accumulation of polyisoprenoid alcohols was up to about 0.3% of dry weight of tissue. In all studied species polyisoprenoid alcohols composed of 12, 13, 14 or 15 isoprene residues formed the main polyrenol family. In the majority of the plants studied lower quantities of an additional polyrenol family were present, in which prenologues composed of 19, 20 or 21 isoprene units were dominating. In one species — Capparis coriacea also the presence of dolichol-like polyrenols with a hydrogenated OH-terminal isoprene unit was documented.

In the organisms studied so far with respect to the presence of polyisoprenoid alcohols, either the accumulation of fully unsaturated poly-cis-pre
nols (as it is the case in the plant kingdom), or the presence (in animal tissues) of α-dihydropolyrenols-dolichols were documented [1]. Plant polyrenols represent a large diversity of the chain length, and variations in polyrenol content. The shortest polyrenols (composed of 6, 7 or 8 isoprene residues) were found in the wood of Betula verrucosa [2]. A number of plants were found to contain longer prenol molecules, e.g. prenol-11, -12, etc., up to prenol-28 and even longer [3, 4]. Polyrenol families with dominating prenologues composed of 10, 11 and 12 isoprene units in leaves of angiosperms and those with dominating -15, -17 and longer prenols are most common in gymnosperm plants. Only very few flowering plant species containing as dominant prenologues prenol-13 and prenol-14 were mentioned in the literature [3]. In the case of animal dolichols the type of structure of the linear polyrenoid is limited to the chain length from C₈₅ to C₁₀₅; in some special cases the normally rather narrow spectrum of dolichols in the natural mixture is somewhat

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broader, but it is never so diverse as shown for plants [3, 4, 5, 6]. All the dolichols are di-trans-poly-cis-isoprenoid alcohols. Many polyprenols isolated from plants contain also two trans-isoprene residues at the ω end, though in the plants accumulating polyprenols composed of 10 to 12 or to 13 isoprene units their structure is rather of a tri-trans type [3, 7]. The presence of dolichols in plants was always postulated because of necessity of these compounds for glycoprotein synthesis in all eukaryotic cells, though it was only in 1984 that the presence of the mixture of fully unsaturated and α-saturated polyisoprenoid alcohols was detected in the seeds of monocotyledons, and the occurrence of α-saturated alcohols in the seeds of dicotyledon plants was documented [8]. In the recent studies performed on plants belonging to the Capparidaceae family only fully unsaturated long chain polyisoprenoid alcohols were found [3, 9]. In this report we describe the simultaneous occurrence of rather large amounts of both polyprenols and their dolicholic analogues in green leaves of Capparis coriacea. We also present the data on the occurrence of long chain polyisoprenoid alcohols in a number of species belonging to the Capparis genus, as its unique chain length of a poly-cis prenol composed of 13 or 14 isoprene units may serve as a chemotaxonomic criterion in the systematics of plants.

MATERIALS AND METHODS

Plant material. Leaves of Capparis coriacea grown in the greenhouse of Botanical Garden of the Charles University in Prague (Czechoslovakia) were kindly given by Dr. Anna Skalnicka. Samples of other species of Capparis were from Botanical Garden in Groningen, The Netherlands, and Caracas, Venezuela.

Chemicals and chromatographic materials. Plates for analytical t.l.c. (E. Merck, Darmstadt, F.R.G.) were coated with silica gel 60 or with RP-18 (0.2 mm thick) with concentrating zone. Lichroprep Si60 for column chromatography and solvents for h.p.l.c. were from the same source. Other chemicals of analytical grade were from POCh, Gliwice, Poland. Standard polyprenols and dolichols were from the “Collection of Polyprenols” of the Institute of Biochemistry and Biophysics, Warsaw, Poland. Thin-layer chromatography (t.l.c.) was performed on silica gel plates using ethyl acetate/benzene, 5:95, v/v (solvent A) and on RP-18 plates using acetone (solvent B). The spots of polyprenols and dolichols were detected with iodine vapors or with anisaldehyde spray reagent [7].

High pressure liquid chromatography (h.p.l.c.) was performed as described by Eggens et al. [10] using the Waters Ass. (U.S.A.) dual pump apparatus, gradient programmer and UV detector set at 210 nm. Peak areas were measured using the Hewlett-Packard integrator. The elution system used for chromatography on the Resolve C18 column (5 μ, 12×0.4 cm, Waters Ass., U.S.A.) was methanol/isopropanol/water (60:40:5, by vol.) and
isopropanol/hexane (30:70, v/v) added from 0% to 50% according to gradient
type “5” (concave). The solvent flow was 1.5 or 0.8 ml/min, and the end of the
gradient was reached after 45 min. N.m.r. spectra were recorded in CDCl₃ with
trimethyl silane (TMS) as internal standard using a Jeol 500 MHz spectrometer.
The assignment of signals was based on the data of Feeney & Hemming [11] and Ibata et al. [12].

Quantitative estimation of polyrenols in plant leaves. The extraction of lipids
and purification of polyrenol fraction were performed as described in the
previous paper [4]. Samples of dried leaves were used for analyses.

Isolation of polyrenols and dolichols from leaves of Capparis coriacea. Dried
leaves (7 g) were extracted exhaustively several times with acetone/hexane, (1:1, v/v) and the extraction was continued with pure hexane. Both extracts were
pooled since on t.l.c. in solvent A no qualitative differences between them were
observed. After evaporation of the solvents the residue (133 mg) was treated
with alkali [7]. On t.l.c. in solvent A, in the unsaponifiable fraction the presence
of two spots giving characteristic colors with anisaldehyde reagent could be
seen. The substance of Rₖ 0.48 resembled dolichols and that of Rₖ 0.54
resembled polyrenols. The fraction of polyrenoid lipids was subjected to
adsorption chromatography on Lichroprep Si60 (1 × 80 cm column) using 10%
ethyl ether in hexane as the eluent, and 5 ml fractions were collected. Fractions
were checked for the presence of polyisoprenoid alcohols by t.l.c. as above. The
substance of Rₖ 0.54 (12 mg) was eluted first from the column and was almost
completely separated from the substance of Rₖ 0.48 (18 mg).

RESULTS

The results of quantitative estimation of long chain polyrenoid alcohols in
9 Capparis species are shown in Table 1. The leaves of the studied plants
contain up to 0.38% polyrenols per dry weight (C. linearis); the lowest value
was found in C. separia (0.02%). In all the species studied except C. afzelii two
groups of polyrenoid alcohols were found; the first one constituting more than
80% and containing prenol - 12, - 13, - 14, - 15 and - 16 as the main prenologues,
and the second containing prenol - 19, - 20, - 21 and - 22 as the main
components. In some plants, e.g. C. pubiflora the second group of longer chain
polyrenols was present only in trace amounts or it was absent (C. afzelii).
A representative h.p.l.c. record for polyrenoid alcohols from C. verrucosa is
shown in Fig. 1. The quantitative results were obtained upon including into the
studied sample a known amount of prenol - 18 as described in the previous
paper [4]. In all the plants studied the polyisoprenoid alcohols were present as
non-esterified compounds. A similar pattern of polyrenols in which the
prenol - 12, - 13, - 14 and - 15 were dominating prenologues, was also observed
in samples of leaves of C. holophylla, C. spinosa and C. zeylanica; however for
these samples only qualitative analysis of polyisoprenoid alcohols was perfor-
### Table 1
The content of polypropens in the leaves of various Capparis species

<table>
<thead>
<tr>
<th>Plant</th>
<th>Total polypropens (% of dry weight)</th>
<th>Content of prenologues (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td><em>C. decidua</em></td>
<td>0.07</td>
<td>2.2</td>
</tr>
<tr>
<td><em>C. odoratissima</em></td>
<td>0.12</td>
<td>1.5</td>
</tr>
<tr>
<td><em>C. coriacea</em></td>
<td>0.27</td>
<td>0.1</td>
</tr>
<tr>
<td><em>C. linearis</em></td>
<td>0.38</td>
<td>0.1</td>
</tr>
<tr>
<td><em>C. afzelii</em></td>
<td>0.07</td>
<td>4.0</td>
</tr>
<tr>
<td><em>C. pachaco</em></td>
<td>0.19</td>
<td>0.1</td>
</tr>
<tr>
<td><em>C. pubiflora</em></td>
<td>0.07</td>
<td>2.3</td>
</tr>
<tr>
<td><em>C. verrucosa</em></td>
<td>0.10</td>
<td>0.1</td>
</tr>
<tr>
<td><em>C. separia</em></td>
<td>0.02</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* Prenologues 13, 14 and 15 are prenols + dolichols (cf. Fig. 2).
med. In the case of *C. coriacea* in which the chromatographic peaks were somewhat broader, a more precise chromatographic analysis has demonstrated the presence of double peaks (Fig. 2). With the use of standard polyprenol and dolichol substances of the given chain length they have been identified as mixtures of prenol-13+dolichol-13, prenol-14+dolichol-14 and prenol-15+dolichol-15. Trace amounts of prenologues composed of 12 and of 16 isoprene residues were also observed. By integrating the areas of polyprenol and dolichol separately in each split peak one can come to the conclusion that in each pair of prenologues the ratio polyprenol/dolichol is constant (approx. 55:45). In none of the other species belonging to *Capparidaceae* (e.g. *C. holophylla*, *C. spinosa*, *C. zeylanica*, etc.) the record of h.p.l.c. has shown the presence of double, split peaks which would indicate the occurrence of larger amounts of dolichol (E. Świeżewska, unpublished). The presence of double, split peaks has not been reported in the studies of other authors performed on *C. spinosa* [9]. It is interesting, however, that in the all plants belonging to *Capparidaceae* so far studied the accumulated polyprenols are form a family in which prenol-13, -14 and -15 are the dominant prenologues. This size of molecules seems to be characteristic for all species belonging to the *Capparidaceae* family and perhaps for some species of the genus *Ziziphus* (W. Jankowski, unpublished). The occurrence of families of polyprenols in which prenol-14 or prenol-15 are dominating was found in some species.
belonging to genus Picea (P. abies, etc.) [3, 6, 13], but it should be added that the polyprenols in plants of this genus occur always in the form of esters and they differ from Capparis polyprenols with respect to the proportion of trans and cis-isoprene residues in the molecule (see below). The polyprenols in all so far studied Capparidaceae as well as polyprenols and dolichols of C. coriacea occur in leaves as free alcohols.

Figure 3 shows the \(^1\text{H}\)-n.m.r. spectra of the polyprenol mixture and dolichol mixture which were separated by preparative column chromatography on Lichroprep Si60 from the polyprenol lipid fraction of C. coriacea. The amounts of each fraction should not be considered as the total amounts of these substances in the material studied as a part of them was lost because of the overlapping of polyprenols and dolichols in the course of the isolation procedure. Figure 3A represents the spectrum of the polyprenol-like substance, giving \(R_F = 0.54\) in solvent A. One can see characteristic signals at 5.45, 4.10 and 1.75 p.p.m. which are common for fully unsaturated polyprenols. The other signals are in agreement with the poly-cis-prenol structure, with a low proportion of trans-isoprene residues. A part of the spectrum in the expanded form is shown as the insert in Fig. 3A. One can calculate from the proportion of the 1.68 p.p.m. and 1.60 p.p.m. signals the exact ratio of cis and trans-isoprene residues, respectively. One can also see clearly a signal of the \(\alpha\)-cis unit at 1.74 p.p.m. Knowing the "average chain length" of the polyprenol mixture (14.6
Fig. 3. $^1$H-n.m.r. spectra of "poliprenol-like" substances (A) and "dolichol-like" substances (B) of *Capparis coriacea*
isoprene units) from integration of peak areas in Fig. 2, the intensities of characteristic signals can be compared with the theoretical values expected for the postulated structure of $\omega$-tri-trans-poly-cis-prenol (Table 2). A di-trans structure which is also common in polyrenols (e.g. Betula verrucosa, several Coniferopsida and many Rosaceae) could be excluded on the basis of the present results (cf. [3]).

In Fig. 3B one can see the spectrum of the “dolichol-like” substance giving $R_F = 0.48$ in solvent A. One can see in the spectrum a characteristic multiplet at 3.65 p.p.m. resulting from the presence of the saturated “OH-terminal” isoprene unit. Only a small signal at 4.10 p.p.m. is visible which evidently results from traces of contaminating fully unsaturated polyrenol that had not been completely removed by column chromatography. Since we were able to calculate the “average chain length” (14.6 isoprene residues, similary as in the case of polyrenols), here also the theoretical proportion of cis- and trans-isoprene residues could be compared with experimental values. The figures presented in Table 2 show that the $\omega$-tri-trans-poly-cis structure can be postulated also for dolichols in C. coriacea.

**Table 2**

*Relative intensities of $^1$H-n.m.r. signals in polyrenols and dolichols from leaves of Capparis coriacea*

The observed and theoretical values for the methyl protons are the number of methyl protons. Theoretical values are in parentheses.

<table>
<thead>
<tr>
<th>Chemical shift S(p.p.m.)</th>
<th>Assignment</th>
<th>Polyprenol fraction (Average chain length = 14.6)</th>
<th>Dolichol fraction (Average chain length = 14.6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.60</td>
<td>CH$_3$ trans</td>
<td>4.0 (4.0)</td>
<td>4.0 (4.0)</td>
</tr>
<tr>
<td>1.61</td>
<td>CH$_3$ trans ($\omega$)</td>
<td>10.5 (10.6)</td>
<td>9.5 (10.6)</td>
</tr>
<tr>
<td>1.68</td>
<td>CH$_3$ cis, cis ($\omega$)</td>
<td>0.8 (1.0)</td>
<td></td>
</tr>
<tr>
<td>1.74</td>
<td>CH$_3$ cis ($\alpha$)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4 shows the $^{13}$C n.m.r. spectra of lipids isolated from C. coriacea leaves. In part A, representing the polyrenol-like substances, one can see the alignment of cis and trans isoprene residues. The signal at 39.78 p.p.m. assigned to the C$_1$-methylene carbon atoms of the trans-isoprene residue in the trans-trans and $\omega$-trans-linkages is present as well as the signals at 32.29 and 32.05 p.p.m. assigned to C$_1$-methylene carbon atoms of the cis-isoprene residue in cis-cis- and trans-cis-linkages. One can also notice the absence of the signal around 40.0 p.p.m. which is characteristic for the cis-trans-linkage; this indicates that the trans isoprene units are incorporated in the $\omega$-trans-trans-trans sequence. The other signals of the spectrum are in accord with the data reported by Ibata et al. [12] for prenol-18. In part B, which
Fig. 4. $^{13}$C-n.m.r. spectra of "polyprenol-like" substances (A) and "dolichol-like" substances (B) of *Capparis coriacea*. 
represents the $^{13}$C-n.m.r. spectrum of the dolichol-like substance, the signals are almost identical with those of polyrenols discussed above. It can therefore be assumed that the distribution of the cis- and trans-isoprene residues along the hydrocarbon chain in polyrenols and dolichols is identical.

**DISCUSSION**

Among the large number of plant species examined so far for the presence of long chain polyrenols, the pattern of the natural mixture of polyisoprenoid alcohols found in leaves of the Capparidaceae family plants is unique. Usually, angiosperm plants accumulate in their leaves variable amounts of fully unsaturated polyrenols with chain length of 10, 11, 12 or 18, 19, 20 or with even longer chains [3, 4, 7].

Most of the studied Capparidaceae contained, as the main group of polyisoprenoid alcohols, prenologues composed of 12, 13, 14 or 15 isoprene residues. This is in agreement with the recent data for C. spinosa [9]. Small amounts of longer chain prenologues, composed of 19, 20, 21 and 22 isoprene units, were also present. C. coriacea studied in this paper contained a mixture of prenol-13, -14 and -15 with trace amounts of shorter and longer prenologues and also almost equal of corresponding $\alpha$-dihydroypolyrenols (dolichols). The characteristic pattern of polyrenol family with prenol-13, -14 and -15 as dominating prenologues was found also in other tested species of Capparidaceae (Table 1) and C. separia, C. spinosa and C. zeylanica (E. Świeżewska, unpublished). In none of them, however, measurable amounts of dihydropolyrenols were found when the polyrenol fraction was analysed by adsorption thin-layer chromatography. In studies on polyisoprenoid compounds from leaves of Sorbus suecica one could notice traces of dolichol-like compounds accompanying the typical fully unsaturated polyrenols (prenol-19, -20 and -21) (T. Chojnacki, unpublished). This could postulate the presence in this plant of catalytic amounts of dolichol type compounds related to the carrier function of dolichol phosphate in glycoprotein biosynthesis. Accumulation of large amounts of dolichols in leaves of C. coriacea seems not to be related to glycosylation processes. The reason for accumulation of fully unsaturated polyrenols in C. coriacea and in a number of other plants remains obscure. Apart from the small numbers of species of Capparidaceae mentioned above, in only a few other plants similar polyrenol mixtures with dominating prenol-12, -13 and -14 were observed (Nephelium chinensis, family Nephelidaceae; Celtis audibersiana, family Tiliaceae [3]). Polyrenols of the chain length starting from 13, 14 isoprene residues occur in numerous gymnosperm plants; in Pinaceae family, especially in several species belonging to genus Picea, the accumulation of relatively short polyrenols, mainly prenol-14, -15 and -16 has often been found [6, 13, 14]. The presence in plant material of two groups of polyrenols differing in the chain length has
previously been found in some gymnosperms [6, 7] and in genus Potentilla of family Rosaceae [4]. This phenomenon may be more common than expected in the plant kingdom as with the advent of more sophisticated chromatographic methods it is being found more frequently. It should be stressed that polyprenols and dolichols in C. coriacea are of tri-trans type, while the polyprenols accumulated in gymnosperms are di-trans compounds [5, 12, 14]. The tri-trans structure seems to be characteristic for all medium chain length polyprenols, e.g. prenol-10, -11 and -12 [7, 15]. In contrast to the leaves of angiosperm plants, seeds of dicotyledon plants contain exclusively dolichols and seeds of monocotyledon plants contain mixtures of dolichols and fully unsaturated polyprenols, but in no case a distinct accumulation of these substances was noticed [8, 16]. In the above mentioned materials the presence of dolichols may be connected with their role in glycoprotein synthesis as they occur in low amounts and are efficiently converted to phosphorylated forms during germination [17]. The uniqueness of the occurrence in leaves of C. coriacea both of fully unsaturated polyprenols and corresponding dolichols will require further studies; it will be necessary to confirm this phenomenon using plants from a natural environment. The occurrence of dolichols in leaves, as documented in our studies and those of Ravi et al. [8] points to the necessity of careful identification of polyisoprenoid alcohols isolated from plant tissues.

The cooperation of Prof. J. Dąbrowski, Prof. U. Lüttege, Dr. A. Ejchart, Mrs J. Hertel, Dr. A. Skalnicka and Dr. E. Świeżewska in designing and performing our studies is gratefully acknowledged.

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