THE OCCURRENCE OF POLYISOPRENOIDS IN THE LEAVES OF STERCULIA RUPESTRIS BENTH.

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Polyisoprenyl acetates with an average number of isoprene residues of 9-12 were isolated from the leaves of Sterculia rupestris Benth. (Sterculiacaeae). Spectroscopic analysis revealed that all the polyisoprenyl acetates were long-chain homologues with the following sequence of isoprene residues: α-trans, two-trans, five to eight cis and cis-α-terminal.

INTRODUCTION

In the course of investigation on the constituents of Sterculia rupestris Benth., we previously reported the presence of flavonoid glycosides in the leaves. Further investigation of the leaves of the plant resulted in the isolation of a series of four polyisoprenoids. The arrangement of the two trans (α-trans-trans) and cis residues indicates that the polyisoprenyl acetates are long-chain homologues of betulaprenols previously reported in gymnosperms. These polyisoprenoids have been used as starting materials for the synthesis of mammalian dolichols (2, m = 12-19, mainly 14-16) which are recognized as one of the most important materials in the biosynthesis of glycoproteins.

In a research for a source of dolichols, many authors have investigated the chain-length distribution of polyprenols in several species belonging to different families. This work is concerned with the identification and structural determination of four polyisoprenyl acetates in the leaves with the structure 1b, where m = 6-9.

EXPERIMENTAL

General

IR spectra are measured in KBr pellets (Berkin-Elmer, 457). 1H NMR spectra were determined in CDCl3 at 90 MHz (FX 90Q JEOl) from Bruker) using TMS as int. standard.

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\(^3\)CNMR spectra were recorded at 100 MHz (CDCl\(_3\), TMS); GX 400 (Jeol) from Bruker. FD-MS were recorded at 70 eV; Finnegan 4000. Reversed phase HPLC: UV detector, Lichrosor RP-18. CC: alumina (Merck). TLC: silica gel G (Merck), cyclohexane-EtOAc (9:1).

**Plant material**

Air-dried powdered leaves of *S. rupestris* (3 kg) were collected from the plants cultivated in the Experimental Station, Faculty of Agriculture, Assuit University, Assuit. The plant was identified by Prof. A. Fayed, Dept. of Plant Taxonomy, Faculty of Science, Assuit University.

**Isolation and purification**

The dried leaves were extracted with cyclohexane. After concentration under reduced pressure, 70 g of the residue was chromatographed on an alumina column (4x75 cm x 750 g) using cyclohexane-EtOAc (9:1) as eluent. An oily fr. 370 mg, R\(_f\) 0.50 (sprayed with 10% H\(_2\)SO\(_4\), giving violet colour) was chromatographed by HPLC after unsuccessful trials for isolating its components by normal chromatographic methods. Reversed-phase prep. RP-18 column, MeOH-EtOH (3:1), flow rate 3 ml min\(^{-1}\), UV detector at 215 nm. The obtained compounds are oily in nature, yellowish in colour with faint odour, oily taste and having the same R\(_f\) value of the mixture.

**RESULTS AND DISCUSSION**

IR spectral data showed the following absorption bands: 1715 (CO), 1665 (C=O), 1005 (C=CH) and 840 cm\(^{-1}\) (C-C-H).

FD-mass spectroscopy of isolated compounds showed a fragmentation pattern characteristic for polyisoprenoids, viz. fragment at \(m/z\) 135, 202, 271, 339, 407, 543, etc., indicating successive losses of isoprene units from the original compounds. The \([M]^+\) of 1-4 appeared at \(m/z\) 672, 740, 808 and 876, corresponding to polypropenyl-9 to -12 acetates of the general formula 1b (m= 6-9). On saponification of the isolated compounds with alcoholic potassium hydroxide, the resulting polyisoprenols gave \([M]^+\) of \(m/z\) 630, 698, 766 and 834 for 1-4 respectively, corresponding to the general formula 1a (m= 6-9).

\(^3\)HNMR spectral data of 1-4 showed allylic methyl signals at \(\delta\) 1.59, 1.67 and 1.75, assignable to methyl groups of the internal trans-isoprene carbon chain, a methyl group of the internal cis-isoprene residue and a terminal methyl group of the \(\alpha\)-terminal cis-isoprene residue respectively.

\(^3\)HNMR spectra of the isolated compounds were similar to those of the polyisoprenols produced by saponification except that the \(-\text{CH}_2\text{OAc}\) and \(-\text{CH}_2\) protons in the \(\alpha\)-terminal residue exhibited signals at \(\delta\) 5.35 (t, \(J = 7.3\) Hz) and 4.55 (d, \(J = 7.3\) Hz) respectively; these signals were shifted to \(\delta\) 5.44 and 4.08 after saponification of the isolated polyisoprenyl acetates. It is evident that \(^3\)HNMR spectral data has been used for chain-length determination using integration. The relative intensities of signals in the isolated compounds
Table 1: Relative intensities of $^1$HNMR signals of isolated compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of isoprene units</th>
<th>[M]$^+$ m/z</th>
<th>Molecular formula</th>
<th>Chemical shift ($\delta$-values) and assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.59 Me trans, $\omega$(trans)</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>672</td>
<td>C$<em>9$H$</em>{16}$O$_2$</td>
<td>2.94* (3)</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>740</td>
<td>C$<em>{10}$H$</em>{18}$O$_2$</td>
<td>3.19* (3)</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>808</td>
<td>C$<em>{11}$H$</em>{22}$O$_2$</td>
<td>3.24* (3)</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>876</td>
<td>C$<em>{12}$H$</em>{19}$O$_2$</td>
<td>3.17* (3)</td>
</tr>
</tbody>
</table>

Theoretical values in parentheses.

Observed and theoretical values for Me protons are the number of methyl groups.
were in good agreement with the theoretical one for structure 1b. This indicates that the principle polyisoprenyl acetates 1-4 were composed of 2 identical trans residues, two to nine internal cis residues and a cis-α-terminal residue.

$^{13}$CNMR spectral analysis is essential for the determination of the internal cis- and trans-isoprene residue alignment; the α-terminal residue exhibited signals at 32.4 (C-1), 142.4 (C-2), 119.3 (C-3), 61.0 (C-4), 170.6 (Me-CO) and 20.9 (Me-CO). The C-1 methylene carbons exhibited signals around δ 32-40 reflecting the linkage of the cis- and trans-isoprene residues where carbon atoms are designed as follows: the signal at δ 39.7 is assigned to C-1 methylene carbons of the trans-isoprene residue in the trans-trans and trans linkage. The signals at δ 32.2 are assigned to C-1 methylene carbons of the cis-isoprene residue in cis-cis and trans-cis linkage, respectively. The absence of signals around δ 40, which is characteristic for a cis-trans linkage, indicates that the trans-isoprene units are incorporated in an α-trans-trans linkage. The presence of the α-trans linkage is also confirmed by close inspection of the characteristic C-2 signal of the terminal unit at δ 131.1 (the α-trans linkage in model compounds showed a signal around δ 131.0-131.3 while the ω-cis-linkage showed a signal around δ 131.5-131.6). The relative intensities of the C-1 methylene signals of the isolated compounds were in good agreement with the theoretical values obtained according to the general structure 1b (Table 2). This indicates that the ω-terminal, two trans residues, five to eight cis residues and cis-α-terminal are aligned in that order.

The present study is the first report of polyisoprenyl acetates of the betulaprenol-type, having 9-12 isoprene units in their skeletons in Sterculia rupestris.

Acknowledgement

The authors are grateful to the Institute of Pharmaceutical Chemistry, Erlangen, Germany, for performing the HPLC isolation and carrying out the spectral analysis of the isolated compounds.

Table 2: Relative intensities of C-1 methylene carbon signals in the isolated compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of isoprene residues</th>
<th>Chemical shift [δ-values] and assignment</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>32.0</td>
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<td>1</td>
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<td>2</td>
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<td>1.11(1)</td>
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<tr>
<td>3</td>
<td>11</td>
<td>0.94(1)</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>1.20(1)</td>
</tr>
</tbody>
</table>

Theoretical values in parentheses.
REFERENCES