Coumarins from the Seeds of *Poncirus trifoliata* L.

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The coumarinic components of *Poncirus trifoliata* seeds were investigated. Two coumarins aurapten and 6-methoxy-7-geranyloxycoumarin were isolated and identified.

The occurrence of furocoumarinic compounds in *Poncirus trifoliata* L. seeds already has been described, but to our knowledge little is known about the coumarinic components.

In a general research program on furocoumarins and coumarins from this plant we recently described the identification of the five furocoumarins, bergapten, imperatorin, isopimpinellin, prangenin and prangenin hydrate in the seeds obtained from ripe fruits of plants cultivated in the area of Padua. We are now reporting the isolation of two coumarin derivatives, i.e. 7-geranyloxycoumarin (aurapten) and 6-methoxy-7-geranyloxycoumarin from these seeds.

The main component eluted with petroleum-ether/benzene (85/15) was a blue fluorescent compound identified as 7-geranyloxycoumarin (aurapten). The identification was based on elemental analysis and molecular weight for C_{20}H_{24}O_{4}, UV spectrum characteristic of a 6,7-dialkoxycoumarin, unchanged by alkali addition. Hydrolysis of aurapten gave a compound which, by direct comparison with synthetic samples of the two possible isomers 6-methoxy-7-hydroxyxocoumarin ( scopoletin) and 6-hydroxy-7-methoxyxocoumarin, proved to be scopoletin. These data suggested for compound aurapten the iso-meric structures 6-methoxy-7-geranyloxycoumarin or 6-methoxy-7-neryloxycoumarin. In the H'-NMR spectrum at 60 MHz the C_H vinyl proton appears as a triplet at 5.50 ppm (J = 6.7 c/s), whereas at 90 MHz each peak splits into quartets (J = 1.2 c/s). The ratio between the absorption at 2.14 and 2.09 ppm (2H each, C_H - H_2 and C_H - H_2) is about 1.5:2.5, in agreement with a C_H - C_H trans conformation in the alkenyl moiety. All the evidence suggests for the compound aurapten the formula 6-methoxy-7-geranyloxycoumarin.

The same compound was recently identified in *Thapsia garganica* (Umbelliferae) by Larsen et al., who reported m.p. 84 — 84.5 °C in contrast to the synthetic 7-neryloxycoumarin with m.p. 45 — 46 °C. It was also isolated by Talapatra et al. in *Feronia elephantum* (Rutaceae).

Further fractions eluted from the silica-gel column with benzene/ethyl acetate (1/1) and ethylacetate gave a residue that we did not completely resolve. Preliminary investigations showed the presence of other coumarins in very small amounts, as well as limonoids.

By increasing the polarity of the eluent the already described furocoumarins bergapten, imperatorin and isopimpinellin were separated. The main component eluted with benzene/ethyl acetate (9/1) was a blue fluorescent compound identified as 6-methoxy-7-geranyloxycoumarin. aurapten and 6-methoxy-7-geranyloxycoumarin were isolated and identified.

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Experimental Part

Melting points were determined in open capillary and were not corrected. The UV spectra were recorded on an Optica CF4 instrument; IR spectra (KBr pellets) on Perkin-Elmer 457; H-NMR spectra (TMS internal standard, chemical shifts expressed in ppm) on Perkin-Elmer R-12 and Bruker 90 instruments. For TLC Merck 5715 silica-gel plates were used, moving solvent cyclohexane/ethyl acetate (65/35). The fluorescent spots were localized by exposure to UV light (Philips HPW 125, 365 nm).

Extraction of seeds and chromatographic fractionation

The seeds obtained from ripe fruits were dried in an oven at 60 °C to constant weight and finely ground. The powder (3.3 kg) was exhaustively extracted with petroleum ether 30 – 50 °C. The extract was concentrated and the solid crystalline material, separated by standing, filtered off. This procedure was repeated until all the crystallizable material was removed. The viscous residue (130 g), obtained by evaporation of the solvent, was applied to a column (ø 7.8 cm) of 1100 g deactivated silica-gel (5% water p/p) and eluted with a mixture of solvents of increasing polarity. Fractions of 200 ml were collected and reduced to a small volume by evaporation under vacuum. They were examined by TLC and those showing a similar chromatographic pattern pooled according to the scheme shown in Table 1.

7-[(3',7'-dimethyl-2',6'-octadienyl)oxy]coumarin (aurapten)

The pooled fractions 222 – 245 when concentrated gave a solid. This was crystallized from n-exane, resulting in 920 mg of white needles (compound 1): m.p. 71 °C, R_f 0.75, UV (ethanol 95 °C) \( \lambda_{max} \) nm (log e): 323 (4.22); \( \lambda_{min} \) 260 (3.19).

Elemental analysis:

\[
\text{C}_{19}\text{H}_{22}\text{O}_3 \quad \text{Found: C 76.43; H 7.34,} \\
\text{Calcd: C 76.48; H 7.43.}
\]

Molecular weight (osmometric method in benzene), found: 302.9, calcld: 298.37.

The compound 1 (400 mg) was hydrolized in a mixture of acetic acid (2 ml) and sulfuric acid (0.2 ml). The precipitate obtained by standing at room temperature for two hours was filtered off and crystallized from ethyl acetate-n-exane: m.p. 221 – 222 °C, elemental analysis C_{19}H_{23}O_3. This compound, when methylated with diazomethane in ether, gave a compound that, crystallized from methanol, melted at 114 °C, elemental analysis C_{10}H_{18}O_3.

From these data, together with further evidence obtained from mmp, UV and IR spectra and TLC behaviour, we could identify the hydrolysis product of 1 as 7-hydroxycoumarin (umbelliferon) and the methylated compound as 7-methoxycoumarin (erniarin).

Table I. Separation by chromatography on silica gel column (ø 7.8 cm; 1100 g; H_2O 5%) of the mother-liquors of Citrus trifoliata seeds extract.

<table>
<thead>
<tr>
<th>Fractions [200 ml]</th>
<th>Solvent</th>
<th>TLC Fluorescence</th>
<th>R_f</th>
<th>Substances present</th>
<th>Recovery [after recrystallization] [mg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 221</td>
<td>Petroleum ether</td>
<td>violet</td>
<td>0.9</td>
<td>unidentified substance</td>
<td>–</td>
</tr>
<tr>
<td>222 – 245</td>
<td>Petroleum ether 30 – 50 °C/ Benzene (85/15)</td>
<td>blue</td>
<td>0.75</td>
<td>aurapten</td>
<td>920</td>
</tr>
<tr>
<td>246 – 287</td>
<td>Petroleum ether 30 – 50 °C/ Benzene (70/30)</td>
<td>blue</td>
<td>0.75</td>
<td>aurapten</td>
<td>–</td>
</tr>
<tr>
<td>288 – 304</td>
<td>Petroleum ether 30 – 50 °C/ Benzene (70/30)</td>
<td>yellow</td>
<td>0.52</td>
<td>bergapten</td>
<td>60</td>
</tr>
<tr>
<td>305 – 340</td>
<td>Petroleum ether 30 – 50 °C/ Benzene (50/50)</td>
<td>yellow</td>
<td>0.57</td>
<td>imperatorin</td>
<td>145</td>
</tr>
<tr>
<td>341 – 353</td>
<td>Benzene</td>
<td>yellow</td>
<td>0.57</td>
<td>imperatorin</td>
<td>–</td>
</tr>
<tr>
<td>354 – 370</td>
<td>Benzene/ethyl acetate (9/1)</td>
<td>blue</td>
<td>0.45</td>
<td>isopimpinellin</td>
<td>27</td>
</tr>
<tr>
<td>371 – 384</td>
<td>Benzene/ethyl acetate (1/1)</td>
<td>blue</td>
<td>0.43</td>
<td>6-methoxy-7-geranyloxy coumarin</td>
<td>195</td>
</tr>
<tr>
<td>385 – 460</td>
<td>Ethyl acetate</td>
<td>violet-blue</td>
<td>0.25</td>
<td>unidentified substance</td>
<td>–</td>
</tr>
</tbody>
</table>


6-methoxy-7-[3',7'-dimethyl-2',6'-octadienyl]oxy]coumarin

The pooled fractions 354–370 by concentration gave a solid which was crystallized from 95% ethanol, 195 mg white plates (2): m.p. 87.5–88 °C, Rf 0.43 UV (ethanol 95%), λmax nm (log ε) 345.5 (4.11); 296 (3.78); 252 (3.79); 257.5 (shoulder, 3.74); 230 (4.26) and λmin 306.5 (3.68); 269.5 (3.32).

Elemental analysis:
C29H24O4 Found: C 73.21; H 7.26; -OCH3 9.51, 
Calcd: C 73.14; H 7.37; -OCH3 9.44.

Molecular weight (osmometric method in benzene); found: 330.8, calcld 328.3. H NMR spectrum: 6.26, C3—H doublet, J = 9.5 c/s; 7.64, C4—H doublet, J = 9.5 c/s; 6.89, C5—H singlet; 6.80, C8—H singlet; 3.90, C6—OCH3 singlet; 1.60 and 1.65, C7'—(CH3)2 singlets; 1.78, C7'—CH3 singlet; 2.09 and 2.14, C4'—H2 and C5'—H2 broad singlets; 4.68, C1'—H2 doublet, J = 6.5 c/s; 5.08, C6'—H broad multiplet unresolved; 5.50, C2'—H broad triplet, J = 6.7 c/s (each peak splits at 90 MHz in a quadruplet J 1.2 c/s); the relative areas of the peaks were consistent with their assignments.

The compound 2 (100 mg) was hydrolized in acetic acid (2 ml) and sulfuric acid (0.2 ml). The mixture was neutralized with NaHCO3 after two hours and extracted exhaustively with chloroform. After removal of the solvent the residue, crystallized from methanol, melted at 211 °C without depression in mixture with a synthetic sample of 6-methoxy-7-hydroxycoumarin (scopoletin) 7.

Elemental analysis:
C16H16O4 Found: C 62.56; H 4.20; -OCH3 16.45, 
Calcd: C 62.50; H 4.20; -OCH3 16.10.

We wish to thank Prof. A. Pietrogrande for the elemental analysis and Mr. G. Pastorini for helpful technical assistance.