Coumarins from the Seeds of *Poncirus trifoliata* L.

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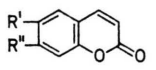
Coumarin, *Poncirus trifoliata*, Rutaceae

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 [1–4], 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 [4] 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 coumarinic derivatives, *i. e.* 7-geranyloxycoumarin (aurapten) and 6-methoxy-7-geranyloxycoumarin from these seeds.



1 R' = H R'' = $C_{10}H_{17}O$

2 R' = CH_3O R'' = $C_{10}H_{17}O$

For the isolation of these compounds the seeds were extracted with petroleum-ether, the less soluble furocoumarins separated out by filtration and the mother-liquors containing coumarins fractionated on a silica-gel column.

The main component eluted with petroleum-ether/benzene (85/15) was a blue fluorescent compound, identified as 7-geranyloxycoumarin (**1**) (aurapten). The identification was based on elemental analysis ($C_{19}H_{22}O_3$), m.p. 71 °C, IR, H'-NMR and UV spectra [5, 6]. Further evidence was based on the hydrolysis product of **1**, identified as 7-hydroxycoumarin (umbelliferon) which, by methylation, gave 7-methoxycoumarin (erniarin).

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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 (**2**). **2** was crystallized from ethanol: m.p. 87.5 – 88 °C, 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 **2** gave a compound which, by direct comparison with synthetic samples of the two possible isomers 6-methoxy-7-hydroxycoumarin [7] (scopoletin) and 6-hydroxy-7-methoxycoumarin [8], proved to be scopoletin. These data suggested for compound **2** the isomeric structures 6-methoxy-7-geranyloxycoumarin or 6-methoxy-7-neryloxycoumarin. In the H'-NMR spectrum at 60 MHz the $C_{2'}$ 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, $C_{4'}-H_2$ and $C_{5'}-H_2$) is about 1.5 : 2.5, in agreement with a $C_{2'}-C_{3'}$ *trans* conformation in the alkenyl moiety [6, 9]. All the evidence suggests for the compound **2** the formula 6-methoxy-7-geranyloxycoumarin.

The same compound was recently identified in *Thapsia garganica* (Umbelliferae) by Larsen *et al.* [10], who reported m.p. 84 – 84.5 °C in contrast to the synthetic 7-neryloxy isomer with m.p. 45 – 46 °C. It was also isolated by Talapatra *et al.* [11] 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.

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 Brucker 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 (⌀ 7.8 cm) of 1100 g of 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 chromatografic pattern pooled according to the scheme shown in Table I.

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) λ_{max} nm (log ε): 323 (4.22); λ_{min} 260 (3.19).

Elemental analysis:

$C_{19}H_{22}O_3$ Found: C 76.43; H 7.34,
Calcd: C 76.48; H 7.43.

Molecular weight (osmometric method in benzene), found: 302.9, calcd: 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 $C_9H_6O_3$. This compound, when methylated with diazomethane in ether, gave a compound that, crystallized from methanol, melted at 114 °C, elemental analysis $C_{10}H_8O_3$.

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) (⌀ 7.8 cm; 1100 g; H_2O 5%) of the mother-liquors of *Citrus trifoliata* seeds extract.

Fractions [200 ml]	Solvent	TLC Fluorescence	TLC R_f	Substances present	Recovery [after recrystallization] [mg]
1 – 221	Petroleum ether	violet	0.9	unidentified substance	—
222 – 245	Petroleum ether 30 – 50 °C /Benzene (85/15)	blue	0.75	aurapten	920
246 – 287	Petroleum ether 30 – 50 °C /Benzene (70/30)	blue	0.75	aurapten	—
		yellow	0.52	bergapten	60
288 – 304	Petroleum ether 30 – 50 °C /Benzene (70/30)	yellow	0.57	bergapten	—
		yellow	0.52	imperatorin	—
305 – 340	Petroleum ether 30 – 50 °C /Benzene (50/50)	yellow	0.57	imperatorin	145
341 – 353	Benzene	yellow	0.57	imperatorin	—
		orange-yellow	0.45	isopimpinellin	27
354 – 370	Benzene/ethyl acetate (9/1)	blue	0.43	6-methoxy-7-geranyloxycoumarin	195
		violet-blue	0.25	unidentified substance	—
371 – 384	Benzene/ethyl acetate (1/1)	blue	0.43	6-methoxy-7-geranyloxycoumarin	—
		violet-blue	0.25	unidentified substance	—
		violet-blue	0.23	unidentified substance	—
385 – 460	Ethyl acetate	violet-blue	0.25	unidentified substance	—
		violet-blue	0.23	unidentified substance	—

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, R_F 0.43 UV (ethanol 95%), λ_{max} nm (log ε) 345.5 (4.11); 296 (3.78); 252 (3.79); 257.5 (shoulder, 3.74); 230 (4.26) and λ_{min} 306.5 (3.68); 269.5 (3.32).

Elemental analysis:

$C_{20}H_{24}O_4$ Found: C 73.21; H 7.26; $-OCH_3$ 9.51,
Calcd: C 73.14; H 7.37; $-OCH_3$ 9.44.

Molecular weight (osmometric method in benzene); found: 330.8, calcd 328.3. H′NMR spectrum: 6.26, C_3-H doublet, J = 9.5 c/s; 7.64, C_4-H doublet, J = 9.5 c/s; 6.89, C_5-H singlet; 6.80, C_8-H singlet; 3.90, C_6-OCH_3 singlet; 1.60 and 1.65, $C_{7'}-(CH_3)_2$ singlets; 1.78, $C_{3'}-CH_3$ singlet; 2.09 and 2.14, $C_{4'}-H_2$ and $C_{5'}-H_2$ broad singlets; 4.68, $C_{1'}-H_2$ doublet, J = 6.5 c/s; 5.08, $C_{6'}-H$ broad multiplet unresolved; 5.50, $C_{2'}-H$ 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 $NaHCO_3$ 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:

$C_{10}H_8O_4$ Found: C 62.56; H 4.20; $-OCH_3$ 16.45,
Calcd: C 62.50; H 4.20; $-OCH_3$ 16.10.

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