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Short Communications

Chalcones from the Fronds of *Pityrogramma chrysophylla*, var. *heyderi*

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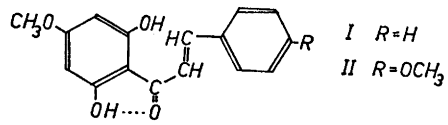
In continuation of the studies on frond pigments of the "ceraceus" ferns [1-3] the pigments of *Pityrogramma chrysophylla* (Sw.) Link, var. *heyderi* (Lauche) Domin, have now been investigated. This fern has an orange-coloured coating on the back of the fronds.

P. chrysophylla Kaulf. and *P. sulphurea Desv.* have been investigated by Zopf [4] (the genus then was given as *Gymnogramme*). He isolated a red material, m.p. 159°, which analysed for $C_{18}H_{18}O_5$. This "gymnogrammen" on boiling with ethanol was converted into colourless "gymnogrammidin", m.p. 114–115° (no analysis).

A similar material was obtained from *P. chrysophylla* var. *heyderi* in the present investigation. It proved to be a mixture which was separated by chromatography to give two main coloured components. Both pigments had indefinite melting points around 160°, which were not improved by recrystallisation. On vacuum distillation both were converted into colourless compounds, m.p. 100–102° and 119–120°. These rearrangement products were found to be flavanones and comparison with authentic material showed them to be (±)-5-hydroxy-7-methoxyflavanone and (±)-5-hydroxy-4′,7-dimethoxyflavanone, respectively.

The infrared and ultraviolet spectra of the pigments clearly indicate that these are the corresponding chalcones: 2′,6′-dihydroxy-4′-methoxychalcone (I) and 2′,6′-dihydroxy-4,4′-dimethoxychalcone (II). Of these only I has been observed before — it was obtained by Lindstedt [5] in a small amount on acidification of an alkaline solution of the corresponding flavanone and was found to cyclise very readily. Appreciable amounts of the flavanones were also isolated from the extracts.



I R=H
II R=OCH_3

In the *Pityrogramma* species hitherto investigated all pigments on the back of the fronds are of the $C_6C_3C_6$-type. Whereas in *P. chrysophylla* var. *heyderi* the orange colour is due to the chalcones I and II the white coating of var. *marginata* contains the corresponding dihydrochalcones [3]. In *P. triangularis* on the other hand the yellow pigment, although closely related to the chalcone I, is 3′,3′-dimethylated preventing aromaticity of the phloroglucinoid nucleus [1,2].

The gymnogrammen described by Zopf could be identical with the chalcone II.

Experimental. Melting points were determined on a Kofler block. The infrared spectra were recorded on Perkin Elmer No. 21 instrument (sodium chloride prism, potassium bromide discs) and the ultraviolet spectra on a Beckman DK-2 spectrophotometer (ethanol solutions).

Isolation. The samples of *P. chrysophylla* var. *heyderi* were collected and identified in the Royal Botanic Gardens at Kew in the autumn 1958. Fresh fronds and stems (147 g) were extracted with ether and the material obtained partitioned between dimethyl sulphoxide and *cyclo*hexane as described earlier [3]. A wax fraction (1.7 g, m.p. 40–75°) and a pigment fraction (4.9 g, m.p. 130–140°) were

obtained. Recrystallisation from benzene gave a bright red, crystalline material, m.p. 140—156°. Paper chromatography (*iso*propylether, dimethyl sulphoxide, EDTA treated paper [3]) showed the presence of two phenolic components, R_F 0.26 and 0.18. The mother liquors contained small amounts of a further slow-moving component (R_F 0.15) and two rapid components (R_F 0.57 and 0.38). The latter gave a brown colour with the benzidine reagent only after addition of sodium carbonate.

Separation of the mixtures as described for the dihydrochalcones [3] gave the following components: R_F *0.57*, 0.3 g; *0.38*, 0.2 g; *0.26*, 0.3 g and *0.18*, 1 g.

The R_F 0.57 product was recrystallised from methanol and sublimed, colourless plates, m.p. 100—102° identical with (±)-5-hydroxy-7-methoxyflavanone [3] (mixed m.p. and IR).

The R_F 0.38 product was recrystallised from methanol and sublimed to give colourless needles, m.p. 119—120°, identical with (±)-5-hydroxy-4′,7-dimethoxyflavanone [3] (mixed m.p. IR).

The R_F 0.26 product was recrystallised from benzene giving red prisms, m.p. 156—167°. The melting point range was not improved by repeated crystallisations. The infrared spectrum showed a broad band between 3 200 and 2 400 cm^{-1} and absorptions at 1 635, 1 610 and 825 cm^{-1}. The ultraviolet spectrum showed a broad maximum at 355 mμ (log ε 4.70). On attempted sublimation (130°, 0.005 mm) a colourless oil distilled. It solidified on cooling and proved to be identical with (±)-5-hydroxy-4′,7-dimethoxyflavanone (mixed m.p., IR). On standing, particularly in solution, the pigment was slowly converted into the flavanone.

The R_F 0.18 product on recrystallisation from benzene gave red prisms with an indefinite melting point, 156—162°. Relevant infrared absorptions were observed at 3 200—2 400, 1 635, 745 and 685 cm^{-1}. The ultraviolet spectrum showed a broad absorption at 340 mμ (log ε 4.74). Thermal rearrangement as described above gave (±)-5-hydroxy-7-methoxyflavanone in almost quantitative yield (identified by mixed m.p. and IR).

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Gel Filtration Chromatography in the Separation of Human Serum

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It has been reported by Alridge [1], Mounter and Whittaker [2], Augustinsson [3], and Marton and Kalow [4] that human plasma contains arylesterase activity.

In connection with investigations of cytolytic serum factors [5,6], it was noticed that the arylesterase activity of various fractions obtained in continuous (courtain) flow electrophoresis of human serum had such values that the presence of two arylesterases is indicated.

It can now be reported that two different arylesterases have been partly separated in fractions from human serum. As found in the investigations cited [5,6], there is one heat labile (56°C for 30 min.) arylesterase in human serum which is not influenced by EDTA and one heat stabile arylesterase which is completely inactivated by EDTA.

Experimental. Normal human serum from healthy blood donors (10 ml) was fractionated at +4°C with ammonium sulphate at pH 5.2. The sediments from 0—50, 50—60, and 60—70 % saturation were dissolved in 5 ml portions and for desalting filtered through Sephadex [7] G-25 columns 2 × 28 cm, equilibrated with 0.9 % NaCl. The supernatant from 70 % saturation was filtered too. The different solutions were at the desalting by Sephadex filtration collected in 3 ml fractions and the optical density at 280 mμ was measured as well as protein nitrogen and arylesterase activity. All fractions filtered were free from ammonium