The Pigments of the Yellow Iris (*Iris pseudacorus*)

By P. J. DRUMM AND W. F. O'CONNOR, *Department of Chemistry, University College, Cork*

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It is of interest from the standpoint of phytochemical processes to record variations in the nature of the pigments among flowers of the same genus. Thus the yellow petals of *Crocus sativus* and the stigmas of the violet *C. neapolitanus* contain the carotenoid crocetin whereas the petals of the blue *C. evernus* owe their colour to an anthocyanin. A number of blue and purple varieties of the *Iris* family have been shown by Robinson & Robinson (1934) to contain specific anthocyanins whilst the present authors have already referred (O'Connor & Drumm, 1941) to the carotenoids of *I. pseudacorus*, the detailed examination of which forms the subject of the present communication.

I. pseudacorus, more commonly known as the waterflag, is found in marshy ground and flowers in June–July. The only recorded observation in the chemical literature concerning the pigments of this flower is that of van Wisselingh (1917). Using the well-known microchemical crystallization method of Molisch (1896), he established the carotenoid nature of the colouring matter but did not attempt the isolation of any individual pigment.

In the present work, a light-petroleum extract of the dried petals, after saponification, was fractionated between light petroleum and aqueous methanol, the pigments being resolved into hydrocarbons and a mixture of xanthophylls. By chromatographic resolution of the former, β -carotene was obtained in crystalline form. The xanthophylls of the methanol phase were separated by repeated adsorption on calcium carbonate into two crystalline pigments, that occupying the top zone being identified as violaxanthin, first isolated by Kuhn & Winterstein (1931) from the petals of the yellow pansy. The other pigment, occupying the middle zone, was identified as lutein (Kuhn, Winterstein & Lederer, 1931). These pigments may be present as esters in the flower. Spectroscopic evidence of the presence of yet a third xanthophyll, probably zeaxanthin, was obtained but the amount available was not sufficient for complete characterization.

EXPERIMENTAL

Yellow iris petals (2 kg.), collected in the month of June, were dried at 50°, powdered and sieved, giving a golden yellow powder (500 g.). This was covered with 2 l. of light petroleum (Analar, b.p. 30–50°) and allowed to stand overnight in an atmosphere of nitrogen. The light-petroleum

extract was filtered off and the above treatment repeated until the extract was only faintly coloured. The combined orange-yellow extracts (6 l.) were concentrated to 2 l. at 40° *in vacuo*. The concentrated extract was then saponified by adding a solution of sodium ethoxide in ethanol (120 ml. of 10%), then sufficient ethanol (100 ml.) to make the solution homogeneous, and setting aside at room temperature for 24 hr. Methanol (90%) was added until a separation into two layers occurred. The lower layer was drawn off, and washed three times with light petroleum (100 ml.), the final washing being almost colourless.

The united light-petroleum extracts (Extract A) were next washed, once with 90% methanol (100 ml.), and then with a further two portions of 50 ml. each, all these washings being added to the main methanol layer. This methanol phase was then covered with a thin layer of light petroleum (b.p. 60–80°) and water carefully added until a distinct cloudiness appeared. On vigorous shaking, a solid began to separate and after the extract had stood overnight an orange-red solid had collected at the interface. This was collected, dissolved in carbon disulphide, the orange-red solution washed with water to remove traces of methanol, dried over anhydrous sodium sulphate, and chromatographed in portions on calcium carbonate columns (15 × 5 cm.). Developed with carbon disulphide the completed chromatograms exhibited the following zones:

Zone	Colour	Absorption maxima (CS ₂) (m μ)
Top	Yellow	502, 470, 442
Middle	Orange-yellow	509, 476, 444
Bottom	Orange	515, 482, 450

TOP ZONE. The eluate from this was brought to dryness *in vacuo*, the residue again dissolved in carbon disulphide and further resolved by reabsorption on calcium carbonate. The resulting top band was eluted with methanol, the eluate evaporated to small bulk, covered with light petroleum and water added until a cloudiness resulted. The mixture was left overnight in the ice-chest and the solid which separated at the interface was collected and recrystallized several times from carbon disulphide, from which it was obtained as reddish brown needles, m.p. 198–199°; absorption maxima in carbon disulphide, 501, 469, 441 m μ and in chloroform 482, 451, 424 m μ . When dissolved in ether it gave a very intense blue colour reaction with 25% hydrochloric acid. With cold glacial acetic acid the crystals gave a grass-green colour, with dichloroacetic acid a blue, and when a drop of 70% perchloric acid was added to their solution in glacial acetic acid a deep blue colour was obtained. This pigment is therefore violaxanthin.

MIDDLE ZONE. This was eluted with carbon disulphide containing ethanol and the latter then removed by repeated

washing with water. The washed solution was dried over anhydrous sodium sulphate and readsorbed on calcium carbonate. The completed chromatogram exhibited three definite bands, the uppermost of which was faintly yellow (violaxanthin). The middle orange-yellow band, which contained the main portion of the colouring matter, was eluted, and the eluate crystallized first from methanol and subsequently from carbon disulphide, the pigment being obtained as orange-red crystals, m.p. 194°; absorption maxima in carbon disulphide 511, 478, 446 m μ , in chloroform 487, 456, 427 m μ . The solution in concentrated acetic acid developed a yellow colour and in dichloroacetic acid the pigment dissolved to a greenish solution. This pigment is therefore lutein. The third pigmented zone at the base of the column was spectroscopically identical with the pigment contained in the bottom zone of the original chromatogram, to which it was therefore added.

BOTTOM ZONE. The eluate from this, which contained very little colouring matter, was readsorbed on calcium carbonate with the result that a separation from a further quantity of lutein was effected, but the quantity of pigment obtained was not sufficient for the isolation of any colouring matter; absorption maxima in carbon disulphide 516, 483, 451 m μ . It was probably zeaxanthin.

Extract A. The light-petroleum extract was washed free from methanol, dried over anhydrous sodium sulphate and adsorbed on aluminium oxide (Brockmann), the chromatogram being developed with light petroleum (b.p. 40–60°). The deep orange-red zone at the top of the column was eluted with benzene-ethanol and the solution concentrated to small bulk. After standing in the ice-chest for 24 hr. the precipitated pigment was repeatedly extracted with boiling ethanol to remove colourless impurities. Recrystallization of the residue from benzene gave reddish metallic-glancing prisms, m.p. 184°; absorption maxima in CS₂, 520, 484, 452 m μ and in petroleum ether, 485, 452, 424 m μ . This pigment is therefore β -carotene.

Ether extract. The deep red solution, obtained by extraction with diethyl ether of the methanolic mother liquors remaining after precipitation of the pigments by water, was washed free of alkali, dried over anhydrous sodium sulphate and the solvent removed *in vacuo*. The residual dark red oil product was dissolved in benzene and adsorbed on aluminium oxide, the chromatogram being developed with a 1:1 mixture of benzene and chloroform and finally with pure chloroform. The chromatogram contained two orange-red bands on top and a reddish diffuse band underneath. This latter band had no distinct absorption maxima and was not further examined. The top band, on elution, gave a dark red waxy solid; it showed absorption maxima in CS₂ at 497, 466, 437 m μ , in close agreement with the spectroscopic data for the unidentified lipochrome pigment isolated from *Viola tricolor* by Kuhn & Winterstein (1931).

The middle band of the above chromatogram had absorption maxima in CS₂ at 502, 470, 440 m μ . The pigment from this was dissolved in carbon disulphide and readsorbed on calcium carbonate. It gave two yellow bands very near the top of the column, the upper of which had absorption maxima in CS₂ at 497, 466, 437 m μ (identical with those of the waxy material above). The second and main band had absorption maxima at 501, 469, 440 m μ . The eluate from this gave the characteristic violaxanthin reaction with 25 % hydrochloric acid.

SUMMARY

1. *Iris pseudacorus* has been examined and found to contain β -carotene and the xanthophylls violaxanthin and lutein.

2. A third xanthophyll, present in minute quantities, was spectroscopically identical with zeaxanthin.

3. In addition an uncrystallizable waxy carotenoid was present, similar in properties to that isolated by Kuhn & Winterstein (1931) from *Viola tricolor*.

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Addendum to paper by C. R. Harington & Rosalind V. Pitt Rivers (see p. 164)

The following information about the hydrates of *l*(+)-di-iodotyrosine is worth recording. Di-iodotyrosine crystallizes from water, either by separation from the hot solution or by neutralization of a solution in aqueous acid as the dihydrate which forms glistening plates, which retain their water of crystallization on air-drying. (Found: I, 53.9%; calc. for C₉H₉O₃NI₂·2H₂O: I, 54.1%.)

On drying the above dihydrate over P₂O₅ *in vacuo* the water of crystallization is lost. (Found: I, 58.0%; calc. for C₉H₉O₃NI₂: I, 58.6%.)

Separation of di-iodotyrosine by evaporation of an ammoniacal solution at a bath temperature $\pm$ 40° yields long hair-like needles of the monohydrate. (Found: I, 56.1%; calc. for C₉H₉O₃NI₂·H₂O: I, 56.3%.)

When di-iodotyrosine separates slowly from 60 % acetic acid it forms large prisms, containing one molecule of acetic acid, which are stable to desiccation *in vacuo* over P₂O₅. (Found: I, 51.5%; calc. for C₉H₉O₃NI₂·C₂H₄O₂: I, 51.5%.) When the separation is rapid the form obtained is the monohydrate described above.