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PIGMENTS OF CENTROSPERMAE—III.
BETAXANTHINS FROM *BETA VULGARIS* L.

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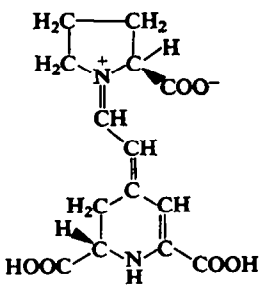
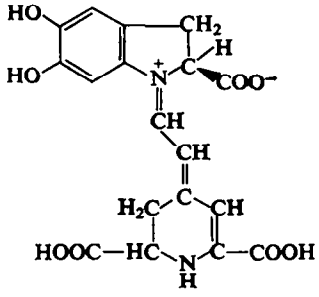
Abstract—Two betaxanthins (vulgaxanthin-I and vulgaxanthin-II) have been isolated from beetroot (*Beta vulgaris* L.). From consideration of physical data and chemical properties, structures (III) and (IV) are proposed for these two pigments.

BETAXANTHINS, originally called flavocyanins, are yellow pigments occurring in plants belonging to the order Centrospermae. They have properties in common with the betacyanins, the red–violet nitrogenous pigments whose distribution in nature is restricted to the same plant order.[1–4] Both are insoluble in organic solvents and, in electrophoresis, migrate as anions even at pH's as low as 2·4. Thanks to these characteristics they can easily be differentiated from the common flavonoid pigments. Chemical investigation of the pigments of the Centrospermae has hitherto been focused on the betacyanins. It is only lately that the isolation and structural determination of indicaxanthin, a betaxanthin from *Opuntia ficus-indica* fruits, has been reported.[5] Its structure (I) is closely related to that of betanidin (II),[6] which, on the basis of what is known to date, is held to be the aglycone of all the betacyanins.[7,8]



(I)



(II)

In pursuing our research on the Centrospermae pigments, we have studied the betaxanthins in red beets. This vegetable material contains a complex mixture of pigments, of which

[1] H. REZNIK, *Z. Botan.* **43**, 499 (1955).
[2] H. REZNIK, *Planta* **49**, 406 (1957).
[3] T. J. MABRY, A. TAYLOR and B. L. TURNER, *Phytochem.* **2**, 61 (1963).
[4] W. RAUH and H. REZNIK, *Botan. Jaarb.* **81**, 95 (1961).
[5] M. PIATTELLI, L. MINALE and G. PROTA, *Tetrahedron* **20**, 2325 (1964).
[6] H. WYLER, T. J. MABRY and A. S. DREIDING, *Helv. Chim. Acta* **46**, 1745 (1963).
[7] H. WYLER and A. S. DREIDING, *Experientia* **17**, 23 (1961).
[8] M. PIATTELLI and L. MINALE, *Phytochem.* **3**, 547 (1964).

betanin, a glucoside of betanidin, is the main component. Besides other betacyanins, several yellow pigments are present, the number of which—according to Reznik[2]—depends on the variety examined. In the one we studied ("piatta d'Egitto") at least six betaxanthins were found, all of them in minute amounts.

In the present paper we report the isolation and structural elucidation of the two betaxanthins present in somewhat greater amounts, which we have named vulgaxanthin-I and vulgaxanthin-II. They were isolated by a method similar to the one we used for indicaxanthin.[5] However, because of the greater complexity of the pigment mixture present in beets, it proved necessary to adopt experimental conditions which gave rise to considerable losses, due mainly to the use of eluents of lower pH and to the longer time required for the procedure. The method is based on the non-ionic absorption of betacyanins and betaxanthins onto cation exchange resin, followed by elution with water. The resulting mixture of pigments was separated by chromatography on polyamide powder, using a citrate buffer

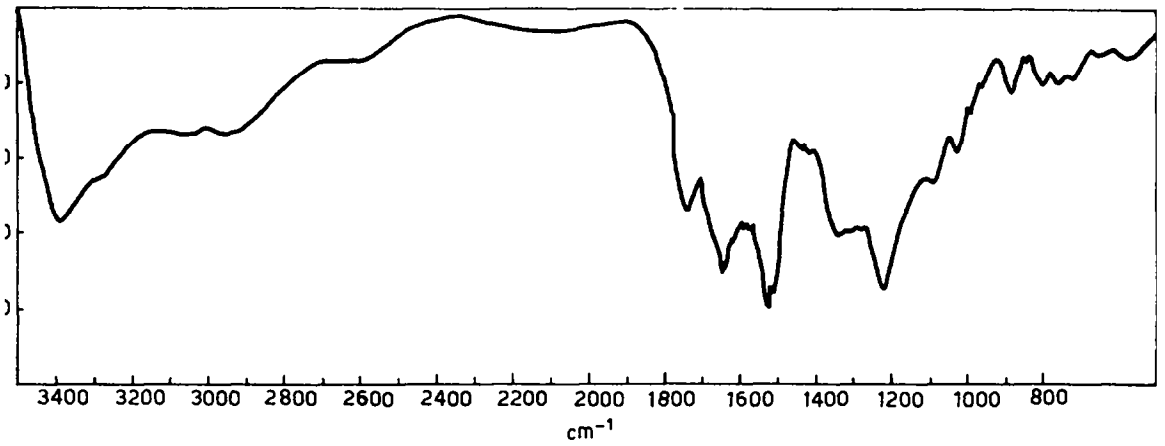


FIG. 1. THE I.R. SPECTRUM OF VULGAXANTHIN-I.

(pH 4) as eluent. Under these conditions three fractions containing betaxanthins were obtained. The first fraction, which in paper electrophoresis showed the presence of three pigments, was not present in sufficient amount to allow further purification. From the second fraction a small quantity of vulgaxanthin-I was obtained in crystalline form. The third fraction yielded vulgaxanthin-II in the amorphous state after separation by preparative paper electrophoresis from another yellow pigment present in a lesser amount. On account of the small quantities obtained, no attempt was made at crystallization of vulgaxanthin-II. The i.r. spectrum of vulgaxanthin-I (Fig. 1) is similar to that of indicaxanthin,[5] but, beyond this fact in itself, no further useful information is to be had from it. Since the u.v. spectra of vulgaxanthins are very similar (Fig. 2) to that of indicaxanthin, it was deduced that the same chromophore must be present in all these pigments. Furthermore, just as indicaxanthin by alkaline fusion yielded 4-methylpyridine-2,6-dicarboxylic acid and an amino acid (proline), both vulgaxanthins yielded 4-methylpyridine-2,6-dicarboxylic acid and an amino acid (glutamic acid). These results indicate that the structures of the two vulgaxanthins are closely related to that of indicaxanthin, the only difference being due to the nature of the amino acid bound to the dihydropyridine moiety. On hydrolytic fission in hot acid, glutamic acid was produced from both vulgaxanthins. The difference between the two pigments was

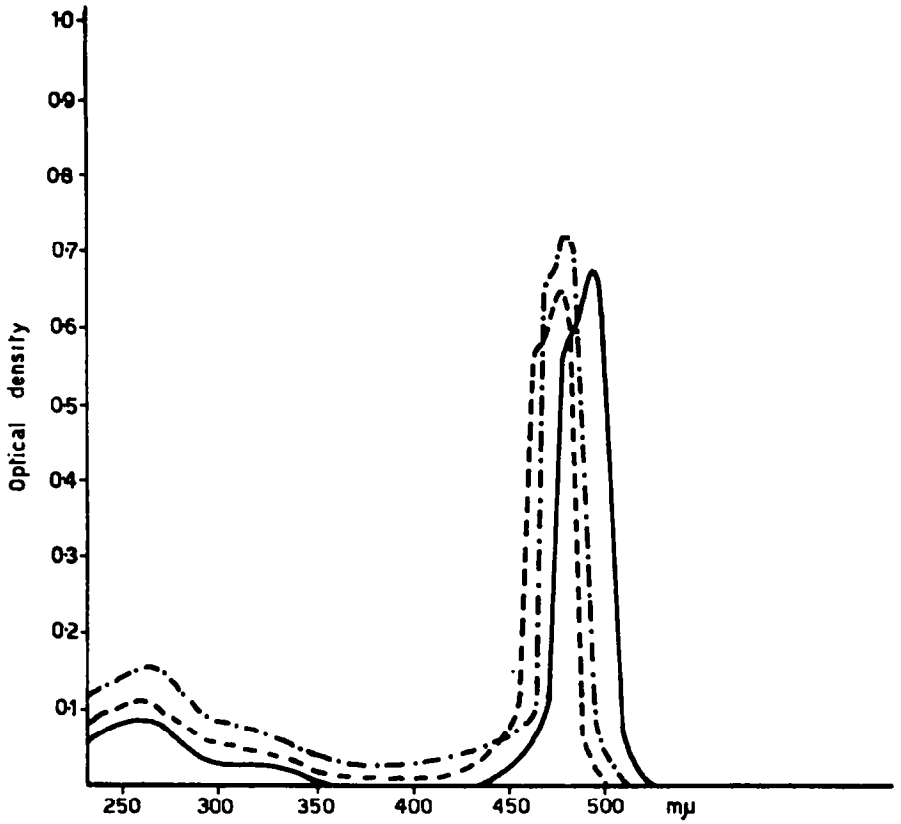
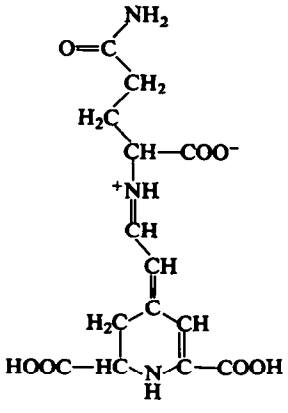
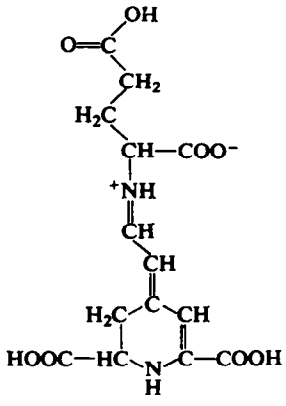


FIG. 2. THE U.V. SPECTRA OF INDICAXANTHIN (——), VULGAXANTHIN-I (-------) AND VULGAXANTHIN-II (-·-·-·-·-·-·-).

brought out by acid degradation under as mild conditions as possible, whereupon vulgaxanthin-I gave glutamine and vulgaxanthin-II yielded glutamic acid. Formulas (III) and (IV) account for all the above data on vulgaxanthin-I and vulgaxanthin-II, respectively. The yield of the two pigments was too small, however, for further experiments to be carried



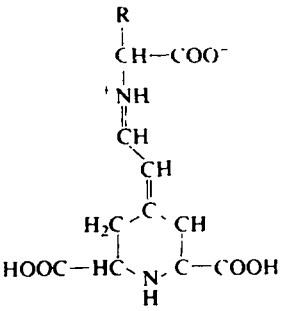
(III)



(IV)

out in order to confirm the above two structures and determine the configuration of their asymmetric centres. It is to be hoped that a systematic research on the distribution of the betaxanthins in Centrospermae may furnish some clues toward a more suitable source.

The most striking feature of these structures is the cyanine chromophore which is reminiscent of the similar system in betanidin (II) and indicaxanthin (I). Therefore, betacyanins and betaxanthins very likely belong to a single class of naturally occurring pigments, closely related, which can be represented by the expression (V).



(V)

EXPERIMENTAL

The i.r. spectra were taken on a Beckmann IR 9 instrument in KBr pellets and the u.v. spectra on a Unicam SP 500 spectrophotometer. Chromatograms were carried out on Whatman No. 1 paper (descending technique) and on silica gel thin-layer plates. The solvent systems used, prepared on a v/v basis, are as follows: BAW, *n*-butanol:acetic acid:water (12:3:5); EAW, ethanol:33% ammonia:water (20:1:4); MP, methanol:water:pyridine (20:5:1); PW, phenol:water (4:1); CMA, chloroform:methanol:17% ammonia (2:2:1). Electrophoretograms were run on Whatman No. 1 paper for about 1 hr at 16 V cm in a horizontal apparatus in phosphate buffer 0·05 M (pH = 6·8). The amino acids were detected on air-dried chromatograms by ninhydrin. Tentative identification of degradation products was always substantiated by co-chromatography with authentic samples.

Isolation of Vulgaxanthin-I and Vulgaxanthin-II

Red beets (cultivar "piatta d'Egitto") (1 kg) were macerated in a blendor with ice-water (1·5 l.). The homogenate was filtered through cheese-cloth and the residue re-extracted with water. The combined extracts, acidified to pH 3 with N HCl and clarified by centrifuging, were passed through a column, kept at 5°, of Dowex 50W-X2 (25 × 5 cm; H^+ form). In these conditions, betacyanins and betaxanthins were absorbed by the resin non-ionically. After washing with 0·1% HCl (1·6 l.), they were eluted with water. The eluate (4·5 l.) was concentrated under reduced pressure at 30° (bath temperature) to a volume of 150 ml. The concentrated solution was applied on top of a column (35 × 5 cm), cooled to 5°, of polyamide powder (polyexamethyleneadipamide). Development of the column with a 50:50 (v/v) mixture of 0·2% citric acid and 0·2% sodium citrate in water yielded three fractions, A, B and C, which emerged from the column after about 0·5 l., 0·9 l. and 1·25 l. respectively.* The fractions, adjusted to pH 3 with N HCl were freed from the buffer by resin treatment.

* The betacyanins had a much greater retention volume than the betaxanthins.

Fraction A, in paper electrophoresis, showed the presence of three yellow substances E_l 0·67 (main component), 0·94 and 1·20 respectively.† This fraction was investigated no further due to its small quantity.

Fraction B, containing mainly a yellow pigment E_l 0·94, was concentrated to a volume of 2 ml and allowed to stand overnight at 4°. The crystals formed were filtered off, washed with cold water (0·5 ml) and dried to constant weight (2·1 mg) *in vacuo* over P_2O_5 (λ_{max} 477 mμ, $E_{1\%}^{1cm} = 750$). The mother liquor, evaporated to dryness, gave 8·4 mg of crude vulgaxanthin-I.

Paper electrophoresis of fraction C showed the presence of two yellow pigments E_l 0·91 and 1·20 respectively. By preparative electrophoresis the two pigments were separated and the fraction containing the main pigment (E_l 1·20) was desalted by resin treatment. The solution was taken to dryness under reduced pressure thus obtaining 0·95 mg of amorphous vulgaxanthin-II (λ_{max} 478 mμ). On account of the small quantity of the pigment obtained, crystallization could not be achieved. The minor pigment of fraction C (E_l 0·91) was not investigated any further since it was present in minute amounts.

Alkaline Fusion of Vulgaxanthin-I and Vulgaxanthin-II

Vulgaxanthin-I (7 mg) was added in a stream of nitrogen to a boiling mixture of NaOH (0·25 g) and water (0·1 ml). Just after addition of the pigment, the mixture was cooled and dissolved in water (2 ml). The solution, freed from alkali by passing through a column of Amberlite IRC-50 (H^+ form), was extracted with ether. In the aqueous layer, glutamic acid was identified by paper chromatography in the solvents BAW, EAW, MP and PW and by paper electrophoresis. The ether extract, evaporated to dryness, yielded a residue in which 4-methylpyridine-2,6-dicarboxylic acid was identified by paper chromatography in the solvents BAW, EAW, MP and by paper electrophoresis (spray reagent: $FeSO_4$ 5% aq. solution).

Vulgaxanthin-II (3·5 mg), when degraded with alkali in the experimental conditions as above, yielded glutamic acid and 4-methylpyridine-2,6-dicarboxylic acid.

Degradation of Vulgaxanthin-I and Vulgaxanthin-II with Hot Acid

A solution of vulgaxanthin-I (3 mg) in N HCl (5 ml) was refluxed for 30 min and then evaporated to dryness under reduced pressure. Glutamic acid was identified in the residue by paper chromatography in the solvent systems BAW, EAW, MP, PW and thin-layer chromatography on silica gel (solvent systems PW and CMA alone and in combination). Glutamic acid was also obtained from vulgaxanthin-II, degraded with acid as above.

Degradation of Vulgaxanthin-I and Vulgaxanthin-II with Cold Acid

A solution of vulgaxanthin-I (3 mg) in N HCl (5 ml) was allowed to stand at room temperature for 24 hr and then evaporated to dryness *in vacuo* at a temperature no higher than 30°. The residue was taken up in water and chromatographed as before. Glutamine and glutamic acid were identified as breakdown products of vulgaxanthin-I and vulgaxanthin-II respectively.

†E_l equals migration on paper electrophoresis relative to indicaxanthin.