Phytochemistry, 1965, Vol. 4, pp. 817 to 823. Pergamon Press Ltd. Printed in England

PIGMENTS OF CENTROSPERMAE—V.
BETAXANTHINS FROM *MIRABILIS JALAPA* L.

M. PIATTELLI, L. MINALE and R. A. NICOLAUS

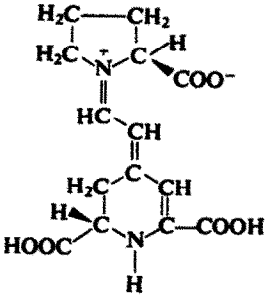
Centro Nazionale di Chimica delle Sostanze Organiche Naturali del C.N.R., sezione III.
Istituto di Chimica Organica dell'Università di Napoli, Italy

(*Received* 17 *May* 1965)

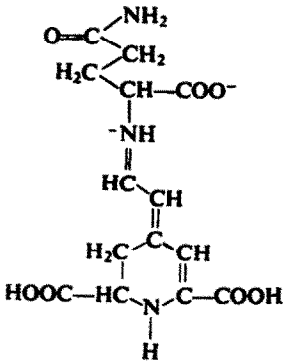
Abstract—Eight betaxanthins have been isolated from *Mirabilis jalapa* flowers. Two of them were identified as indicaxanthin (I) and vulgaxanthin-I (II), respectively. Structures IV–VII were assigned to four other pigments (miraxanthin-I, -II, -III and -V). The remaining two compounds (miraxanthin-IV and -VI) were not completely characterized. The synthesis of betaxanthins from betanin is also described.

INTRODUCTION

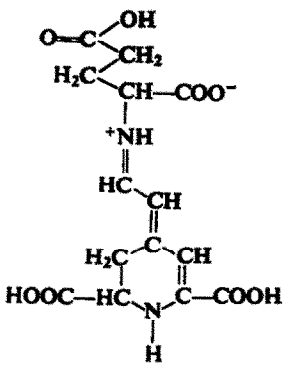
PREVIOUS work on the nitrogenous yellow pigments of Centrospermae (betaxanthins) led to the isolation and characterization of three substances: indicaxanthin (I) from *Opuntia ficus-indica*,[1] vulgaxanthin-I (II) and vulgaxanthin-II (III) from *Beta vulgaris*.[2]



(I)



(II)



(III)

This paper deals with the isolation and characterization of betaxanthins present in the flowers of *Mirabilis jalapa*.

RESULTS

Nine pigments have been isolated from the flowers of *Mirabilis jalapa*. Due to the great sensitivity of these pigments to chemical reagents and due to the numerous steps of chromatographic and electrophoretic separation necessary to isolate them, they could not be obtained in more than milligram amounts. The electrophoretic mobilities and the spectral and chromatographic properties of these compounds are listed in Table 1.

[1] M. PIATTELLI, L. MINALE and G. PROTA, *Tetrahedron* **20**, 2325 (1964).
[2] M. PIATTELLI, L. MINALE and G. PROTA, *Phytochem.* **4**, 121 (1965).

The characterization of one of these pigments (A3), having spectral properties quite different from the others, is still in progress. Of the other eight pigments, two (A1.1 and A1.3) are known compounds, viz., indicaxanthin (I) and vulgaxanthin-I (II). Identification was based on direct comparison with authentic samples by absorption spectra, electrophoretic and chromatographic behaviour, and on degradation studies.

TABLE 1. PROPERTIES OF THE PIGMENTS OF *Mirabilis jalapa* FLOWERS

Pigment	$\lambda_{max.}$ in water (mμ)		R_f		E_i‡	
	Alone	+HCl conc. (1 drop/3 ml)	EA*	EW†	pH 6·8§	pH 8·6**
A1.1 (indicaxanthin)	484·5 (s 464)	471	0·17	—	1·00	1·00
A1.2 (miraxanthin-I)	475 (s 462)	465	0·10	—	0·96	—
A1.3 (vulgaxanthin-I)	477 (s 462)	465	0·08	—	0·96	—
A2 (miraxanthin-II)	477 (s 462)	467	—	—	1·20	—
A3	505	—	—	—	0·47	0·50
B1 (miraxanthin-III)	473·5 (s 458)	458·5	—	0·50	0·61	0·54
B2 (miraxanthin-IV)	473·5 (s 458)	458·5	—	0·48	0·61	0·54
B3 (miraxanthin-V)	475·5 (s 458)	458·5	—	0·46	0·61	0·84
B4 (miraxanthin-VI)	477	467	—	0·42	0·61	0·84

* Ethanol:0·3% aq. sodium acetate (4:1). † Ethanol:water (4:1). ‡ Migration in paper electrophoresis relative to indicaxanthin. § 0·05 M phosphate buffer. ** 0·2 M borate buffer.

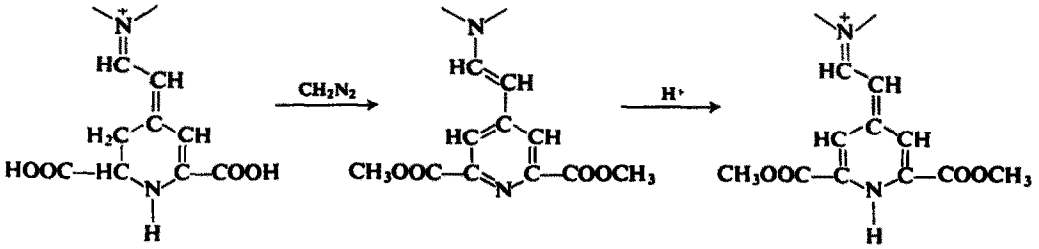
The other six pigments, all new compounds, have similar spectral properties in neutral and acidic solution to the known betaxanthins.[2] Thus, the same chromophore must be present in all these pigments; furthermore, on alkaline fusion all of them yielded 4-methylpyridine-2,6-dicarboxylic acid, which was similarly obtained from indicaxanthin and

TABLE 2. SPECTRAL PROPERTIES OF METHYLATED PIGMENTS

Methylation products of pigments	$\lambda_{max.}$ in methanol (mμ)	
	Alone	+HCl conc. (1 drop/3 ml)
A1.1	361	437
A1.2	359	435
A1.3	359	433
A2	359	433
B1	355	433
B2	355	433
B3	363	437
B4	345	441

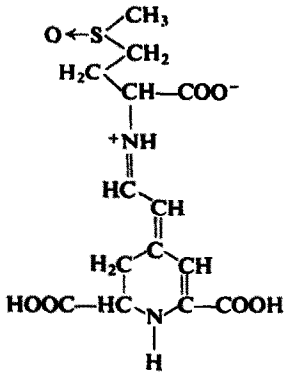
vulgaxanthin-I and -II.[1,2] It follows that all these compounds must have the same dihydropyridine moiety. The presence of the nitrogenous polymethine chromophore in all these pigments was confirmed by the observation that methylation with diazomethane gave derivatives with absorption maxima at 340–360 mμ, which in acidic solution exhibit batho-

chromic shifts of 80–100 mμ (see Table 2). Such shifts are also shown by the derivatives of neobetanidin, obtained by treatment of betanidin derivatives with diazomethane.[3] Scheme I shows the changes in the chromophore following reaction with diazomethane and subsequent protonation.

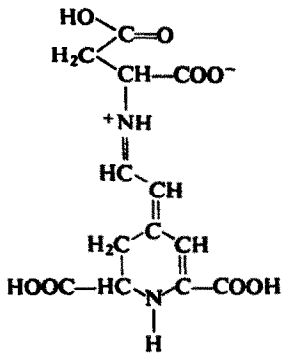


SCHEME I.

A1.2 is a tricarboxylic acid, since methylation, with methanol catalyzed by boron fluoride, yields a monomethyl, a dimethyl and finally a trimethyl ester. By acid hydrolysis, methionine sulphoxide was obtained, hence structure IV can be assigned to pigment A1.2 (miraxanthin-I).



(IV)



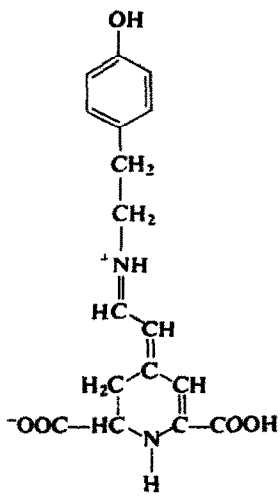
(V)

A2, which on paper electrophoresis (phosphate buffer, pH 6·8) has a similar mobility to the tetracarboxylic acid vulgaxanthin-II (III), yielded aspartic acid upon acid hydrolysis; hence, structure V can be assigned to pigment A2 (miraxanthin-II).

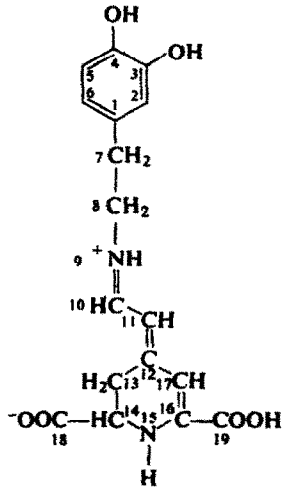
When B1, B2, B3 and B4, isolated as the hydrochlorides, were methylated with methanol (boron fluoride as catalyst), monomethyl and, subsequently, dimethyl esters were formed. Therefore, in each of these pigments, only two carboxyl groups are present so that the dihydropyridine moiety cannot be bound to an amino acid, as in the betaxanthins hitherto described. As expected, these pigments failed to give any amino acid by acid hydrolysis. B1 (miraxanthin-III) yielded tyramine, and B3 (miraxanthin-V) dopamine; thus structures VI and VII respectively were assigned to these pigments.* Structure VII, proposed for miraxanthin-V, which was the only pigment obtained in sufficient amount for further investigation, was confirmed by its NMR spectrum (Table 3).

* An examination of the phenols occurring in the flowers of *Mirabilis jalapa* led to the identification by chromatography and electrophoresis of both tyramine and dopamine.

[3] T. J. MABRY, H. WYLER, G. SASSU, M. MERCIER, I. PARIKH and A. S. DREIDING, *Helv. Chim. Acta* **45**, 640 (1962).



(VI)



(VII)

B2 (miraxanthin-IV) on acid hydrolysis yielded a basic substance, not yet identified, which couples with diazotized sulphanilic acid and shows a chromatographic and electrophoretic behaviour very similar to that of tyramine.

B4 (miraxanthin-VI), which from its electrophoretic mobility in borate buffer seems to be an *o*-dihydric phenol, yielded on hydrolysis a basic substance (as yet unidentified) which couples with diazotized sulphanilic acid and gives a green colour with ferric chloride.

TABLE 3. ASSIGNMENT OF NMR SIGNALS OF MIRAXANTHIN-V

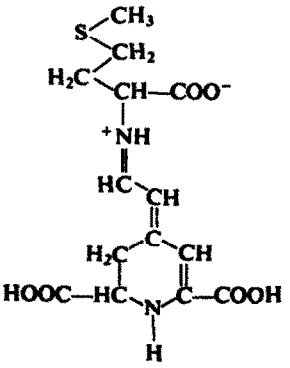
τ	Multiplicity	Assignment
1·20	Doublet (J 13)	H at C-10
3·03	Multiplet	3H at C-2, C-5 and C-6
3·50	Singlet	H at C-17
3·65	Doublet (J 13)	H at C-11
5·42	Multiplet	H at C-14
5·92	Multiplet	2H at C-8
6·88	Multiplet	4H at C-7 and C-13

The structures of all betaxanthins described in the present paper were confirmed by synthesis. Since all the nitrogenous pigments of the Centrospermae so far characterized possess the same dihydropyridine fragment, their interconversion by exchanging the amino acid (or amine) moiety was studied and the readily available betanin was used as a convenient starting material. Betanin was dissolved in water and the solution saturated with sulphur dioxide; when the violet colour completely disappeared, sulphur dioxide was removed *in vacuo* and an excess of the requisite amino acid (or amine) was added and the solution adjusted to pH 9. The resulting betaxanthin was isolated and its electrophoretic, chromatographic and spectral properties determined.*† All the synthetic pigments were shown to be identical to the corresponding natural compounds.

* Further investigation on the interconversion of the nitrogenous pigments of Centrospermae is in progress.

† Recently, Wyler, Wilcox and Dreiding synthesized indicaxanthin from betanin using different experimental conditions. (*Helv. Chim. Acta* **48**, 361 (1965).)

As methionine is readily oxidized to methionine sulphoxide, it was thought that methionine sulphoxide obtained by acid hydrolysis of miraxanthin-I (IV) might be an artifact, and that the real structure was (VIII). This possibility was ruled out by synthesizing the betaxanthin VIII, which separated from miraxanthin-I on paper chromatography.



(VIII)

EXPERIMENTAL

Ultra-violet spectra were measured with a Beckmann DB spectrophotometer. The NMR spectrum of miraxanthin-V was recorded in CF_3COOH immediately after preparation of the solution (15% w/v) with tetramethylsilane as internal reference, on a Varian A–60 spectrometer. Paper chromatography was carried out on Whatman No. 1 paper by the descending technique in the following solvent systems: BAW, *n*-butanol:acetic acid:water (12:3:5); EAW, ethanol:33% ammonia:water (20:1:4); MP, methanol:water:pyridine (20:5:1); EW, ethanol:water (4:1); EA, ethanol:0·3% aq. sodium acetate (4:1). Thin layer chromatographic analyses were carried out with silica-gel plates using the following solvent systems: PW, phenol:water (4:1); CMA, chloroform:methanol:17% ammonia (2:2:1); BuHCl, *n*-butanol:HCl conc. (9:1). Electrophoretograms were run on Whatman No. 1 paper for about 1 hr at 16 V/cm in a horizontal apparatus in the following electrolytes: A, phosphate buffer 0·05 M (pH 6·8); B, borate buffer 0·2 M (pH 8·6). The following sprays were used: 1, ninhydrin (0·2% solution in acetone); 2, isatin (0·2% solution in acetone); 3, diazotized sulphanilic acid followed by N NaOH; 4, ferric chloride (3% solution in ethanol). Tentative identifications of degradation products were always substantiated by co-chromatography and co-electrophoresis with authentic samples.

Extraction and Isolation of Pigments

Fresh petals of yellow flowers of *Mirabilis jalapa* (3 kg) were macerated in 500-g batches in a blender with 5 l. cold methanol. The macerate was filtered through several layers of cheese-cloth and the residue was re-extracted with a 3:1 (v/v) methanol–water mixture (4 l.). The combined extracts after centrifuging were concentrated *in vacuo* to 700 ml. The solution, after standing overnight at 4°, was again centrifuged and the supernatant concentrated *in vacuo* and finally absorbed onto Whatman cellulose powder. This powder, dried in a vacuum desiccator over P_2O_5, was placed on top of a cellulose column (5 × 60 cm) and then eluted with methanol. After removing the first fraction (2 l.) containing a number of phenolic

compounds,* the yellow pigments were collected in about 5 l. of eluate. This fraction was evaporated to dryness under reduced pressure. The residue was taken up in water (50 ml) and applied on top of a column of polyamide powder (4·2 × 50 cm), cooled to 5°. Elution with water gave a fraction (A) of yellow pigments with a retention volume of 600 ml. Subsequent elution with 0·15% aq. sodium acetate gave a second fraction of yellow pigments (B) which emerged from the column after 1·2 l. Fraction A was adjusted to pH 3 with N HCl and the pigments were absorbed onto a column of Dowex 50W–X2 (H^+ form, 3 × 20 cm), kept at 5°. The column was washed with 0·1% HCl (2 l.) and the washing discarded. The betaxanthins were eluted with water and the eluate (3 l.) was taken to dryness under reduced pressure at 30° (bath temp.), giving 150 mg of residue. A 100-mg portion of this residue was taken up in water (20 ml) and chromatographed on polyamide (5 × 30 cm). Development with 0·015% aq. sodium acetate produced two fractions (A1 and A2), which emerged from the column after about 1·5 l. and 1·9 l., respectively. The resolved bands, adjusted to pH 3 with N HCl, were desalted with resin. Further separation of fraction A1 was accomplished by chromatography on a column of cellulose powder (4 × 30 cm), using a 4:1 (v/v) mixture of ethanol–0·3% aq. sodium acetate. Three yellow fractions (A1.1, A1.2 and A1.3) emerged from the column after 660, 960 and 1280 ml respectively. The resolved bands were freed from sodium acetate by resin treatment and the eluates taken to dryness under reduced pressure at 30° to give 25·6 mg of A1.1, 9·6 mg of A1.2 and 7·2 mg of A1.3. Fraction A2, homogeneous in paper electrophoresis and paper chromatography, after evaporating to dryness *in vacuo* weighed 10·8 mg. During the chromatography of fraction A on polyamide powder a labile red pigment was observed, which was completely destroyed before reaching the bottom of the column. This pigment (A3) was isolated from the remaining 50-mg portion of fraction A by chromatography on Whatman 3 MM paper using a 4:1 (v/v) mixture of ethanol–water as the developing solvent.

Fraction B was passed through a column of Amberlite IRC–50 (H^+ form). The eluate was evaporated to about 5 ml and streaked on sheets of Whatman 3 MM paper, which were then developed in 4:1 (v/v) mixture of ethanol–water (time of flow: 36 hr). Four yellow bands were clearly visible with R_f values of 0·50 (B1), 0·48 (B2), 0·46 (B3), and 0·42 (B4). The bands were cut out, eluted with water and the eluates evaporated to dryness. The separated bands were further purified by preparative paper electrophoresis (electrolyte A) in order to remove minor components. The main bands were cut out and eluted with water. The eluates were taken to dryness, the residues were dissolved in methanol containing 1% HCl and to the filtered solutions ether was added. The precipitates were collected and dried; the yields were 8·5 mg of B1, 11·2 mg of B2, 98·5 mg of B3 and 23·5 mg of B4.

Authentic Pigments

Indicaxanthin and vulgaxanthin-I were obtained from *Opuntia ficus-indica* and *Beta vulgaris*, respectively, as previously reported.[1,2]

Alkaline Fusion of the Pigments

Each pigment (5 mg) was added to 5 ml of a 50% (w/w) aq. KOH under nitrogen and after 5 min refluxing, the solution was cooled, acidified and continuously extracted with ether. The ether extract was concentrated to a residue which was dissolved in 0·1 M ammonia and the resulting solution was analyzed for 4-methylpyridine-2,6-dicarboxylic acid by paper

* Tyramine and dopamine were identified in this eluate, on the basis of R_f values, electrophoretic mobilities and co-chromatography with authentic samples.

chromatography (solvent systems: BAW and EAW) and paper electrophoresis (electrolyte A) (spray reagent: 5% aq. $FeSO_4$).

Esterification of the Pigments with Methanol

Pigment (0·5 mg) was dissolved in methanol (5 ml) and boron fluoride etherate (3 drops) was added. Samples were examined by paper electrophoresis (electrolyte A) at 12-hr intervals. The successive formation of three yellow compounds with E_f* values 0·68, 0·35 and −0·67 respectively was observed during the esterification of A1.2. Esterification of B1–B4 proceeded with the initial formation of a yellow compound (E_f value for all four pigments 0·35), followed by the formation of a second yellow pigment with E_f −0·67 (also in this case the E_f value is identical for all pigments).

Diazomethane Methylation of the Pigments

To the pigment (0·2 mg) dissolved in methanol (3 ml) diazomethane was added. After 1 hr the solution was evaporated to dryness. The spectral properties of the methylated pigments are reported in Table 2.

Degradation of the Pigments with Acid

The pigments, except A3, were degraded with N HCl (1 mg in 10 ml of HCl) at 35°. The pigments A1.1, A1.2, A1.3 and A2 were completely degraded after 24 hr; B1–B4 required 5 days' treatment. At the end of the reaction the solution was evaporated to dryness under reduced pressure, excess HCl was removed and the residue was taken up in water (0·1 ml). The amino acids or amines present were identified by paper chromatography in BAW, EAW and MP, by thin layer chromatography in PW, CMA and BuHCl and by paper electrophoresis in electrolytes A and B.

Synthesis of Betaxanthins

Betanin (5 mg) was dissolved in water (10 ml) and the solution saturated with sulphur dioxide. After 5 hr the colourless solution was concentrated *in vacuo* to 0·1 ml and L-proline (20 mg), dissolved in water (3 ml), was added. The mixture was adjusted to pH 9 by adding 1 N NH_4OH. After standing 1 hr at room temperature, the yellow solution was adjusted to pH 3 with 1 N HCl and passed through a column of Dowex 50W–X2. After washing with 0·1% HCl (50 ml) the indicaxanthin was eluted with water and the eluate evaporated to dryness (0·9 mg).

Analogous procedures were used for the synthesis of the other betaxanthins. Miraxanthin-III and -V, which were not eluted with water from the Dowex column, were recovered by elution with 1% aq. sodium acetate. The eluates were desalted by passing through a column of Amberlite IRC-50 and evaporating to dryness *in vacuo*. The spectral, chromatographic and electrophoretic properties of the synthetic pigments were identical to those of the corresponding natural betaxanthins.

Acknowledgement—The NMR spectra were performed at the Institute of Organic Chemistry of University of Rome; we are very grateful to Professor L. Panizzi for facilities put at our disposal.

* E_f equals migration on paper electrophoresis relative to indicaxanthin.