Phytochemistry, Vol. 24, No. 7, pp 1475–1477, 1985.
Printed in Great Britain

0031–9422/85 $3.00 + 0.00

CHANGED BETAXANTHIN PATTERN IN VIOLET FLOWERS OF *PORTULACA GRANDIFLORA* AFTER THE FEEDING OF DOPA

ERIKA RINK and HARTMUT BÖHM

Institut für Biochemie der Pflanzen der Akademie der Wissenschaften der DDR, 4010 Halle (Saale), Weinberg 3, G.D.R.

(*Received* 8 *October* 1984)

Key Word Index—*Portulaca grandiflora*; Portulacaceae; betalains; betaxanthins; L-3,4-dihydroxyphenylalanine.

Abstract—The administration of DOPA to violet flowers of *Portulaca grandiflora* led to the biosynthesis of betaxanthins not present in untreated plant material. Among them vulgaxanthin II but no dopaxanthin could be identified. DOPA serves as a precursor of the dihydropyridine moiety of the new betaxanthins. Its role as an elicitor of betaxanthin biosynthesis is discussed.

INTRODUCTION

The seedlings of *Amaranthus caudatus* are used widely in studies of the regulation of betalain metabolism [1, 2]. As a result, the regulation of betaxanthin metabolism has been neglected because *A. caudatus* plants normally form betacyanins only [3].

In future experiments in this field, it appears important to investigate also a plant species that synthesizes betacyanins (**2** in Fig. 1) as well as betaxanthins (**3** in Fig. 1). *Portulaca grandiflora* may be a candidate since it produces both pigment types characteristic of the order Centrospermae [4, 5] and is suitable as experimental material [6, 7].

The small number of regulatory investigations with *A. caudatus* seedlings which were not confined to the betacyanins, however, led to a surprising result since, besides betacyanins, betaxanthins were produced, under the influence of external factors or agents such as intense light and DOPA [8–11]. As this phenomenon needed to be studied in more detail, we have examined the response of violet *P. grandiflora* flowers to DOPA feeding.

RESULTS AND DISCUSSION

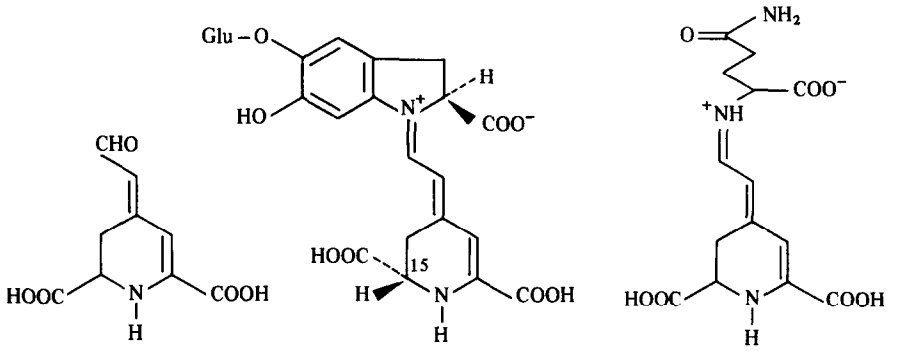
Untreated violet flowers of *P. grandiflora* line H [7] exhibit a white colour in the basal parts of their petals. After the application of a substantial amount of DOPA (0.75 mg) to a stem tip with a flower bud, the bottom of the perianth shows a golden yellow pigmentation extending into the violet areas. This can be seen even before anthesis. Crude extracts from DOPA-fed petals are brick-red in colour in contrast to the violet extracts of untreated flowers. This difference is caused by a qualitative change of the betalain composition of flowers fed DOPA due to the synthesis of additional betaxanthins.

After DOPA application, the separation of a petal extract by DEAE–Sephadex A-25 CC [12] yields betalain fractions in the following order: (a) a very small amount of betaxanthin; (b) the feeding-depending betaxanthin; (c) the main betacyanin; (d) betaxanthin; and (e) a very small amount of betacyanin. In untreated flowers as well as after the feeding of ascorbic acid, only fraction b is absent. Betalamic acid (**1** in Fig. 1) is never detectable.

The question now arose as to whether or not DOPA acted as a precursor and/or as an elicitor of betaxanthin biosynthesis. On feeding L-3,4-dihydroxyphenyl[3-^{14}C]alanine, a significant amount of radioactivity was detectable in fraction b (Fig. 2). After alkaline hydrolysis [13] of this fraction and subsequent CC [12, 14], radioactively labelled betalamic acid was identified. The amino acid portion could not be characterized by this procedure. The results show that, as expected, flowers of *P. grandiflora* incorporate exogenously supplied DOPA into the dihydropyridine moiety of betalains. This finding agrees with the report [15] on betalain biosynthesis in *Opuntia* fruits when, after D,L-3,4-dihydroxyphenyl[2-^{14}C]alanine administration, more than 90% of the betanin radioactivity was found in the betalamic acid obtained by hydrolysis. Since the main betacyanin of *P. grandiflora* flowers also contains radioactivity after feeding L-3,4-dihydroxyphenyl[3-^{14}C]alanine, we assume that this amino acid reaches the internal DOPA pool. No further investigations on betacyanins were carried out.

If a betaxanthin molecule is subjected to acid hydrolysis [16], the amino acid moiety is split off. By this procedure our fraction b afforded two amino acids suggesting that it is composed of two betaxanthins. One of the amino acids showed the same R_f value as glutamic acid in two solvent systems. Therefore, the parent betaxanthin is vulgaxanthin II [16] (**3** in Fig. 1). Attempts to identify the second amino acid were unsuccessful. However, we can exclude the presence of DOPA and dopamine, the 'upper' parts of dopaxanthin and miraxanthin V, respectively [17, 18]. After DOPA application to *Amaranthus* cotyledons Giudici de Nicola *et al.* [9] were unable to find any dopaxanthin but vulgaxanthin II and miraxanthin II. A number of compounds, including vulgaxanthin II, were reported by Colomas [10].

These results show that, in violet flowers of *P grandiflora* line H as in *A. caudatus* seedlings, DOPA administration elicits the formation of betaxanthins which are absent in untreated plants. Since dopaxanthin is one of the main betaxanthins in yellow *P. grandiflora* flowers [4, 5] the presence of this pigment is to be expected after DOPA feeding. But the experiments with *P. grandiflora* confirm the absence of dopaxanthin observed in *A. caudatus* after



1 Betalamic acid 2 Betanin 3 Vulgaxanthin II

Fig. 1. Betalamic acid (1) is a precursor of all betalains and represents the chromophore of these pigments In betacyanins, e.g. betanin (2), it is always linked to cyclo-DOPA whose glycosidation and acylation lead to *ca* 50 known betacyanins. In betaxanthins, e.g. vulgaxanthin II (3), betalamic acid is attached to an amino acid or an amine without any glycosidation.

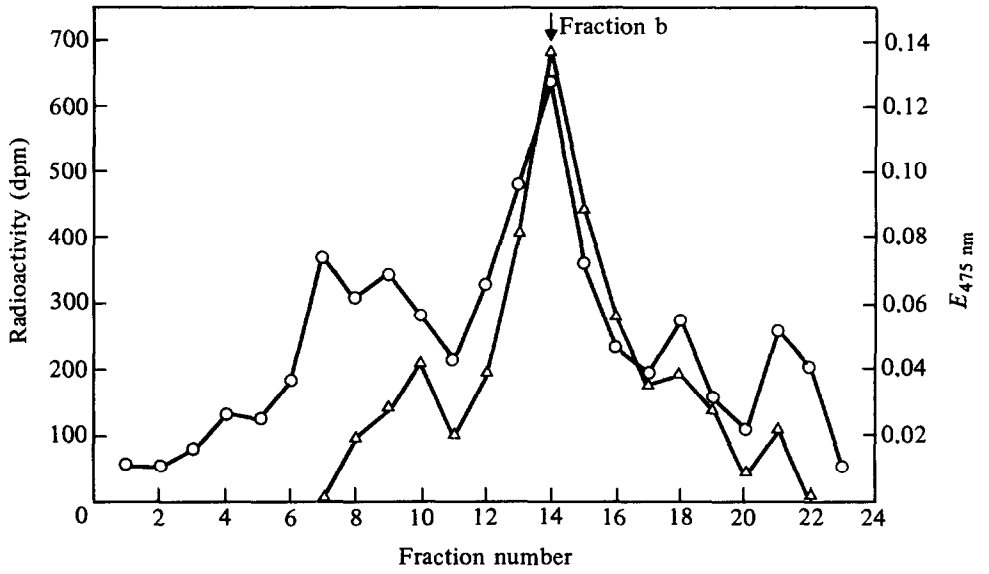


Fig 2. DEAE-Sephadex A-25 CC of the [^{14}C]betaxanthin fractions isolated from petals fed L-3,4-dihydroxyphenyl[3-^{14}C]alanine. (○) Radioactivity; (△) E_{475nm}.

DOPA application. Obviously, the feeding of a certain amino acid cannot induce the biosynthesis of the respective betaxanthin. This is in agreement with the finding that proline application to a yellow flowering *Celosia plumosa* variety does not result in the formation of indicaxanthin [19].

It is noteworthy that the basal parts of violet *P. grandiflora* flowers exhibit a yellow colour in the afternoon of very sunny days. Separation of the petal extract on DEAE-Sephadex A-25 [12] shows the presence of fraction b. This observation supports the assumption that the effect of DOPA is not restricted to its precursor function.

It now needs to be clarified whether the betaxanthin synthesis found under the influence of different conditions leads to identical patterns and whether these betaxanthins occur in untreated yellow flowering *P. grandiflora* plants.

EXPERIMENTAL

Plant material Plants of the violet flowering inbred line H of *Portulaca grandiflora* Hook [7] were grown in a greenhouse under long day conditions

Feeding method Apical stem parts of 2 cm length with flower buds were transferred into 0.3 ml 13 mM aq. DOPA soln containing an equimolar amount of ascorbic acid to inhibit oxidation. After uptake of the feeding soln, the stem tips were kept in H_2O up to anthesis. Controls were fed with 13 mM aq. ascorbic acid soln only In some cases, 130 μl (= 280 μg) L-3,4-dihydroxyphenyl[3-^{14}C]alanine (1.4 mCi/mmol) was administered per stem tip.

Isolation and fractionation of betalains. Petals of *P. grandiflora* were collected 1 or 2 days after DOPA application and stored at −15° until required. The frozen petals were homogenized with quartz sand in a mortar and extracted with H_2O. After centrifugation (15000 rpm for 10 min) the supernatant was passed through a DEAE–Sephadex A-25 column [12]. Pigments were eluted by a discontinous gradient of NaCl (from 0 to 0.6 M) and determined by their absorption spectra. In some cases most of the betacyanin fraction of petal extracts was separated by a polyamide column [14] (solvent, 0–2% aq. citric acid) before DEAE–Sephadex CC of betaxanthins.

Alkaline hydrolysis. After application of [3-^{14}C]DOPA, the [^{14}C]betaxanthin fraction b was isolated by polyamide and subsequent DEAE–Sephadex A-25 CC. An aq. soln of this fraction was de-aerated and the pH adjusted to 9–10 by addition of 0.6 M NH_3 under a stream of N_2 [13]. Under these conditions betaxanthins (λ_{max} nm: 475) were split and the resulting betalamic acid (λ_{max} nm: 430) was isolated by DEAE–Sephadex A-25 CC. All fractions were collected and their absorption maxima as well as radioactivity were determined.

Acid hydrolysis. The betaxanthin soln was applied to the top of a Dowex 50 × 8 (H^+) column in order to bind free amino acids. The betaxanthins were eluted with H_2O, reduced to dryness on a rotary evaporator and allowed to stand with 2 ml 1 M HCl at 30°–35° for 24–48 hr [16]. When the cleavage of betaxanthins was complete the reaction mixture was chromatographed on Dowex 50 × 8 (H^+). After careful washing of the column with H_2O, the amino acids were eluted with 10% NH_3. The eluate was evaporated to dryness. The residue was dissolved in a few drops of 50% aq. EtOH and subjected to PC.

Paper chromatography of amino acids Performed on Schleicher & Schüll 2043 b M. Solvent systems: *n*-BuOH–HOAc–H_2O (12:3:5) and PhOH (H_2O satd).

Radioactivity measurements. Carried out in a Packard Scintillation System Tri-Carb 2660.

Chemicals. DEAE–Sephadex A-25 was a product of Pharmacia Fine Chemicals AB, Uppsala, Sweden, and Polyamide powder of M Woelm, Eschwege, West Germany. L-DOPA and Dowex 50 × 8 were obtained from Serva, Heidelberg, West Germany and L-3,4-dihydroxyphenyl[3-^{14}C]alanine (10.9 mCi/mmol) from the Radiochemical Centre, Amersham, U.K.

Acknowledgements—The skilled technical assistance of Mrs H. Nixdorf is gratefully acknowledged. Mrs. W. Schröder kindly provided the seeds of *P. grandiflora* line H.

REFERENCES

1 Mabry, T. J. (1980) in *Encyclopedia of Plant Physiology Secondary Plant Products* (Bell, E. A. and Charlwood, B. V., eds) Vol. 8, p. 513. Springer, Berlin.
2. Piattelli, M. (1981) in *The Biochemistry of Plants: Secondary Plant Products* (Conn, E. E., ed) Vol. 7, p. 557 Academic Press, London.
3. Reznik, H. (1981) in *Pigments in Plants* (Czygan, F.-C., ed.) p 370. Akademie, Berlin.
4. Strack, D. and Reznik, H. (1979) *Z. Pflanzenphysiol.* **94**, 163.
5 Adachi, T and Nakatsukasa, M. (1983) *Z. Pflanzenphysiol* **109**, 155.
6. Konar, R. N. (1978) *Z. Pflanzenphysiol.* **86**, 443
7. Schröder, W. and Böhm, H. (1984) *Plant Cell Rep.* **3**, 14
8. French, C. J., Pecket, R. C and Smith, H. (1974) *Phytochemistry* **13**, 1505.
9. Giudici de Nicola, M., Amico, V., Sciuto, S. and Piattelli, M. (1975) *Phytochemistry* **14**, 479.
10. Colomas, J. (1977) *Z. Pflanzenphysiol.* **85**, 227.
11. Bianco-Colomas, J. (1980) *Planta* **149**, 176
12. Döpp, H. and Musso, H. (1973) *Chem Ber.* **106**, 3473.
13. Wyler, H., Wilcox, M. E and Dreiding, A. S. (1965) *Helv. Chim. Acta* **48**, 361.
14. Piattelli, M. and Minale, L. (1964) *Phytochemistry* **3**, 307.
15. Miller, H. E., Rösler, H., Wohlpart, A., Wyler, H., Wilcox, M. E., Frohofer, H., Mabry, T. J. and Dreiding, A. S. (1968) *Helv. Chim Acta* **51**, 1470.
16. Piattelli, M., Minale, L and Prota, G. (1965) *Phytochemistry* **4**, 121.
17 Impellizzeri, G., Piattelli, M. and Sciuto, S. (1973) *Phytochemistry* **12**, 2293.
18. Piattelli, M., Minale, L. and Nicolaus, R. A. (1965) *Phytochemistry* **4**, 817.
19. Sciuto, S., Oriente, G., Piattelli, M., Impellizzeri, G. and Amico, V. (1974) *Phytochemistry* **13**, 947.