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NEW CHEMICAL INDUCERS OF CAROTENOID BIOSYNTHESIS*

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Key Word Index—*Citrus paradisi*; Rutaceae; Marsh seedless grapefruit; carotenoid biosynthesis; lycopene; [β-(diethylamino)-ethoxy]-benzene; [γ-(diethylamino)-propoxy]-benzene; [δ-(diethylamino)-butoxy]-benzene; 4-[β-(diethylamino)-ethoxy]-benzaldehyde; diethylaminoethyl anisolate.

Abstract—Five compounds having a striking effect on the color of Marsh seedless grapefruit are reported. The compounds, namely [β-(diethylamino)-ethoxy]-benzene, [γ-(diethylamino)-propoxy]-benzene, [δ-(diethylamino)-butoxy]-benzene, 4-[β-(diethylamino)-ethoxy]-benzaldehyde, and diethylaminoethyl anisolate caused a 5- to 12-fold increase in the carotene content. Lycopene, not normally accumulated, became the major pigment. The mode of action appears to be similar to that of CPTA.

INTRODUCTION

PREVIOUSLY, the ability of 2-(4-chlorophenylthio) triethylamine hydrochloride (CPTA) to induce lycopene in a wide variety of carotenogenic tissues has been reported.[1–4] In this paper we report on 5 new compounds, [β-(diethylamino)-ethoxy]-benzene (I), [γ-(diethylamino)-propoxy]-benzene (II), [δ-(diethylamino)-butoxy]-benzene (III), 4-[β-(diethylamino)-ethoxy]-benzaldehyde (IV), and diethylaminoethyl anisolate (V), which induce lycopene accumulation in Marsh seedless grapefruit. These compounds also appear to have profound effects on carotenoid formation in other higher plant tissues and microorganisms. These latter effects will be reported in later papers.

RESULTS AND DISCUSSION

As in the case of CPTA, the response pattern within the Marsh seedless grapefruit is determined essentially by the depth of penetration of the compound in question. In contrast to treatment with CPTA in aqueous solution, compounds I–V were applied as the free amine forms in isopropanol. This gave a more uniform color response. The untreated grapefruit had the normal light yellow color. After treatment with any of the compounds, the fruit developed an intense red color in the flavedo. There was no color enhancement evident in the endocarp. In this paper, only the results from the flavedo are reported.

* Part V in the series "Chemical Regulation of Carotenoid Biosynthesis". For Part IV see *Phytochemistry* **11**, 2985 (1972).

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[1] COGGINS, JR., C. W., HENNING, G. L. and YOKOYAMA, H. (1970) *Science* **168**, 1589.

[2] YOKOYAMA, H., COGGINS, JR., C. W., and HENNING, G. L. (1971) *Phytochemistry* **10**, 1831.

[3] YOKOYAMA, H., COGGINS, JR., C. W. HENNING, G. L. and DEBENEDICT, C. (1972) *Phytochemistry* **11**, 1721.

[4] HSU, W. J., YOKOYAMA, H. and COGGINS, JR., C. W. (1972) *Phytochemistry* **11**, 2985.

TABLE 1. EFFECT OF I, II AND III AT 5% (W/V) ON CAROTENE CONTENT OF FLAVEDO OF MARSH SEEDLESS GRAPEFRUIT (μg/g dry wt)

			Treated	
	Control	I	II	III
Phytofluene	24·8	26 0	47·3	49 8
ζ-Carotene	1·2	11·2	46	61·7
Neurosporene	3·6	5 1	19·4	17·7
Lycopene	—	208	275	199
γ-Carotene	—	1·1	2·7	2·3
α-Carotene	0·7	0·6	0·7	0·9
β-Carotene	1·0	—	—	—
Total	31·3	252	391 1	331·4

Examination of the flavedo of the treated fruit showed that lycopene accumulated as the major pigment in each case (Tables 1 and 2). Lycopene was not detected in the untreated fruits; it is not normally detected in the mature grapefruit.[5] A significant increase in the amount of ζ-carotene was also observed.

TABLE 2. EFFECT OF I, IV AND V AT 10% (W/V) ON CAROTENE CONTENT OF FLAVEDO OF MARSH SEEDLESS GRAPEFRUIT (μg/g dry wt)

			Treated	
	Control	I	IV	V
Phytofluene	23·8	22·1	16·1	27·2
ζ-Carotene	0·8	6·5	2·0	7·3
Neurosporene	0·9	2·8	0·6	0·4
Lycopene	—	151	121	149
β-Zeacarotene	—	—	—	0·5
δ-Carotene	—	—	0 5*	—
γ-Carotene	0·8	2·3	0·6	0 9
α-Carotene	0·3	—	0·7	0 6
β-Carotene	—	0·2	0·3	0·5
Total	26·6	184·9	141·8	186·4

* Tentative identification.

All the test compounds gave a several-fold increase in the total amount of carotenes present. The largest increase, 12·5-fold, was caused by II. The major cause of the increase was the large accumulation of lycopene. For I (5% and 10%), IV (10%) and V (10%) lycopene was 80–85% of the total carotenes. A more detailed comparison of the effectiveness of these compounds in the carotenogenic molds will be reported later. For II (5%) and III (5%), lycopene was 70 and 60% respectively. There was a larger increase in phytofluene, ζ-carotene, and neurosporene for fruits treated with II and III which accounts for the decrease in the percentage of lycopene. The increase in ζ-carotene resulting from treatment with II and III was 38- and 51-fold, respectively. This response pattern is similar to that of

[5] YOKOYAMA, H. and WHITE, M. J. (1967) *J. Agr. Food Chem* **15**, 693.

Marsh seedless grapefruit treated with CPTA.[3] It also resembles the increase of carotenes observed in the fully mature citrus hybrid *Sinton citrangequat* when treated with CPTA. Apparently the mode of action of the 5 compounds (I–V) is similar to that of CPTA, which has been suggested as an inhibitor of the cyclase(s) and as a derepressor of a gene regulating the synthesis of a specific enzyme(s) in the lycopene pathway.[4]

EXPERIMENTAL

Fruit samples. The fruit samples of Marsh seedless grapefruit were harvested at the fully mature stage. The fruit used in the 5 and 10% test were harvested from different locations at different times. Each sample for the 5% test consisted of 6 fruits. The samples for the 10% test consisted of 5 fruits.

Post-harvest treatment of fruit. The test compounds were applied to the fruit as 5 or 10% (W/V) solutions in isoPrOH. For the control, pure isopropanol was used. The solution was poured over the fruit in such a manner as to cover the entire surface. The fruit were allowed to drain and then removed to a clean surface to air dry for several hours. The fruit were then stored at room temp. (about 21°) for 2 weeks in polyethylene bags.

Extraction, isolation and quantitative determination of pigment. The carotenoid pigments were isolated and separated as described previously.[5] The method of Davies[6] was used for quantitative determinations. A portion of the ground flavedo was dried *in vacuo* to obtain the sample dry wt.

Identification of pigments. The pigments were identified by their visible and UV spectra and adsorption behavior relative to known compounds.

Materials. I,[7] II[8] and III[8] were synthesized according to literature methods. Bromides were used to synthesize II and III. IV is available from Aldrich Chemical Company, Inc. V was prepared from anisoyl chloride and 2-diethylaminoethanol.

[6] Davies, B. H. (1965) *Chemistry and Biochemistry of Plant Pigments* (Goodwin, T. W., ed.), p. 489, Academic Press, London.
[7] D'Amico, J. J. and Weiss, A. G. (1962) *Chem. Abstr.* **57**, 2625d.
[8] Foldeak, S., Czombos, J., Matkovics, B. and Porszasz, J. (1964) *Chem. Abstr.* **61**, 1194e.