Phytochemistry, 1972, Vol. 11, pp. 1721 to 1724. Pergamon Press. Printed in England.

SHORT COMMUNICATION

INDUCED COLOR CHANGES IN GRAPEFRUIT AND ORANGE*

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(*Received* 28 *October* 1971)

Abstract—The fruits of Marsh seedless grapefruit and navel orange do not normally accumulate lycopene. After treatment with 2-(4-chlorophenylthio)triethylamine hydrochloride (CPTA) both fruits accumulate large amounts of lycopene. CPTA appears to stimulate the lycopene pathway. It does not, however, appear to influence the terpenoid flavoring constituents present in the peel oil.

INTRODUCTION

AS PART of our research program on the chemical regulation of carotenoid biosynthesis in plant tissues, we have been studying the effects of the lycopene inducing compound, 2-(4-chlorophenylthio)triethylamine hydrochloride, (CPTA)[1] on color development in citrus fruits. We recently reported that CPTA caused a major alteration of the carotenoid composition in the citrus hybrid Sinton citrangequat by inducing the accumulation of lycopene and inhibiting the formation of the methylketone carotenoids normally present.[2]

In continuing these studies, we investigated the effect of CPTA on carotenogenesis in two additional citrus fruits, the Marsh seedless grapefruit and navel orange. These citrus fruits exhibit dissimilar patterns of carotenoid development during maturation. In the grapefruit, net synthesis of carotenoid pigments ceases when the decrease in chlorophyll occurs, resulting in a relatively low concentration of carotenoid pigments and lightly colored fruits.[3] In contrast, loss of chlorophyll in the ripening fruit of the navel orange is accompanied by the rapid accumulation of carotenoid pigments[4] thus resulting in a much higher concentration of carotenoids and, consequently, a much deeper color. We also extended our investigations to include the possible influence of CPTA on the terpenoid flavoring constituents present in the peel oil. In view of the report that nicotine hydrochloride causes lycopene to accumulate in *Mycobacterium marinum*,[5] it was examined on citrus.

* Part III in the series "Chemical Regulation of Carotenoid Biosynthesis".

[1] C. W. COGGINS, JR., G. L. HENNING and H. YOKOYAMA, *Science* **168**, 1589 (1970).
[2] H. YOKOYAMA, C. W. COGGINS, JR. and G. L. HENNING, *Phytochem.* **10**, 1831 (1971).
[3] H. YOKOYAMA and M. J. WHITE, *J. Agric. Food Chem.* **65**, 693 (1967).
[4] E. V. MILLER, J. K. WINSTON and H. A. SCHOMER, *J. Agric. Res.* **60**, 259 (1940).
[5] C. D. HOWES and P. P. BATRA, *Biochem. Biophys. Acta* **222**, 174 (1970).

RESULTS AND DISCUSSION

The response pattern within the citrus fruits appeared to be determined essentially by depth of penetration of CPTA. In the citrus hybrid, Sinton citrangequat, the peel is thin and the response as evidenced by lycopene accumulation was also observed in the endocarp.[2] In the thicker peeled Marsh seedless grapefruit and navel orange, the effectiveness of CPTA was apparent only in the peel; no enhancement of color was evident in the endocarp. However, on injection of CPTA into the interior of the fruit, increased color response was observed in the endocarp of both the grapefruit and orange. The results presented here will be from the flavedo only.

The untreated fruits of Marsh seedless grapefruit and navel orange reflected their normal colors. The grapefruit retained the usual yellow color, and the navel orange was light orange in color. After treatment with CPTA, both citrus fruits developed the same degree of intense red color, much like that of a ripe red variety of tomato.

Examination of the carotenoids of the flavedo of mature fruits which were treated with CPTA indicated the accumulation of lycopene in major amounts in both the Marsh seedless grapefruit (Table 1) and navel orange (Table 2). Lycopene was not detected in the untreated fruits; it is not normally detected in the mature grapefruit[3] and navel orange.[6]

TABLE 1. CPTA EFFECT ON CAROTENOID CONTENT OF FLAVEDO OF MARSH SEEDLESS GRAPEFRUIT (μg/g dry wt*)

	Control	CPTA Treated		
		Immature green	Mature green	Fully mature
Phytoene	6·2	72·6	85·2	81·2
Phytofluene	2·9	20·2	24·6	22·6
ζ-Carotene	0·2	67·7	72·6	75·5
Neurosporene	Trace	51·7	52·8	54·9
Lycopene	—	249	272	266
β-Zeacarotene	Trace	Trace	Trace	Trace
γ-Carotene	—	19·8	24·2	22·8
β-Carotene	Trace	—	—	Trace
Carotenols including epoxides	2·3	0·8	2·9	2·5

* Freeze dried.

Additionally, increased synthesis of phytoene, phytofluene in grapefruit, ζ-carotene- neurosporene, and γ-carotene were observed. There was no net synthesis of the usual secondary carotenoids in either cultivar subsequent to treatment of mature fruit with CPTA.

Fruits of navel orange treated at the immature green and mature green stages of maturity attained the same degree of deep red color when harvested at full maturity. The carotenoid composition was completely altered (Table 2). Lycopene accumulated in large amounts but little or no accumulation of secondary carotenoids occurred. A similar stimulation of lycopene and other hydrocarbon carotenoids and an inhibition of formation of secondary carotenoids has been observed when immature Sinton citrangequat fruit were treated with CPTA.[2]

However, in the grapefruit treated with CPTA at the mature green stage, and to a lesser extent at the immature green stage, the concentration of the secondary carotenoids

[6] A. L. CURL and G. F. BAILEY, *J. Food Sci.* **26**, 422 (1961).

TABLE 2. CPTA EFFECT ON CAROTENOID CONTENT OF FLAVEDO OF NAVEL ORANGE (μg/g dry wt*)

	Control	CPTA treated Immature* green	CPTA treated Fully mature
Phytoene	37·2	78·8	82·1
Phytofluene	22·6	24·1	26·2
ζ-Carotene	25·4	70·1	68·2
Neurosporene	—	41·2	42·3
Lycopene	—	264	240
β-Zeacarotene	—	—	—
γ-Carotene	—	22·3	17·8
β-Carotene	0·6	Trace	Trace
Carotenols including epoxides	284	20·1	339

* Freeze-dried.
† The results for mature green fruits were similar.

approached that observed in the untreated fruits. This can be plausibly attributed to the dissimilar pattern of carotenoid development in the two fruits. In the navel orange carotenoid synthesis continues during the whole maturation process, whereas in the grapefruit net synthesis of carotenoids ceases after the loss of chlorophyll. Thus, most of the secondary carotenoids normally present in the grapefruit were already formed at the stage of maturity at which the fruits were treated with CPTA.

The pigments of both citrus fruits treated at the immature green and mature green stages of maturity were essentially hydrocarbon in nature. The main pigment was lycopene. Phytoene, phytofluene, ζ-carotene, neurosporene and γ-carotene constituted the remainder of the major hydrocarbons.

We investigated the influence of CPTA on the isoprenoid pathway involved in the formation of the important terpenoid flavoring constituents which are found in the peel oils. The comparison of gas chromatographic profiles of peel oils from untreated and treated fruits indicates CPTA does not affect the peel oil constituents. The GLC profiles were identical in every respect. It suggests that CPTA acts essentially at the C-40 level of the carotenoids in the citrus.

It is apparent that CPTA regulates the formation of carotenoids in the fruits of Marsh seedless grapefruit and navel orange, much in the manner previously observed in the citrus hybrid Sinton citrangequat.[2] Treatment with CPTA causes a shift in the type of carotenoids produced. A general pattern of large accumulation of lycopene with concomitant increase in synthesis of phytoene, phytofluene, ζ-carotene and neurosporene and of γ-carotene was noted in both citrus fruits.

It is of particular interest to note the effect of CPTA on carotogenesis in the lighter colored Marsh seedless grapefruit. Normally the grapefruit attains a light yellow color at full maturity. Previous studies[3] have shown that in the ripening grapefruit an accumulation of the primary C-40 precursor phytoene was observed after the decrease in chlorophyll. This suggested that an inhibition of the dehydrogenation step in the sequential pathway occurs in the grapefruit. However, on treatment with CPTA the fruit develops a deep red color, resulting from the large accumulation of lycopene. The pattern of large accumulation of the acyclic lycopene accompanied by smaller accumulation of the monocyclic γ-carotene and general inhibition of the formation of the bicyclic carotenoids strongly

suggests an additional role for CPTA in carotenogenesis in citrus. CPTA appears to inhibit the cyclization reaction, particularly the second cyclization reaction leading to the formation of β-carotene. It does not appear to completely inhibit the initial cyclization reaction by which γ-carotene is formed at the 5000 ppm concentration of CPTA.

We also investigated the effect of nicotine hydrochloride on the formation of carotenoid in citrus, since it had previously been shown to promote the formation of lycopene rather than β-carotene in the bacterium *Mycobacterium marinum*.[5] Mature grapefruit and navel orange fruit tissue did not accumulate lycopene in response to the compound; thus, nicotine does not appear to be active on a broad spectrum of plants.

EXPERIMENTAL

Fruit samples. The fruit samples of Marsh seedless grapefruit and navel orange were all harvested at the fully mature stage of coloring from trees located at the University of California at Riverside. Each sample consisted of twelve fruits.

Treatment with CPTA. The fruits were immersed for 30 sec in a solution containing 5000 ppm of CPTA and 0·05% wetting agent (Multi-Film X-77). The fruits were treated at three stages of maturity: Stage 1, immature green, treated 13 October 1969; Stage 2, mature green, the fruits had attained nearly full size but still retained the chlorophyll, treated 5 November, 1969; Stage 3, mature, fruits essentially free of chlorophyll, treated 11 December, 1969. The untreated fruits were allowed to reach full color on the trees and then all of the samples were collected on 26 January, 1970. Fruits were treated postharvest with nicotine hydrochloride (5000 ppm) and stored for two weeks at 30° at 80–85% relative humidity. Such a treatment with CPTA would result in high concentration of lycopene.

Extraction, isolation and quantitative determination of pigment. The carotenoid pigments were isolated and separated as described previously.[3] In the development and elution of columns, a continuous gradient system was employed. Wherever possible the individual carotenoid was isolated in the crystalline state. The method of Davies[7] was used for quantitative determinations. The carotenol content including epoxides was estimated from the spectrophotometrically determined amount of in ether extract using $E^{1\%}_{1\,cm} = 2700$ at 450 nm.

Identification of pigments. For the pigments isolated from CPTA treated fruit samples, our structural assignment and identification rested largely on TLC, IR, UV and NMR spectral comparisons with authentic samples. Where authentic samples were not available, the identification was made on UV, IR and NMR spectra in accord with literature values. For extremely small samples, NMR spectra were obtained by the spectrum accumulation technique. The minor carotenol constituents including epoxides present only in trace quantities were not subjected to stringent identification procedures.

The pigments of Marsh seedless grapefruit[3] and navel orange[6] were tentatively identified earlier. In the present studies, the pigments from the untreated fruit samples were isolated in insufficient amounts for unambiguous identification. The identification procedures used for the pigments from the untreated samples specified in Tables 1 and 2 were much less rigorous than those employed for the major pigments from the treated samples. Identity was based on UV spectral and TLC comparisons with authentic samples. No attempts were made to identify the secondary carotenoids except to note that they were similar on TLC to the carotenoids normally present.

Extraction of peel oil. 20–30 g sample of peel was extracted with two 100-ml portions of CH_2Cl_2 in an Omni-Mixer, and concentrated by distillation on a steam bath to give a sample of peel oil.

GLC. GLC profiles of the citrus peel oil were obtained by employing the system of MacLeod *et al.*[8] GLC operations were carried out on a Perkin–Elmer hydrogen flame ionization gas chromatograph, Model 226, equipped with a 300 ft long 0·010 in. i.d. stainless steel open tubular column coated with a mixture of 5% w/w IGEPAL Co-880 (nonylphenoxypolyoxyethylene ethanol) in Apiezon L. The injector block containing the annular stream splitter was maintained at 200°. A 0·5–1·0 μl sample of peel oil was injected into the chromatograph and split 100:1. The oven temperature was programmed from 80 to 160° at 1·5°/min with a helium flow of 5 ml/min. A total of 3 hr was required.

Acknowledgement—CPTA was supplied by Amchem Products, Inc., Ambler, Pa.

[7] B. H. DAVIES, *Chemistry and Biochemistry of Plant Pigments* (edited by T. W. GOODWIN), Academic Press, New York (1963).
[8] W. D. MACLEOD, W. H. MCFADDIN and N. M. BUIGUES, *J. Food Sci.* **31**, 591 (1966).

Key Word Index—Citrus; Rutaceae; grapefruit; navel orange; carotenoid synthesis; lycopene; effect of 2-(4-chlorophenylthio)triethylamine hydrochloride.