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THE EFFECT OF 2-(4-CHLOROPHENYLTHIO)-TRIETHYLAMINE HYDROCHLORIDE ON THE FORMATION OF CAROTENOIDS IN CITRUS*

HENRY YOKOYAMA

Fruit and Vegetable Chemistry Laboratory,† Pasadena, California 91106, U.S.A.

and

C. W. COGGINS, JR. and G. L. HENNING

Department of Horticultural Science, University of California, Riverside, California 92507, U.S.A.

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Abstract—2-(4-Chlorophenylthio)triethylamine hydrochloride (CPTA) has a profound effect on the formation of carotenoids in the fruit of the trigeneric citrus hybrid *Sinton citrangequat*. When CPTA is applied to the immature green fruit and the fruit is allowed to ripen to full maturity and color, lycopene accumulates as the main pigment and is responsible for the rich color in the flavedo. Synthesis of methylketone carotenoids which normally produce the red color in the flavedo is inhibited. Treatment with CPTA after the fruits had attained near full maturity and color results in the accumulation of lycopene as a major pigment; no response is apparent in the previously formed methylketone carotenoids. Treatment with CPTA at immature green, mature green or nearly mature stages of fruit development, stimulates the synthesis of lycopene as the principal pigment or as a major pigment depending on stage of development.

INTRODUCTION

IN A PRELIMINARY communication[1] we reported on the accumulation of lycopene induced by 2-(4-chlorophenylthio)triethylamine hydrochloride (CPTA) in a wide array of carotenogenic tissues. Among the citrus fruits tested and responding to CPTA was the trigeneric hybrid *Sinton citrangequat*. The report of the discovery of the induction of lycopene synthesis by CPTA mentioned only the accumulation of lycopene. In this paper we report in full our results on the effect of CPTA on carotenoid formation in the fruit of the citrus hybrid *Sinton citrangequat*.

The carotenoid mixture in this citrus hybrid is extremely complex.[2] However, it was selected for further detailed study of the response to CPTA because the fruit normally produces unique alicyclic methylketones which are responsible for the rich color in the flavedo of mature fruits. It became of interest to determine the response when fruits were treated with the compound prior to the formation of the methylketones and allowed to ripen to full maturity.

RESULTS AND DISCUSSION

The carotenoids of the *Sinton citrangequat* were reported earlier.[2] The fruit normally produces the methylketone carotenoids in major amounts, principally reticulataxanthin and

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† A laboratory of the Western Utilization Research and Development Division, Agricultural Research Division, U.S. Department of Agriculture.

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

[2] H. YOKOYAMA and M. J. WHITE, *Phytochem.* **5**, 1159 (1966).

citranaxanthin in the fully mature fruit at full color, and in addition, in the late season, sintaxanthin and 3-hydroxysintaxanthin.[3] These methylketones are responsible for the deep orange-red color of the fruit. Lycopene is not normally detected at any stage of development of the fruit.

Initially, the response to CPTA was studied on fruits which had already attained deep orange-red color prior to treatment. Postharvest and preharvest treatment were equally effective. In the former case the highest storage temperature investigated (30°) gave the greatest response. When fruits were held at 30° for 2 weeks subsequent to treatment, accumulation of lycopene throughout the peel was observed. The response pattern within the fruit appeared to be determined essentially by depth of penetration of the compound. The fruit of the *Sinton citrangequat* is small and the peel is thin. In a number of fruits the entire endocarp responded to the treatment. This response demonstrates the effectiveness of the compound in the tissues of the endocarp; however, in the endocarp it varied enough due to penetration problems to preclude the use of the results in this study. In this paper, only the results from the peel are presented.

TABLE 1. EFFECT OF CPTA ON CAROTENOID CONTENT OF PEEL OF POSTHARVEST TREATED FRUIT OF *Sinton citrangequat* (1968–69 SEASON)

	μg/g dry wt.*	
	Control	Treated
Phytoene	trace	62·8
Phytofluene	10 2	24·6
ζ-Carotene	7·5	52·1
Neurosporene	5·8	36·2
Lycopene	—	210
γ-Carotene	4·2	20·2
α-Carotene	2·1	1·2
β-Carotene	26 1	20·1
Sintaxanthin	2·2	2·9
3-Hydroxysintaxanthin	10·1	8 6
Citranaxanthin	71·5	74·2
Reticulataxanthin	325	331
β-Apo-8′-carotenal	3·2	2·8
β-Citraurin	22·8	24·1

* Freeze-dried.

Examination of carotenoid constituents of the peel of treated fruits indicated the accumulation of lycopene in major amounts (Table 1). Additionally, increased synthesis of phytoene, phytofluene, ζ-carotene, neurosporene and γ-carotene were observed. There was no net synthesis of the normally present major carotenoids, the methylketones. The appearance of the treated fruit was enhanced greatly with the accumulation of lycopene. It is apparent that the lycopene pathway is stimulated.

In continuing studies at the beginning of the following season (1969–70), four fruit samples were selected, each consisting of an identical number of fruits. One lot remained untreated. The remaining three lots received preharvest treatments with CPTA at three

[3] H. YOKOYAMA and M. J. WHITE, *J. Org. Chem.* **30**, 3994 (1965).

stages of maturity: (1) immature green; (2) mature green, some evidence of decrease of chlorophyll in the flavedo; (3) nearly mature, appearance of mature color (orange-reddish) and absence of chlorophyll. All the fruits remained on the tree until untreated fruits were fully mature.

The peel of untreated fruits contained the normal methylketones as major carotenoids (Table 2). However, the pattern of distribution of the methylketones differed in the 1969–70 season. In contrast to previous results, 3-hydroxysintaxanthin rather than the usual reticulataxanthin was the main methylketone carotenoid present in the peel of the untreated fruit. Undoubtedly, this was due to seasonal variation.[3,4] Structure of 3-hydroxysintaxanthin had been tentatively identified previously.[2] Details of the complete elucidation of this pigment will be reported in a later paper along with a further elaboration of the biosynthesis of the methylketones. Additionally, β-citraurin and sintaxanthin were present in significant amounts; smaller amounts of reticulataxanthin and citranaxanthin were also detected. Lycopene could not be detected in the untreated fruits.

TABLE 2. EFFECT OF CPTA ON CAROTENOID SYNTHESIS IN PEEL OF FRUIT OF *Sinton citrangequat* TREATED AT DIFFERENT LEVELS OF MATURITY AND ALLOWED TO RIPEN

	μg/g dry wt.*			
	Control	Immature green	Mature green	Fully mature
Phytoene	trace	71·8	76·9	68·6
Phytofluene	9·2	16·7	20·2	18·6
ζ-Carotene	6·2	70·2	65·6	60·2
Neurosporene	4·3	41·2	44·8	43·6
Lycopene	—	266	248	212
γ-Carotene	3·1	22·2	20·1	18·2
α-Carotene	1·6	trace	trace	1·9
β-Carotene	20·1	2·6	3·2	18·9
Sintaxanthin	75·1	1·3	1·8	79·2
3-Hydroxysintaxanthin	201	2·2	2·7	192
Citranaxanthin	25·4	trace	trace	27·6
Reticulataxanthin	42·6	1·1	2·6	43·2
β-Apo-8′-carotenal	31·6	—	—	29·1
β-Citraurin	112	1·9	2·2	106

* Freeze-dried.

The fruits which were treated at the immature green and mature green stages of maturity attained a deep orange-red color, slightly less intense than that of the control, when harvested at full maturity. The carotenoid composition in the treated fruits was completely altered (Table 2). The normally present methylketone carotenoids could not be detected in normal concentration in the peel of the treated fruits. The formation of the methylketone carotenoids was inhibited by treatment of fruit with CPTA at the immature green or mature green stage; the synthesis of precursor carotenoids was inhibited. The small amounts of the methylketones were probably due to the residual precursor carotenoids formed prior to treatment of the green fruit.[5]

The pigments of the treated immature green and mature green fruits were essentially hydrocarbon in nature. The acyclic carotene lycopene was the main pigment. Phytoene,

[4] H. YOKOYAMA and M. J. WHITE, *J. Org. Chem.* **31**, 3452 (1966).
[5] H. YOKOYAMA and M. J. WHITE, *J. Agri. Food Chem.* **15**, 693 (1967).

phytofluene, ζ-carotene, neurosporene and γ-carotene constituted the remainder of the major hydrocarbons. Trace amounts of several xanthophylls were present. However, no attempt was made to structurally identify these constituents because an insufficient amount was isolated. These xanthophylls were undoubtedly those associated with the green fruit prior to treatment.

The major carotenoids found in the peel of the fruit treated near full maturation were similar to those observed earlier. In addition to the usual methylketone carotenoids, increased concentrations of phytoene, phytofluene, ζ-carotene, neurosporene and γ-carotene were found. Lycopene accumulated in major amount.

It is apparent that CPTA has a profound effect on the formation of carotenoids in the fruit of the citrus hybrid Sinton citrangequat. Treatment with CPTA causes a shift in type of carotenoids produced. A general pattern of large accumulation of lycopene with concomitant increase in synthesis of its precursors, phytoene, phytofluene, ζ-carotene and neurosporene, and of γ-carotene was noted. It appears that the synthesis of secondary carotenoids is inhibited.

The accumulation of γ-carotene in the citrus hybrid would seem to indicate that CPTA does not generally inhibit the cyclization reaction. The accumulation of γ-carotene and the role of CPTA in carotenogenesis will be discussed in detail in later papers on the effect of CPTA on biosynthesis of carotenes in *Blakeslea trispora* and mutants of *Phycomyces blakesleeanus*.

EXPERIMENTAL

Fruit Samples

The fruit samples were all harvested in the fully ripe stage of maturity from trees located at the University of California at Riverside. Each sample consisted of 12 fruits. Larger number of fruits were used for structural identification.

Treatment with 2-(4-Chlorophenylthio)triethylamine Hydrochloride (CPTA)

The fruits were immersed for 30 sec in a solution containing 5000 ppm of CPTA and 0·01% wetting agent (Ortho X-77 Spreader). The fruits were treated at three stages of maturity: stage 1, immature green, treated 13 October 1969; stage 2, mature green, the fruits had reached nearly full size but still retained large part of the chlorophyll, treated 5 November 1969; stage 3, nearly mature, treated 8 December 1969. The fruits were allowed to ripen on the trees and all were harvested on 26 January 1970.

Postharvest treated fruits were stored for 2 weeks at about 30° at 80–85% relative humidity.

Extraction and Isolation of Pigments

The carotenoid pigments were extracted from the peels and isolated in the manner described previously.[2] In the development and elution of columns, a continuous gradient system was employed. Wherever possible the individual carotenoid was isolated in the crystalline state.

Quantitative Determination

The usual method used has been described by Davies.[6]

Identification of Pigments

Our structural assignments and identification rested largely on TLC, IR, UV and NMR spectral comparisons with authentic samples, wherever possible. Where authentic samples were not available, the identification was made on NMR spectra in accord with the literature values. For determination of NMR spectra of extremely small samples, a spectrum accumulation technique was employed

In this study, 3-hydroxysintaxanthin was isolated in sufficient amounts for unambiguous structural assignment. Full details will be presented in a separate communication.

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[6] B. H. DAVIES, in *Chemistry and Biochemistry of Plant Pigments* (edited by T. W. GOODWIN), Academic Press, New York (1963).