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Lycopene Accumulation Induced by 2-(4-Chlorophenylthio)-Triethylamine Hydrochloride

Abstract. *After fruits, roots, or the mycelium of certain plants were treated with 2-(4-chlorophenylthio)-triethylamine hydrochloride, lycopene was detected in the tissue. This is the first known success in causing lycopene to accumulate in a wide range of carotenogenic tissues that normally do not accumulate the pigment at some stage of development. The response should be of value in the study of carotenoid biosynthetic pathways and gene control mechanisms.*

We report that 2-(4-chlorophenylthio)-triethylamine hydrochloride (CPTA) (*1*) affects carotenoid biosynthesis in a number of tissues. Where the effect was positive, lycopene accumulated and became the predominant pigment regardless of pigmentation in the untreated samples. We observed that pink areas developed in the pericarp (rind) of Marsh grapefruit after CPTA was applied to the rind of mature fruits before or after the fruit was harvested. The pigment responsible for the pink color was lycopene, as identified by direct spectral (electronic, infrared, nuclear magnetic resonance, and mass spectra) and chromatographic comparisons with an authentic specimen.

Purcell (*2*) reported that lycopene occurs in small but detectable amounts in the immature Marsh grapefruit, but lycopene has not been detected previously in mature fruit of this variety (*2, 3*). The induction of lycopene accumulation in mature fruits of Marsh grapefruit is interesting because some of the red-pigmented grapefruit varieties trace back to the Marsh variety (*4*).

Red-pigmented grapefruit contain appreciable concentrations of lycopene in either the pericarp or the edible portion, or both. The compound CPTA caused the accumulation of higher concentrations of lycopene in the pericarp of Redblush grapefruit. The absence of an influence on lycopene concentration in the edible portion may be due to lack of sufficient migration of CPTA.

When Washington navel orange, Valencia orange, Eureka lemon, Satsuma mandarin, and Sinton citrangequat were treated with the compound, lycopene accumulated as the predominant pigment in the pericarp. Except for immature Marsh grapefruit, red-pigmented grapefruit and pummelo varieties, two orange cultivars, and tangerines (*2, 5*), lycopene has not previously been reported in citrus.

Accumulation of lycopene in citrus occurred within a few days in small irregularly shaped patches of tissue. Frequently the accumulation occurred adjacent to preexisting surface injuries. When fruits were held for several weeks or longer subsequent to treatment, lycopene accumulated throughout the exocarp (flavedo) and also to some extent in the mesocarp (albedo). This response pattern suggests that penetration does not occur readily, that migration of CPTA within the tissue is slow, and that CPTA is reasonably stable. The conclusion that CPTA does not penetrate the rind surface readily is suggested also by comparison of concentration versus response on intact or excised pericarp tissue. For intact fruit the magnitude of the response increased as concentrations were increased to at least 5000 parts per million (ppm), while a maximum response occurred at 200 to 400 ppm when the mesocarp area of excised pieces of pericarp was treated.

Initial microscopic examination suggested that lycopene accumulated as discrete units, probably in plastids. These units were numerous in cells of the exocarp and in the parenchyma cells of vascular bundles, but were less numerous in mesocarp cells. Casual observation of the intact rind surface of treated fruits gave the impression that the red color was concentrated in oil glands. While we cannot discount the possibility that the compound produced changes in composition of the essential oils, we observed that lycopene was abundant in cells that surround oil glands and we suggest that the oil gland acts as a lens system and that the red appearance of the gland is caused by the presence of lycopene in the surrounding cells. Increasing lycopene accumulation in the Marsh grapefruit was associated with higher storage temperatures (range of 4°, 10°, 13°, and 21°C) after the harvested grapefruit was treated with CPTA.

The response of mature, orange-colored Valencia orange fruits to CPTA was altered little if any by prior treatment with the growth regulators gibberellic acid (500 ppm), 2,4-dichlorophenoxyacetic acid (500 ppm), and succinic acid 2,2-dimethylhydrazide (0.5 percent). However, the accumulation of lycopene increased in fruits that were given prior treatment with 2-chloroethylphosphonic acid (Ethrel); this increase may have been due to the production of ethylene from Ethrel (*6*). Since Ethrel alone did not cause lycopene to accumulate, it appears that CPTA enabled the tissue to accumulate lycopene and that Ethrel influenced the magnitude of the response. We anticipated that gibberellic acid would interfere with lycopene accumulation because it reduces the rate of accumula-

tion of many carotenoids during development of the orange color in the rind of navel oranges (*7*). The fact that lycopene accumulation was not reduced by gibberellic acid in Valencia oranges indicates that gibberellic acid does not interfere with the sequential pathway from phytoene to lycopene.

The oxy analog of CPTA caused citrus rind tissue to accumulate lycopene, but, because 29 additional analogs were not effective, a high degree of structural specificity for induction of lycopene accumulation appears to exist among the members of this class of compounds.

We also applied CPTA (5000 ppm) to fruits of apricot, prune, and several varieties of peach, to four strains of tomatoes which do not normally produce lycopene, and to roots of carrot and sweet potato. In all of the foregoing material, lycopene accumulated as a major carotenoid constituent. Similar effects were obtained when mycelia of *Blakeslea trispora* and *Phycomyces blakesleeanus* were grown in media containing 15 ppm of the compound. The quantity of lycopene that accumulated varied from a low of 10 μg per gram (dry weight) of sweet potato root to a high of 4300 μg per gram (dry weight) of *Blakeslea trispora* mycelium. The latter concentration equals or exceeds the quantities found in red varieties of tomato. Whether the difference in magnitude of response observed in treated tissues is related to different degrees of penetration or to other factors was not determined. Apple and pear fruit tissue did not accumulate lycopene in response to the compound.

We know of only one report of success with chemicals in causing an appreciable change in lycopene accumulation by plant tissues. Knypl (*8*) reported that detached pumpkin cotyledons accumulate lycopene when treated with (2-chloroethyl)trimethylammonium chloride (Cycocel). We have not seen similar activity when Cycocel was applied to citrus fruits. Since Cycocel has been widely investigated and since lycopene accumulation has not been reported for other plant tissues, it appears that CPTA, and possibly its oxy analog, are active on a much broader spectrum of plants, including at least two fungi.

The fact that a wide array of carotenogenic tissues accumulate lycopene when treated with CPTA should provide an opportunity for examination of carotenoid biosynthetic pathways and gene control mechanisms. Also, it is possible that other isoprenoid pathways are influenced by the compound.

C. W. Coggins, Jr.
G. L. Henning
Department of Horticultural Science, University of California, Riverside
Henry Yokoyama
USDA Fruit and Vegetable Chemistry Laboratory, Pasadena, California

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Trisporic Acid Synthesis in Blakeslea trispora

Abstract. *Cultures of the (−) strain of* Blakeslea trispora *synthesize trisporic acid C, a sex hormone, from precursors made by cultures of the (+) strain. The precursors are nonacidic compounds isolated from the culture medium of the (+) strain by chloroform extraction. Actidione does not inhibit trisporic acid synthesis from isolated precursors, suggesting that the enzymes involved in the synthesis in cultures of the (−) strain are constitutive.*

Trisporic acids, oxidized unsaturated derivatives of 1,1,3-trimethyl-2-(3-methyloctyl)cyclohexane (*1*), stimulate carotenogenesis in *Blakeslea trispora* (*2–5*) and the formation of zygophores (sex cells) in *Mucor mucedo* (*5–8*). Trisporic acids have been isolated from combined cultures of (+) and (−) strains of *B. trispora* (*1–7*) and *M. mucedo* (*8*) but not from individual (+) and (−) mating-types of these species. Sutter and Rafelson (*3*) reported that synthesis of trisporic acids in combined cultures of (+) and (−) strains of *B. trispora* was proportional to the amount of inoculum of the (+) strain used with excess inoculum of the (−) strain and was largely independent of the amount of inoculum of the (−) strain used with excess inoculum of the (+) strain. They suggested that cultures of the (+) strain made trisporic acids when stimulated by cultures of the (−) strain. Also van den Ende (*5*) observed that trisporic acids were synthesized when *Zygorhynchus moelleri* (a homothallic species) was incubated with cultures of the (+) strain of *B. trispora* but not with those of the (−) strain. I report, however, that cultures of the (−) strain of *B. trispora* synthesized trisporic acid C from precursors made by cultures of the (+) strain.

Cultures (3) were grown in a medium (PGT) composed of 1.25 percent potato extract, 2 percent glucose, and 0.0002 percent thiamine-hydrochloride in 500-ml flasks capped with cotton plugs (*9*) by incubation in darkness at 28°C on a 5-cm stroke New Brunswick gyrotory shaker at 175 rev/min. Trisporic acids were isolated from acidified, mixed culture media of (+) and (−) strains by chloroform extraction (*1*). The chloroform fraction was extracted with 4 percent sodium bicarbonate, and the bicarbonate or acid fraction (after acidification) was extracted again with chloroform. The chloroform was evaporated to dryness, and the residue (trisporic acids) was dissolved in ethanol. Trisporic acid B (maximum absorbance at 321 nm in ethanol) and trisporic acid C (maximum absorbance at 325 nm in ethanol) were separated by thin-layer chromatography on silica-gel plates (Eastman No. 6061) with a mixture of chloroform and acetic acid (*5*). All operations were performed under red fluorescent light (*10*).

The culture medium of the (+) strain stimulated the culture of (−) strain to synthesize trisporic acid C (Table 1). A 5-day old culture medium of the (+) strain, sterilized by filtration, was incubated for 12 hours with and without a 36-hour-old culture of the (−) strain. After incubation, the two media exhibited similar absorbances at 325 nm. However, when acid fractions of the media were isolated and examined, a significant difference in absorbance at 325 nm was observed; the