pening at 32 °C and the total carotenoid content of the control fruits decreased. The reduced lycopene content of controls ripened at 32 °C for 12 days is consistent with earlier reports on the effect of temperature on carotenoid synthesis in ripening tomato (Tomes, 1963; Tomes et al., 1956, 1958).

High-beta fruit treated with CPTA and ripened at 21 °C exhibited a reduction in β-carotene after 6 and 12 days concomitant with an increased synthesis of lycopene and γ-carotene. The results suggest a partial shift from β-carotene to lycopene synthesis in the presence of CPTA. Ripening at 32 °C effectively blocked the CPTA-induced synthesis of lycopene and γ-carotene. The β-carotene content similarly decreased upon ripening at 32 °C. Furthermore, at 32 °C the CPTA-treated high-beta fruits contained greater quantities of lycopene and γ-carotene than the corresponding controls. This increase appears to have occurred at the expense of β-carotene which is slightly lower than the untreated controls. The synthesis of the more saturated acyclic intermediates was not affected by treatment with CPTA when the fruit was ripened at 32 °C.

The high-beta tomato line possesses the dominant gene *B* and the recessive modifier mo_B (Kirk and Tilney-Bassett, 1967). Studies by several workers suggested that in the high-beta genotype the formation of β-carotene from lycopene was enhanced in the presence of the dominant *B* allele (Kohler et al., 1947; Porter and Lincoln, 1950). Tomes (1963) and Tomes et al. (1956) reported that some of the β-carotene in the high-beta tomato could be inhibited by ripening at high temperature while β-carotene synthesis in the normal red fruit was not. The increased biosynthesis of lycopene and γ-carotene at 32 °C in response to CPTA treatment is insufficient to make lycopene the major pigment of the fully ripe high-beta tomato fruit. Thus, the ripe fruit has a yellow-orange color due to the high concentration of β-carotene. Therefore, CPTA fails to overcome a temperature-inhibited step in the conversion of lycopene to β-carotene in the high-beta tomato fruit.

The data presented are in variance with the earlier hypothesis of Rabinowitch and Rudich (1972) and suggest that in both the normal red and high-beta tomato fruits, an inhibition of carotenoid biosynthesis by high temperature cannot be overcome by treatment with CPTA.

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Carotenoid Induction in Orange Endocarp

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The effects of synthetic bioregulators of carotenogenesis on orange endocarp are reported for the first time. Valencia oranges were treated with 2-(4-chlorophenylthio)triethylamine hydrochloride, 2-(*p*-ethylphenoxy)triethylamine hydrochloride, and 4-[β-(diethylamino)ethoxy]benzophenone hydrochloride. There is a large increase in the xanthophyll fraction and a twofold increase in total carotenoids. Bioregulators sprayed on the fruit during preharvest treatment caused no lycopene to accumulate. Postharvest treatment by pressure infiltration caused less lycopene accumulation in the endocarp than usually seen in treated peel.

A large number of bioregulators of carotenoid biosynthesis have been developed (Hsu et al., 1975; Poling et al., 1975; Poling et al., 1976) and shown to have a profound effect on carotenogenesis in a number of organisms (Coggins et al., 1970; Yokoyama et al., 1971; Rabinowitch and Rudich, 1972; Gertman and Fuchs, 1973; Hayman et al., 1974; Elahi et al., 1975; Jahn and Young, 1975; Hayman and Yokoyama, 1976). These compounds cause accumulation of the acyclic carotene, lycopene, and induce increased carotenogenesis. The first effect is caused by an inhibition of the cyclase enzyme(s) which normally functions in most carotenogic systems to produce the cyclic carotenes and xanthophylls from the acyclic precursor

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carotenes such as lycopene. The second effect is believed to result from derepression at the genetic level (Hsu et al., 1972). The carotenoid distribution of citrus fruit is economically important because of both consumer acceptance (color quality) and nutritional value (provitamin A content). Because of penetration difficulties, previous work has dealt with the peel portion of the fruit only. In the present study, the effect of several bioregulators on carotenogenesis in the endocarp or edible portion of Valencia oranges was investigated.

EXPERIMENTAL SECTION

Fruit Samples. Valencia oranges (*Citrus sinensis* (L.) Osbeck) were used throughout the experiment. Fruit were fully mature and chosen for uniform color and size. Fruits used in the postharvest treated experiments were obtained unprocessed from a local packing house (Sept 8, 1976, Riverside County, Calif.). Preharvest treatment was carried out from June through Aug 1976 in Riverside County, Calif.

Chemicals. The bioregulators chosen for this study were 2-(4-chlorophenylthio)triethylamine hydrochloride (**1**) (Schuetz and Baldwin, 1958), 2-(*p*-ethylphenoxy)triethylamine hydrochloride (**2**) (Poling et al., 1975), and 4-[β-(diethylamino)ethoxy]benzophenone hydrochloride (**3**) (Hsu et al., 1975). The bioregulators were dissolved in water, 0.01% (w/v) Ortho X-77 spreader (Chevron Chemical Company, San Francisco, Calif.) was added, and the pH adjusted to 10.

Preharvest Treatment of Fruit. Grove dirt was washed from the fruit first by hand spraying with a detergent solution (Wt-41, Pennwalt Corp., Monrovia, Calif.). Fruits were individually sprayed until the bioregulator solution began to drip off the fruit. Compounds **1**, **2**, and **3** were applied at concentrations of: 7, 4, and 6×10^{-3} M, respectively. Fruits from an adjacent branch were used as controls. Fruits were harvested after 1 month.

Postharvest Treatment of Fruit. Fruits were washed by dipping in detergent. Compound **1** (0.02 M) and compounds **2** and **3** (0.1 M) were pressure infiltrated into the fruit. The addition of numerous fine holes (ca. 50) through the flavedo just into the albedo with a No. 22 syringe needle was necessary for good color induction. The fruit were placed in a closed, thick-walled glass container and submerged beneath a solution of the appropriate bioregulator. The apparatus was then attached to a tank of compressed nitrogen and subjected to three 1-min cycles of 7 lb/in.^{2} pressure. Fruits were held at room temperature in the laboratory for 11 days and then frozen prior to analysis.

Analysis of Pigments. Fruits to be analyzed were peeled and the endocarp was weighed and then blended with an equal volume of methanol. The mixture was filtered through hyflo Super-Cel and the methanol extract was discarded. The residue was exhaustively extracted with acetone. The nonsaponifiable portion of the extract was partitioned two times with an equal volume of 95% methanol. The epiphase was chromatographed on a 2.5 × 10 cm column of 1:1, w/w, MgO–Super-Cel and developed with 1% acetone in P.E. The isolated carotenes from the column and the xanthophylls from the 95% methanol fraction were quantitatively analyzed (Davies, 1965).

RESULTS AND DISCUSSION

The results of preharvest application of the bioregulators are presented in Table I. The total endocarp carotenoid level for fruit treated with compound **1** was more than twice that of the control sample. Compound **3** appeared to have no effect; while compound **2** exhibited an intermediary effect. The increase in total carotenoids was due principally to an increase in xanthophylls. Lycopene did not accumulate.

Table I. Preharvest Application of Bioregulators to Valencia Orange (μg of carotenoid/g of endocarp, fresh weight[a])

Compound	1	2	3	Control
Xanthophyll	14.9	11.5	6.4	7.3
Lycopene				
ζ-Carotene	1.7	1.6	0.9	0.5
β-Carotene	1.0	0.3	0.4	0.3
α-Carotene	0.1	0.1	0.1	0.1
Phytofluene	1.7	0.9	0.6	0.6
Total	19.4	14.4	8.4	8.8

[a] Values represent the mean of two-three replicates (two fruit/replicate) with a range of ±9% the mean for the xanthophylls and ±0–50% of the mean for the carotene fractions.

Table II. Postharvest Application of Bioregulators to Valencia Orange (μg of carotenoid/g of endocarp, fresh weight[a])

Compound	1	2	3	Control
Xanthophyll	16.4	13.5	15.3	9.7
Lycopene	2.6	1.1	1.2	
ζ-Carotene	0.6	0.4	0.4	0.5
β-Carotene	0.2	0.2	0.2	0.3
α-Carotene	0.3	0.1	0.1	0.1
Phytofluene	1.0	0.8	0.7	0.5
Total	21.1	16.1	17.9	11.1

[a] Values represent the mean of two-three replicates (two fruit/replicate) with a range of ±5% of the mean for the xanthophylls and a ±0–100% of the mean for the carotene fractions.

It is characteristic of the bioregulators tested that lycopene should accumulate. Compound **2**, for instance, caused lycopene to accumulate to 84% of the total carotenoids in grapefruit peel (Poling et al., 1975). We were, therefore, surprised that no lycopene accumulated in any of these samples. The lack of lycopene is economically significant. Lycopene possesses a deep red color rather than the deep yellow to orange color preferred, but not always obtained, in oranges and orange products. Additionally, lycopene is acyclic and, therefore, lacks provitamin A activity such as that found in β-carotene and cryptoxanthin.

A postharvest treatment of the endocarp with the same bioregulators was performed. The whole fruit were pressure infiltrated with bioregulator solution. Results from this experiment are given in Table II. Induction of carotenogenesis was again observed. Compound **3** was effective postharvest even though it was not on a preharvest basis. Xanthophylls again accounted for most of the increase in total carotenoids. One difference existed, however, between the two experiments. In postharvest treatment, lycopene accumulated to about 9% of the total carotenoids. The postharvest experiment was curtailed after 11 days due to the poor keeping quality of the treated fruit. The intense red color of the peel of postharvest treated fruit indicated that lycopene accumulated throughout the surface of the fruit.

There are several plausible explanations for induction of carotenoid biosynthesis without lycopene accumulation. Perhaps the bioregulator is less stable in the endocarp than in the flavedo or other carotenogenic systems. Thus, the bioregulator could be translocated into the endocarp where its structure could be altered so that it does not inhibit the cyclase(s) and cause the accumulation of lycopene. Either this altered structure or some secondary inducer synthesized in the endocarp as a result of the bioregulator

might have stimulated carotenogenesis.

Alternately, the secondary inducer might form outside and then translocate into the fruit. Visual observation of halved treated fruit supports this hypothesis. Orange streaks of color emanated from that portion of the albedo just beneath the stem and radiated out through the central core of fruit toward the endocarp. The peel of preharvest treated fruit had red blotches about the stem and along the bottom of the fruit which indicated that lycopene had accumulated in these areas.

The failure of lycopene to accumulate in the endocarp might indicate that the carotenogenic sequence of the endocarp is substantially different from that of other carotenogenic systems.

CONCLUSIONS

Carotenogenesis was induced in orange endocarp by the three synthetic bioregulators tested. Lycopene accumulation was less than normally expected for a postharvest treatment and was not detected on a preharvest basis.

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Relationship between Polyphenols and Browning in Avocado Mesocarp. Comparison between the Fuerte and Lerman Cultivars

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When avocado fruits are cut and exposed to air, the rate of browning of cv. Fuerte is much higher than that of cv. Lerman. A positive correlation was found between the tendency of the fruit to turn brown, total phenols content and polyphenol oxidase (PPO) activity, but not with *o*-dihydroxyphenols (ODHP) content. Reasonable separation of the extracted phenols was achieved by TLC chromatography. Of the large number of spots revealed, leucoanthocyanidine, catechin, and simple phenols (specifically caffeic acid) were identified. Other spots were only partially characterized. Similar phenols were identified in the mesocarp extract of both avocado cultivars. Approximation was made of the endogenous level of ODHP that can potentially be oxidized by avocado PPO. Attempts to identify, in the avocado phenol extract, ODHP which can be specifically oxidized by avocado PPO were unsuccessful, although the sensitivity of the technique was within the estimated range of detection.

We have previously reported that there are pronounced differences in the rate of browning of freshly cut mature ripe fruits of Fuerte and Lerman avocado cultivars (Kahn, 1975; Golan et al., 1977). We also showed that the postharvest behavior of fruits of the two cultivars was the same with regard to ethylene production, respiration, and softening, but that the rate of browning of Fuerte was much higher than that of Lerman throughout the ripening period (Golan et al., 1977).

The browning potential of various fruits, and sometimes in different varieties of the same cultivar, has been shown to be directly related to the phenol level, the polyphenol oxidase (PPO) activity, or a combination of these factors (Mondy et al., 1960; Mapson et al., 1963; Ranadive and Haard, 1971; Chung and Luh, 1972; Mathews and Parpia, 1975). Chubey and Nylund (1969) have demonstrated that the level of total phenols in carrot was more closely associated with the potential browning than was the level of oxidizable *o*-dihydroxyphenols (ODHP). Harel et al. (1970) have shown that both PPO activity and phenol content may determine the rate of browning of apples but that the relative importance of these factors changes at different physiological stages of the fruit. The rate of browning is usually higher in young than in more mature fruit and decreases as the fruit ripens. Likewise, the level of phenolic compounds may change as the fruit matures (Van Buren, 1970; Maier and Metzler, 1962; Ranadive and Haard, 1971).

It has already been shown in our laboratory that the PPO level in commercially mature Fuerte avocado is much higher than that in Lerman (Kahn, 1975). It was interesting to extend this comparison further and to establish whether PPO activity was the sole factor contributing to

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