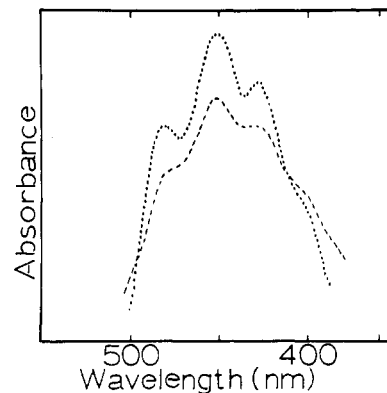**Figure 1.** The infrared spectra from 1200 to 4000 cm⁻¹ (KBr disk) of β-citraurin and β-citraurin myristate isolated from grapefruit. The absorbance values are relative.

Figure 2. The visible spectra (hexane) of β-citraurin myristate (······) and β-citraurin (------) isolated from grapefruit. The absorbance values are relative.

thin-layer plates did not give sufficient material for proper identification.

DISCUSSION

The epiphasic nature of unsaponified carotenoids of Marsh Seedless grapefruit indicates that the xanthophylls in the fruit are esterified. The occurrence of β-citraurin as its myristate ester as a major carotenoid in Marsh Seedless grapefruit is reported for the first time. β-Citraurin myristate and β-citraurin have identical visible spectra (Figure 2), indicating that no color change occurs as a result of esterification. Esterification reduces the adsorption affinity of β-citraurin on silica gel and increases the epiphasic properties. Synthetic carotenoids have limited solubility in neutral fat. Esterification increases the solubility of xanthophylls in fat, and thus can be used to advantage in coloring fat-based foods with natural or synthetic carotenoids. The significance of carotenoid esters in the stability of color and flavor during processing and storage of grapefruit products should be evaluated carefully to determine whether esterification increases the stability of β-citraurin.

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The Nature of Carotenoid Esterification in Citrus Fruits

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The isolation and characterization of laurate esters of cryptoxanthin, violaxanthin, auroxanthin, β-citraurin, and reticulataxanthin from the peel and pulp of Valencia oranges, Temple oranges, and tangelos are described.

Although fruit xanthophylls are known to be esterified (Goodwin, 1965), information on the nature of their esterification is lacking. Philip et al. (1971) isolated capsanthin dilaurate from paprika and extensive esterification of xanthophylls is believed to occur during leaf senescence (Chichester and Nakayama, 1965). Though Curl and Bailey (1956) and Curl (1967) reported the presence of xanthophyll esters in Valencia oranges, they did not determine the nature of esterification. The present investigation is undertaken to study the nature of esterification of xanthophylls in citrus fruits.

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APPARATUS

The visible spectra were recorded in spectral grade solvents with a Perkin-Elmer 202 uv visible spectrophotometer. The infrared spectra were obtained in a KBr disk with a Perkin-Elmer 337 ir spectrometer. The nmr spectra were obtained in CCl₄ with a Varian T-60 nmr spectrometer. The fatty acid analysis was carried out on a Micro-Tek 160 gas chromatograph with FID.

PROCEDURE

Three varieties of citrus fruits, viz., Valencia oranges, Temple oranges, and tangelos (a hybrid of Bowen grapefruit and Dancy tangerine), were obtained in April of 1973 from the University of Arizona Citrus Experiment Station

located at Tempe, Ariz. The fruits were hand peeled, and the peel and pulp were worked up separately.

The peels (5 kg) were blended with 50% methanol (5 l.) in a Waring blender and filtered; the pulp (5 kg) was mixed with an equal volume of 50% methanol and filtered. The cakes were extracted with acetone (10 l.). The acetone extracts were evaporated to dryness (all evaporations were carried out in a rotary evaporator under vacuum below 40°). The residue was dissolved in ethyl ether and the ether layer washed with distilled water. The ether extract was dried over anhydrous sodium sulfate and then evaporated. The experiments were conducted under subdued light and contact with air was avoided as far as possible. The yields of acetone extracts were as follows: Valencia orange peels, 34 g/5 kg; Valencia orange pulp, 25 g/5 kg; Temple orange peels, 43 g/5 kg; Temple orange pulp, 28 g/5 kg; tangelo peels, 48 g/5 kg; and tangelo pulp, 22 g/5 kg.

Carotenoid Separation. The unsaponified carotenoids were subjected to a preliminary purification on a column of neutral alumina (activity 1). The carotenoids were developed with petroleum ether. Two separated zones were observed on the column. The first zone was eluted with petroleum ether containing 2% ethyl ether. This zone was mostly essential oils and carotenes and therefore was discarded. The second zone containing carotenoid esters was eluted with petroleum ether containing 10% ethyl ether. The eluate was evaporated and the residue was subjected to tlc separation on silica gel G using acetone–petroleum ether (5 + 95) for development. The major ester bands were isolated in larger quantities by preparative tlc and were repurified on silica gel G using the same solvent system for development.

Carotenoid Identification. The purified ester was saponified with 10% KOH in methanol (2 hr at room temperature). The saponified carotenoid was extracted with ethyl ether. The ether extract was washed free of alkali, dried over anhydrous sodium sulfate, and evaporated. The saponified carotenoid was purified by tlc on silica gel G using acetone, benzene, and petroleum ether (20:10:70) for development. The purified carotenoid was identified by its physicochemical properties (Jensen and Jensen, 1965).

Ester Determination. The purified ester (1–2 mg) was hydrolyzed with 0.5 ml of 10% KOH in methanol and the fatty acid methylated by the method of Metcalfe *et al.* (1966). The methyl ester was separated on glc (12% DEGS) and identified by comparison of its retention time with authentic compound.

Chemical Tests. Reduction, esterification, and epoxide tests were carried out as described by Philip (1970).

RESULTS

On partition between 95% methanol and petroleum ether, the unsaponified carotenoids from the pulp and peel of Valencia oranges, Temple oranges, and tangelos were epiphasic. The unsaponified carotenoids separate on silica gel G thin-layer plates with a solvent system containing only 5% acetone in petroleum ether, whereas the polarity of the solvent (acetone, benzene, and petroleum ether in the ratio of 20:10:70) had to be increased for the separation of saponified carotenoids. These evidences indicated that the hydroxyl groups are not free in the citrus xanthophylls examined. The absence of –OH vibrations and the presence of ester carbonyl bands at 1725 cm^{-1} in the infrared spectra of the unsaponified carotenoids isolated from the citrus fruits examined unequivocally prove that the xanthophylls are esterified. The –OH vibrations appear and the ester bands disappear in the infrared spectra on saponification.

Thin-layer separations of the major pulp and peel carotenoids were similar except for relative proportions, and the peel contained higher amounts of total carotenoids than the pulp.

Table I. R_f Values (Silica Gel G) of Carotenoid Esters Isolated from Valencia Oranges, Temple Oranges and Tangelos, and Derivatives

	Acetone-petroleum ether, 5 + 95	Acetone-benzene-petroleum ether, 20 + 10 + 70
Cryptoxanthin	0.09	0.51
Cryptoxanthin laurate	0.91	1.00
Cryptoxanthin 5,6-epoxide	0.00	0.48
Cryptoxanthin 5,6-epoxide ester	0.89	1.00
Violaxanthin	0.00	0.20
Violaxanthin dilaurate	0.35	1.00
Auroxanthin	0.00	0.25
Auroxanthin dilaurate	0.37	1.00
β-Citraurin	0.00	0.37
β-Citraurin laurate	0.22	1.00
Reduced β-citraurin	0.00	0.21
Reticulataxanthin	0.00	0.30
Reticulataxanthin laurate	0.21	1.00
Reduced reticulataxanthin	0.00	0.20

The spectral properties of a number of carotenoid esters isolated from the peels of Valencia oranges, Temple oranges, tangelos, and related compounds are described below. The R_f values of the esters and related compounds are given in Table I. The acyl moiety was identified by glc of methyl ester of fatty acid and confirmed by synthesis.

Cryptoxanthin Laurate. This compound (λ_{max} in hexane 428, 453, and 483 nm) was present in all the citrus fruits examined. Cryptoxanthin was identified by λ_{max} in hexane 428, 454, and 482 nm; ir bands at 3300–3600, 2950, 2925, 1550, 1450, 1375, 1350, 1300, 1155, 1105, 1025, 990, and 950 cm^{-1}; nmr signals at 8.03, 8.30, 8.93, and 8.95 τ. Cryptoxanthin isolated from corn and synthesized cryptoxanthin laurate gave identical R_f values with those isolated from citrus fruits.

Cryptoxanthin 5,6-Epoxide Ester. This compound (λ_{max} in hexane 421, 444, and 472 nm) occurs as a minor carotenoid in Valencia oranges. Cryptoxanthin 5,6-epoxide was identified by λ_{max} in hexane 422, 444, and 470 nm and by the positive epoxide test. The ester could not be isolated in sufficient quantity to identify the acyl moiety.

Violaxanthin Dilaurate. This compound (λ_{max} in hexane 395, 417, and 443 nm) was present as a major carotenoid in Valencia oranges. Violaxanthin was identified by λ_{max} in hexane 395, 417, and 443 nm; ir bands at 3300–3600, 3020, 2950, 2850, 1550, 1440, 1360, 1300, 1190, 1150, 1110, 1080, 1030, 980, 950, and 780 cm^{-1}; nmr signals (Barber *et al.*, 1960) at 8.05, 8.25, 8.88 and 9.12 τ and by the positive epoxide test. The synthesized violaxanthin laurate gave an R_f value identical with that isolated from Valencia oranges. The absence of –OH vibration in the infrared spectrum of the ester indicates that both hydroxyl groups are esterified.

Auroxanthin Dilaurate. This compound (λ_{max} in hexane 364, 398, and 423 nm) was present as a minor carotenoid in Valencia oranges. Auroxanthin was identified by λ_{max} in hexane 365, 398, and 423 nm and ir bands at 3300–3600, 3020, 2950, 2850, 1550, 1505, 1440, 1350, 1250, 1150, 1080, 1050, 980, 950, and 780 cm^{-1}. Auroxanthin gave R_f value and visible spectrum identical with that prepared from violaxanthin by treating with HCl in ethanol. The absence of –OH vibration in the infrared spectrum of the ester indicates that both hydroxyl groups are esterified. The synthesized auroxanthin dilaurate gave an R_f value identical with that isolated from Valencia oranges.

Unidentified Laurate. This compound (λ_{max} in hexane 440 nm) was present as a major carotenoid in Valencia oranges. The spectral properties of the hydrolyzed carot-

enoid (λ_{max} in hexane 440 nm; ir bands at 3300–3600, 2950, 2850, 1660, 1550, 1440, 1360, 1250, 1150, 1040, and 955 cm^{-1}; nmr signals at 7.80, 8.01, 8.25, 8.95, and 9.12 τ) indicated a chromophore containing hydroxyl and methyl ketone groups. The visible spectrum gave a broad spectrum lacking in detail. The reduced carotenoid (sodium borohydride) gave a multibanded visible spectrum (λ_{max} in hexane 371, 396, 424, and 450 nm), indicating a mixture of carotenoids which are not separable by tlc with the solvent system studied. The carotenoid is unstable and decomposes rapidly to short-chain green pigments.

β-Citraurin Laurate. This compound (λ_{max} in hexane 435, 455, and 484 nm) was present both in Temple oranges and tangelos. β-Citraurin was identified by λ_{max} in hexane 438, 458, and 486 nm; ir bands at 3300–3600, 2950, 2925, 2850, 1660, 1600, 1555, 1510, 1440, 1400, 1350, 1300, 1260, 1170, 1145, 1030, 990, and 955 cm^{-1}; and nmr signals at 0.6, 8.00, 8.13, 8.23, and 8.93 τ. The hypsochromic shift of the reduced β-citraurin (λ_{max} in hexane 400, 423, and 447 nm) corresponded to one conjugated carbonyl. The synthesized β-citraurin laurate gave an R_f value identical with that isolated from tangelos and Temple oranges.

Reticulataxanthin Laurate. Reticulataxanthin laurate (λ_{max} 467 and 493 nm) was present as a major carotenoid both in Temple oranges and tangelos. Reticulataxanthin (Yokoyama et al., 1965) was identified by λ_{max} in hexane 467 and 495 nm; ir bands at 3300–3600, 2950, 2925, 2860, 1660, 1600, 1510, 1440, 1350, 1240, 1170, 1030, and 955 cm^{-1}; and nmr signals at 7.80, 8.03, 8.24, and 8.93 τ. The hypsochromic shift of the reduced reticulataxanthin (λ_{max} in hexane 425, 445, and 473 nm) corresponded to one conjugated carbonyl group. The synthesized reticulataxanthin laurate gave an R_f value identical with that isolated from Temple oranges and tangelos.

Minor Carotenoid Esters. Several minor carotenoid esters were present in the citrus fruits studied. These esters could not be isolated in sufficient quantities for proper identification. The minor esters from Valencia oranges, tangelos, and Temple oranges were combined separately, and the combined esters on hydrolysis and methylation gave methyl laurate, methyl palmitate, and methyl myristate (identified by comparison of retention time with authentic compounds on gas–liquid chromatography).

Discussion. The epiphasic nature of unsaponified carotenoids of Valencia oranges, Temple oranges, and tangelos indicates that most, if not all, the xanthophylls in these fruits are esterified. The major xanthophylls in these fruits are esterified with lauric acid. Although esterification does not change the visible light absorption properties, esterification increases the solubility of xanthophylls in lipids with which they are associated in nature. Carotenoids impart a pleasing yellow to orange color to citrus juices and the role of carotenoid esters in the stability of color and flavor of citrus frjuices is unknown. The carbonyl-containing carotenoids predominate in the Temple oranges and tangelos, whereas epoxides predominate in Valencia oranges.

ACKNOWLEDGMENT

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