

Nature of Xanthophyll Esterification in Grapefruit

Thomas Philip

The isolation and characterization of β -citraurin myristate from the pulp and peel of grapefruit are described. The partition and adsorption behavior of unsaponified carotenoids of Marsh

Seedless grapefruit indicate that most, if not all, of the xanthophylls are esterified. The presence of β -citraurin in Marsh Seedless grapefruit is reported for the first time.

Fruit xanthophylls are known to occur in an esterified form (Goodwin, 1965). Although Yokoyama and White (1967) studied the carotenoids of Marsh Seedless grapefruit, they did not determine the nature of esterification. Although citrus carotenoids have been studied extensively (Kefford and Chandler, 1970), very little is known about the nature of esterification of xanthophylls in citrus fruits. The present investigation was undertaken to study the nature of esterification of xanthophylls in grapefruit.

PROCEDURE

Carotenoid Extraction. The grapefruits (Marsh Seedless \times Cleopatra mandarin) were obtained in March of 1973 from the University of Arizona Citrus Experiment Station located at Tempe, Ariz. The carotenoids from the pulp and peel of the grapefruit were extracted separately as follows. The natural material (1 kg) was soaked in 50% methanol (2 l.) at room temperature for 2 hr, blended in a Waring blender, and filtered, and the cake extracted exhaustively with acetone. The combined extracts (5 l.) were evaporated under vacuum below 40° and the residue was dried in a desiccator overnight.

Carotenoid Separation. The crude lipids from pulp and peel were partitioned between 95% methanol and petroleum ether. The petroleum ether layer was evaporated and the residue was dissolved in chloroform and subjected to thin-layer chromatography on silica gel G, using acetone in petroleum ether (10 + 90) for development. In this solvent system, triglycerides and hydrocarbons move along with the solvent and the free xanthophylls do not move at all. The separated bands are essentially xanthophyll esters (Philip *et al.*, 1971). One major ester band was isolated in larger quantities and repurified by thin-layer chromatography on silica gel G using the same solvent system.

The ester band was hydrolyzed with 10% methanolic KOH at room temperature for 2 hr. The hydrolyzed pigment was extracted with ethyl ether, washed free of alkali, dried over anhydrous sodium sulfate, and the ether removed under vacuum at room temperature. The hydrolyzed pigment was purified by thin-layer chromatography on silica gel G using acetone, benzene, and petroleum ether (20:10:70) for development.

Carotenoid Identification. R_f values (silica gel G), visible (Perkin-Elmer 202 uv visible spectrophotometer), infrared (Perkin-Elmer 337 ir spectrophotometer), nmr (Varian T60 nmr spectrometer) spectra, and chemical tests were used for the identification of carotenoids.

Gas Chromatography. The β -citraurin ester was methylated by the method of Metcalfe *et al.* (1966) and separated by glc (12% DEGS), and the methyl ester was identified by comparison of its retention time with authentic compounds.

Reduction. β -Citraurin (2-5 mg) isolated from grapefruit was dissolved in ethanol (10 ml) and an excess of sodium borohydride (1 g) was added. When the reduction

was complete (30 min), the mixture was transferred to a separatory funnel containing ethyl ether (50 ml). The mixture, after shaking, was washed with distilled water, and the ether layer was dried over anhydrous sodium sulfate and evaporated. The reduced pigment was purified by thin-layer chromatography on silica gel G using acetone, benzene, and petroleum ether (20:10:70) for development.

Preparation of Myristate. β -Citraurin (2-5 mg) isolated from grapefruit was dissolved in an excess of pyridine (10 ml) and the solution was transferred to a separatory funnel. Myristoyl chloride (10 drops) was added dropwise and the mixture was shaken vigorously for 5 min. Petroleum ether (100 ml) was added and the mixture, after shaking, was washed with dilute HCl (1 + 2) followed by distilled water and the solvent removed under vacuum. The myristate was purified by thin-layer chromatography on silica gel G using acetone and petroleum ether (5 + 95) for development.

RESULTS

On partition between 95% methanol and petroleum ether, the unsaponified carotenoids from the pulp and peel of grapefruit were epiphasic. The unsaponified carotenoids separate well 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 grapefruit xanthophylls.

The infrared spectrum (Figure 1) of the unhydrolyzed pigment isolated from the pulp and peel of grapefruit exhibited strong absorption bands at 1750 cm^{-1} , indicating esterification. The ester moiety was identified as myristic acid by methylation and subsequent gas chromatography.

The pigment ester, R_f 0.37 on silica gel G and acetone-petroleum ether (5 + 95), gave β -citraurin on hydrolysis. β -Citraurin was identified by visible (Yokoyama and White, 1966), infrared, and nmr spectra: λ_{max} (hexane) 425, 452, and 480 nm, (benzene) 458 and 496 nm; ir bands in KBr disk 3400-3500 ($-\text{OH}$), 2900, 1660 (conjugated $\text{C}=\text{O}$), 1600, 1500, 1350, 1250, 1160, 1040, 990, and 950 cm^{-1} ; nmr (CCl_4) τ 0.6 ($-\text{CHO}$), 8.03 (in-chain methyls), 8.12 (vinyl methyl α to $>\text{C}=\text{O}$), 8.27 (cyclohexenyl methyl), and 8.97 (*gem*-dimethyls). The visible spectrum (Figure 2) indicated a chromophore similar to apocarotenals. The ir spectrum showed the presence of conjugated carbonyl (1660 cm^{-1}). The hypochromic shift of the reduced β -citraurin (λ_{max} hexane 394, 428, and 453 nm, R_f 0.20 on silica gel G and acetone-benzene-petroleum ether (20 + 10 + 70)) corresponded to one conjugated carbonyl. The strong $-\text{OH}$ vibration (3400-3500 cm^{-1}) in the ir spectrum indicated the presence of an hydroxyl group.

The synthesized β -citraurin myristate gave an R_f value identical with that of the ester isolated from grapefruit. Only β -citraurin myristate could be isolated in sufficient quantities to study the nature of esterification of xanthophylls in grapefruit. The other ester bands observed on

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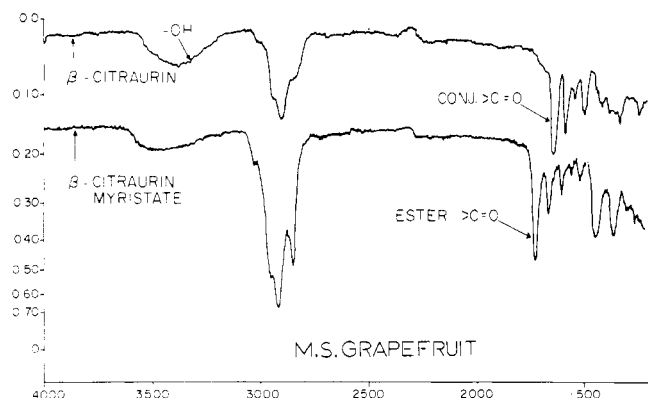


Figure 1. The infrared spectra from 1200 to 4000 cm^{-1} (KBr disk) of β -citraurin and β -citraurin myristate isolated from grapefruit. The absorbance values are relative.

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 de-

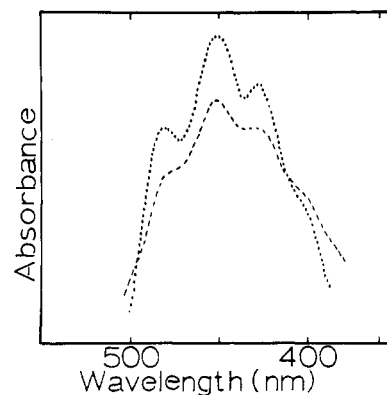


Figure 2. The visible spectra (hexane) of β -citraurin myristate (.....) and β -citraurin (-----) isolated from grapefruit. The absorbance values are relative.

termine whether esterification increases the stability of β -citraurin.

ACKNOWLEDGMENT

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The Nature of Carotenoid Esterification in Citrus Fruits

Thomas Philip

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 CCl_4 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