by nonpolar lipids while the remainder comprised GL and PL in approximately equal concentrations. The chief constituents of the nonpolar lipids GL and PL were pigments, MGDG and PC, respectively (Table I). The major fatty acids were 18:2 and 16:0 except for 18:3 in the MGDG and DGDG (Table VII).

ABBREVIATIONS USED

GLC, gas–liquid chromatography; TLC, thin-layer chromatography; GL, glycolipids; PL, phospholipids; MGDG, monogalactosyl diglycerides; DGDG, digalactosyl diglycerides; SQDG, sulfiquinovosyl diglycerides; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PG, phosphatidylglycerol; TAG, triacylglycerols; DAG, diacylglycerols; MAG, monoacylglycerols; ESG, esterified steryl glycosides. Fatty acids are denoted by the number of carbon atoms followed after colon by number of double bonds.

ACKNOWLEDGMENT

K.S.R. thanks the Council of Scientific & Industrial Research for a Senior Research Fellowship.

LITERATURE CITED

Gopalakrishnan, N.; Kaimal, T. N. B.; Lakshminarayana, G. "Fatty Acid Changes in *Hibiscus esculentus* Tissues during Growth". *Phytochemistry* **1982a**, *21*, 565–568.

Gopalakrishnan, N.; Kaimal, T. N. B.; Lakshminarayana, G. "Fatty Acid Changes in *Althea rosea* Tissues during Growth". *Phytochemistry* **1982b**, *21*, 2205–2208.

Hawke, J. C. "Lipids". In *Chemistry and Biochemistry of Herbage*, 1st ed.; Butter, G. W. Bailey, R. W., Eds.; Academic: London, 1973; Vol. 1, pp 213–263.

Hitchcock, C.; Nichols, B. W. "The Lipid and Fatty Acid Composition of Specific Tissues". In *Plant Lipid Biochemistry*; Academic: New York, 1971: pp 59–95.

Kaimal, T. N. B.; Lakshminarayana, G. "Fatty Acid Compositions of Lipids Isolated from Different Parts of *Ceiba pentandra, Sterculia foetida* and *Hydnocarpus wightiana*". *Phytochemistry* **1970**, *9*, 2225–2228.

Kates, M. "Chromatographic and Radioisotopic Investigations of the Lipid Components of Runner Bean leaves". *Biochim. Biophys. Acta* **1960**, *41*, 315–328.

Kates, M.; Marshall, M. O. "Biosynthesis of Phosphoglycerides in Plants". In *Recent Advances in the Chemistry and Biochemistry of Plant Lipids*; Galliard, T., Mercer, E. I., Eds.; Academic: New York, 1975; pp 115–159.

Klopfenstein, W. E.; Shigley, J. W. "Changes in Fatty Acid Composition of Sulfolipid and Phospholipids during Maturation of Alfalfa". *J. Lipid Res.* **1967**, *8*, 350–358.

Lakshminarayana, G. Pantulu, A. J.; Rao, K. S. "Lipid Class and Fatty Acid Compositions of Young *Amaranthus gangeticus* L. Leaves". *J. Agric. Food Chem.* **1984**, *32*, 1361–1363.

O'Brien, J. S.; Benson, A. A. "Isolation and Fatty Acid Composition of the Plant Sulfolipid and Galactolipids". *J. Lipid Res.* **1964**, *5*, 432–436.

Weenink, R. O. "Acetone-Soluble Lipids of Grasses and other Forage Plants, I—Galactolipids of Red-Clover (*Trifolium pratense*) Leaves". *J. Sci. Food Agric.* **1961**, *12*, 34–38.

Weenink, R. O. "Minor Constituents of the Acetone-Soluble Lipids of Red-Clover (*Trifolium pratense*) Leaves". *Biochem. J.* **1962**, *82*, 523–527.

Wintermans, J. F. G. M. "Concentrations of Phosphatides and Glycolipids in Leaves and Chloroplasts". *Biochim. Biophys. Acta* **1960**, *44*, 49–54.

Zill, L. P.; Harmon, E. A. "Lipids of Photosynthetic Tissue, I. Silicic Acid Chromatography of the Lipids from Whole Leaves and Chloroplasts". *Biochim. Biophys. Acta* **1962**, *57*, 573–583.

Received for review May 6, 1987. Accepted November 16, 1987.

cis-Canthaxanthin and Other Carotenoid-like Compounds in Canthaxanthin Preparations

Susan Taylor Mayne[1] and Robert S. Parker*

Canthaxanthin is a nonprovitamin A carotenoid used as a food coloring agent, oral skin-coloring agent, and research tool in the investigation of biological effects of carotenoids. Reversed-phase HPLC analyses of extracts of canthaxanthin beadlets, capsules, and tablets showed the presence of four closely eluting compounds with absorption maxima ranging from 450 to 474 nm. One peak cochromatographed with and had an absorption spectrum identical with that of *all-trans*-canthaxanthin, and another had an absorption spectrum identical with that of *cis*-canthaxanthin. The ratio of *all-trans*- to *cis*-canthaxanthin in these preparations was approximately 3:1. *cis*- and *all-trans*-canthaxanthin and the two unknown carotenoid-like compounds were absorbed by chicks when fed in beadlet form, as evidenced by the presence of all four compounds in extracts of chick liver and hepatic membranes. The four compounds had different photosensitivities and may have different biological effects; therefore, determination of isomeric composition may be of importance in the interpretation of studies involving canthaxanthin.

Canthaxanthin (4,4′-diketo-β-carotene) is a naturally occurring carotenoid pigment found in several marine species, birds, edible mushrooms, and algae (Klaui and Bauernfeind, 1981). Canthaxanthin is also used in synthetic form as a color additive in human foodstuffs, as an over-the-counter oral skin-coloring (tanning) agent for human use, as a poultry feed additive to obtain a desired color in body fat and egg yolks, and for a number of research purposes. Canthaxanthin is very similar in structure to the well-known provitamin A carotenoid β-carotene but has no vitamin A activity as both β-ionone rings are substituted with oxygen (Figure 1). Since canthaxanthin cannot be metabolized to retinol, it is a potentially valuable research tool for differentiating carotenoid effects from effects associated with vitamin A.

Canthaxanthin, like most carotenoids, is lipid-soluble and susceptible to oxidative degradation. However, a stabilized beadlet form has been developed that is dispersible in aqueous solutions. These beadlets contain

Division of Nutritional Sciences, Cornell University, Ithaca, New York 14853.

[1] Present address: Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT 06510.

0021-8561/88/1436-0478$01.50/0 © 1988 American Chemical Society

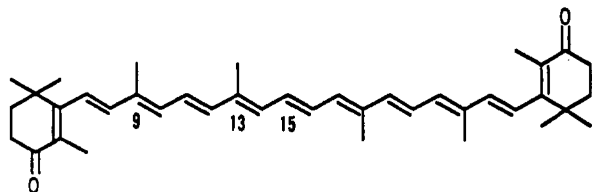

Figure 1. *all-trans*-Canthaxanthin (4,4′-diketo-β-carotene). Sterically unhindered carbon numbers are indicated.

emulsified canthaxanthin encapsulated in a gelatin or starch matrix and may contain a variety of antioxidants such as ascorbyl palmitate, α-tocopherol, butylated hydroxytoluene, and butylated hydroxyanisole. Canthaxanthin beadlets are used commercially for coloring tomato products, water-based products, and simulated meat products (Klaui and Bauernfeind, 1981). Due to the ease of incorporation of beadlets into diets, several investigators are currently using beadlet preparations of canthaxanthin and β-carotene, in addition to carotenoid-free placebo beadlets, in a variety of research areas (Mathews-Roth, 1982; Tyczkowski and Hamilton, 1986; Wamer et al., 1985; Mathews-Roth et al., 1977; Heywood et al., 1985; Parker, 1985; Alam and Alam, 1983; Mathews-Roth and Krinsky, 1984, 1985; Epstein, 1977; Bendich and Shapiro, 1986).

Canthaxanthin is also available over the counter in tablet and capsule forms. Canthaxanthin tablets and capsules have been used extensively in Canada and Europe as oral skin-coloring (tanning) products. To achieve desired skin pigmentation, individuals generally consume 120 mg of canthaxanthin/day (Boudreault et al., 1983; Gupta et al., 1985). Canthaxanthin has not been approved for use as a skin-coloring agent in the United States.

In the course of our studies we found that high-performance liquid chromatographic (HPLC) analyses of extracts of canthaxanthin beadlets monitored at 460 nm showed the presence of four closely eluting compounds. In this report we describe (i) the spectral characteristics of these compounds to aid in their identification, (ii) the effect of UV irradiation on their formation and degradation, (iii) the occurrence of these compounds in various types of canthaxanthin preparations, and (iv) the incorporation of these substances into tissues following oral administration.

MATERIALS AND METHODS

Apparatus. The HPLC system employed consisted of a Beckman 421 controller, Beckman 110A pump, a 4.6 × 250 mm column of 5-μm Ultrasphere ODS (Beckman Instruments, Altex Division, San Ramon, CA), and a Hitachi 100-10 UV/vis variable-wavelength detector. Eluting peaks were plotted and quantified by a Hewlett-Packard 3390A plotter-integrator, used with a full-scale voltage deflection at 63 μV. *all-trans*-Canthaxanthin was partially identified by comparison of retention time with that of a standard (Hoffmann-La Roche, Inc., Nutley, NJ). Spectral analyses of the carotenoid-like beadlet constituents were done on line with the HPLC using a variable-wavelength photodiode-array detector (Hewlett-Packard 1040A) coupled to a Hewlett-Packard 85B data processor. Spectra corresponding to each of the four peaks were plotted from 250 to 550 nm, and adsorption maxima (±1 nm) were calculated by the data processor. The mobile phase used for all HPLC analyses was methanol–water (95:5, v/v) at a flow rate of 2.3 mL/min. Samples and standards were injected with ethanol–water (95:5) as the solvent.

Reagents. HPLC-grade solvents were used for all HPLC analyses. Crystalline *all-trans*-canthaxanthin (>90%), placebo (carotenoid-free) beadlets, β-carotene beadlets (10% carotenoid by weight), and α-tocopherol-free canthaxanthin beadlets (Roxanthin Red 10; 10% carotenoid by weight; Lot 677073) were provided by Hoffmann-La Roche. Canthaxanthin tablets (Horizon, Horizon Natural Products, Soquel, CA) and canthaxanthin capsules (Natrol, Chatsworth, CA) were purchased locally. All other chemicals were reagent grade or better.

Procedures. *Sample Extraction.* Placebo, β-carotene, and canthaxanthin beadlets were briefly solubilized in 50 °C distilled water, extracted initially with hexane–2-propanol (3:2, v/v) containing 0.02% of each of the antioxidants butylated hydroxytoluene, butylated hydroxyanisole, and pyrogallol and then extracted repeatedly with hexane until the aqueous phase was colorless. Canthaxanthin capsules were similarly extracted after the contents were removed from the gelatin capsule. Canthaxanthin tablets were ground in a mortar and pestle, solubilized in water, and then extracted as described above.

Liver samples were obtained from white leghorn chicks fed semipurified carotenoid-free diets (Mercurio and Combs, 1985) containing either placebo beadlets or canthaxanthin beadlets (5.0 g of beadlets/kg diet) from hatching. At 5 weeks of age, chicks were killed by cervical dislocation, and livers were removed, weighed, and frozen at −70 °C pending analysis. Livers were partially thawed at room temperature; 2.0-g portions were removed, homogenized, and extracted three times with hexane–2-propanol (3:2, v/v) containing antioxidants as specified above. Extracts were then saponified in 4% ethanolic potassium hydroxide at 55 °C for 8 min, then reextracted twice with hexane, and analyzed by HPLC as described above. Hepatic membrane extracts were prepared by homogenizing the postnuclear 105000*g* pellet in ice-cold 0.1 M potassium phosphate buffer (pH 7.4) and extracting three times with the extraction solvent described above. All steps were performed in dim light.

Irradiation. Crystalline canthaxanthin was diluted with methanol to an initial concentration of 4.21 μg of *all-trans*-canthaxanthin/mL (HPLC analysis) and portioned into 2.0-dram (approximately 7.5 mL) screw-cap glass vials sealed with Teflon-lined caps under an atmosphere of air. For irradiation, a phototherapy unit (Blue Light 2002, Dr. Hönle, Munich, West Germany) with a high-pressure 400-W metal halide lamp was used. Emitted light was filtered so that the emission spectrum was primarily in the UV A range. UV A irradiation measurements were made with a calibrated National Biological Corp. spectral radiometer (Model LMA-302), and UV B was measured with a calibrated International Light radiometer (Model IL-443). Aliquots were irradiated for varying periods of time. Following irradiation, aliquots were immediately placed in the dark on ice and then analyzed by HPLC at 460 nm as described above.

RESULTS AND DISCUSSION

HPLC analyses of extracts of canthaxanthin beadlets run at 460 nm showed the presence of four peaks (Figure 2A), one of which (peak 2) eluted with a retention time identical with that of *all-trans*-canthaxanthin. With the aid of a photodiode array detector, spectra of the four peaks were obtained (Figure 3). The spectrum of the peak identified as *all-trans*-canthaxanthin was identical with that of the *all-trans*-canthaxanthin standard showing a single absorption maximum at 474 nm, consistent with reported spectra of *all-trans*-canthaxanthin (Nelis et al., 1984). The spectrum of peak 3 indicates that this component is a cis form of canthaxanthin, showing a characteristic cis absorption maximum at 364 nm, as well as an 8-nm hypsochromic shift in the absorption maximum from

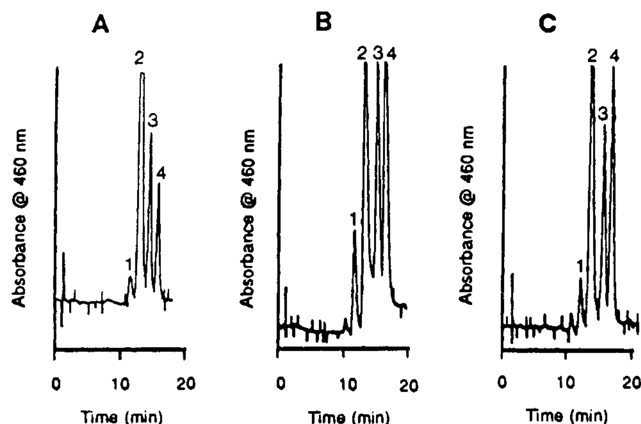

Figure 2. Representative HPLC chromatograms showing *all-trans*-canthaxanthin (peak 2), *cis*-canthaxanthin (peak 3), and the two unknown compounds (peaks 1 and 4) in (A) canthaxanthin beadlet extraction, (B) liver extract from canthaxanthin-fed chick, and (C) hepatic membrane (postnuclear 105000*g* pellet) extract from canthaxanthin-fed chick. Peak areas (peak 1 to *all-trans*-canthaxanthin to *cis*-canthaxanthin to peak 4) relative to *all-trans*-canthaxanthin for the three samples are as follows: (A) 3:100:23:8; (B) 19:100:77:117; (C) 9:100:38:58.

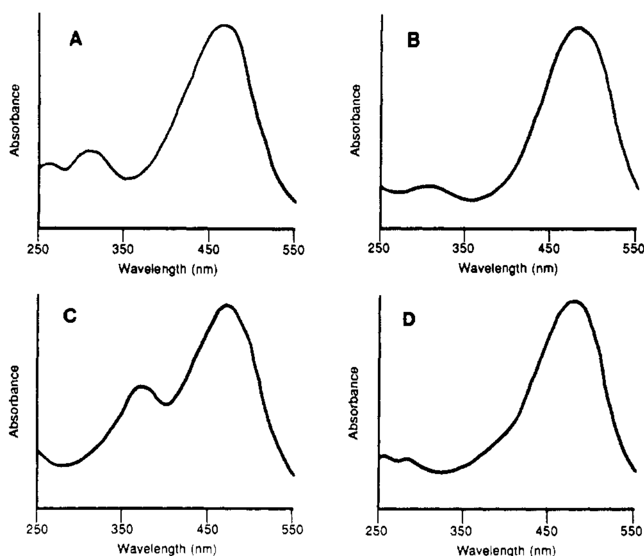

Figure 3. Spectra of *cis-* and *all-trans*-canthaxanthin and the two unknown compounds: (A) peak 1, absorption maximum 450 nm; (B) peak 2, identified as *all-trans*-canthaxanthin, absorption maximum 474 nm; (C) peak 3, identified as *cis*-canthaxanthin, absorption maxima 466 and 364 nm; (D) peak 4, absorption maximum 468 nm. All spectra were automatically expanded to full-scale.

474 nm (all-trans maximum) to 466 nm, consistent with the report of Nelis et al. (1984). All-trans carotenoids are known to show longer wavelengths of maximum absorption than any of their cis isomers (Zechmeister, 1962). Further evidence confirming that peak 3 is a cis form of canthaxanthin is that the wavelength difference between the cis absorption maximum (364 nm) and the absorption maximum of *all-trans*-canthaxanthin (474 nm) is 110 nm, identical with the value reported by Zechmeister (1962). It is possible that peak 3 is a collective cis peak, containing unresolved mono-cis isomers of canthaxanthin, such as 9-*cis*-, 13-*cis*, and 15-*cis*-canthaxanthin (Nelis et al., 1984, 1986), which are the most probable cis isomers as these positions are sterically unhindered (Zechmeister, 1962). The two unidentified peaks (1, 4) also appear to be carotenoid-like, showing absorption maxima at 450 and 468 nm, respectively. Since peaks 1 and 4 have shorter wavelengths

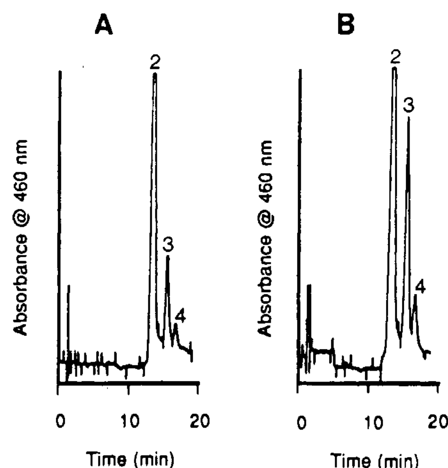

Figure 4. HPLC chromatograms of (A) crystalline canthaxanthin freshly diluted in 95% ethanol and (B) diluted crystalline canthaxanthin stored in 95% ethanol at −20 °C in the dark for 2 months. For (A), the area ratios of *all-trans*-canthaxanthin (peak 2) to peaks 3 and 4 are 8.7 and 26.5, respectively; for (B) the corresponding ratios are 3.4 and 10.8.

of maximum absorption than *all-trans*-canthaxanthin, but similar characteristics, this suggests that peaks 1 and 4 are also cis isomers of canthaxanthin. The absence of a cis absorption maximum at 364 nm does not preclude peaks 1 and 4 from being cis isomers, as this cis absorption maximum is primarily associated with certain highly bent mono-cis structures, such as 13- or 15-cis carotenoids (Zechmeister, 1962). However, for the purposes of this discussion, we will restrict the term *cis*-canthaxanthin to refer to peak 3, fully recognizing that peaks 1 and 4 are likely to be *cis*-canthaxanthin isomers.

To eliminate the possibility that the compounds were unique to the manufacturer's lot analyzed, we examined a different canthaxanthin beadlet preparation containing α-tocopherol as an antioxidant (Dry Canthaxanthin, 10% SD, Lot 915055, Hoffmann-La Roche). HPLC analysis showed the presence of *cis-* and *all-trans*-canthaxanthin and the unknown compounds in similar proportions to those previously observed in the α-tocopherol-free beadlets. This implies that the presence or absence of α-tocopherol in beadlets did not affect the occurrence of these compounds. Extracts of placebo and β-carotene beadlets were also analyzed by HPLC for the presence of these non-*all-trans*-canthaxanthin chromophores. None were found, further suggesting that the compounds were related to canthaxanthin.

Crystalline *all-trans*-canthaxanthin freshly diluted in 95% ethanol showed traces of *cis*-canthaxanthin and peak 4, but not of peak 1 (Figure 4A). When the diluted crystalline canthaxanthin solution was stored at −20 °C in the dark for 2 months, increases in *cis*-canthaxanthin and peak 4 relative to *all-trans*-canthaxanthin were observed (Figure 4B). These results further suggest that peak 4 may be an isomer of canthaxanthin and that a solution containing primarily *all-trans*-canthaxanthin may isomerize to form *cis*-canthaxanthin during storage. Nelis et al. (1984) also reported stereoisomerization of *cis*-canthaxanthin to an equilibrium level of 40% *cis*-canthaxanthin and 60% *all-trans*-canthaxanthin. This relative isomeric content was calculated from HPLC peak heights, correcting for different molar absorption coefficients of *cis-* and *all-trans*-canthaxanthin (the molar absorption coefficient for the all-trans form is roughly 1.5-fold greater than the molar absorption coefficient of the cis form; Nelis et al., 1984). We performed similar calculations on our data

Table I. Recovery of *cis*- and *all-trans*-Canthaxanthin and Peak 4 following Irradiation

irradiation, J	% remaining		
	all-trans-canthaxanthin	*cis*-canthaxanthin	peak 4
0	100	100	100
5	74	47	100
10	85	31	109
20	66	21	135

using peak areas and found that the freshly diluted crystalline canthaxanthin (Figure 4A) contained approximately 93% *all-trans*-canthaxanthin and 7% *cis*-canthaxanthin; after 2 months of freezer storage the canthaxanthin solution contained approximately 84% *all-trans*- and 16% *cis*-canthaxanthin. Assuming that this solution was not at equilibrium, these data are also consistent with that of Zechmeister (1962), who showed that carotenoids in general undergo spontaneous isomerization to an equilibrium level of 40–60% unchanged all-trans carotenoid.

To determine whether the formation of peaks 1, 3, and 4 could be catalyzed by light, crystalline canthaxanthin (containing *all-trans*- and *cis*-canthaxanthin and peak 4; see Figure 4A) was diluted with methanol and aliquots were irradiated for varying periods of time. Peak 4 increased slightly with irradiation (Table I), further suggesting that peak 4 is an isomer of canthaxanthin. Irradiation resulted in a net loss of *cis*-canthaxanthin, and of *all-trans*-canthaxanthin to a lesser extent. The loss of *cis*- and *all-trans*-canthaxanthin as a result of irradiation is not surprising, as photodegradation of carotenoids competes with photostereoisomerization, especially when samples are in the presence of air (Zechmeister, 1962). Light may have catalyzed the formation of cis isomers or other compounds that were then rapidly photodegraded. A separate irradiation experiment involving canthaxanthin beadlet extracts showed that peak 1 was very unstable to UV light, with none detected after 5 J of irradiation (data not shown). The apparent relative stability of the four compounds to UV light in the presence of air can thus be summarized as follows: peak 4 > *all-trans*-canthaxanthin > *cis*-canthaxanthin > peak 1. Others have reported increased photosensitivity of cis carotenoids, especially mono-cis isomers, which have a highly bent, unstable molecular shape (Zechmeister, 1962) relative to all-trans forms.

The investigation of these four compounds was extended by analyzing extracts of over-the-counter canthaxanthin capsules and tablets. HPLC analyses of extracts of Natrol and Horizon products showed that both preparations contained all four compounds in nearly identical proportions as follows: *all-trans*-canthaxanthin contributed 73%, *cis*-canthaxanthin 19%, peak 4 6%, and peak 1 2% of the total area units detected at 460 nm. Again, correcting for differences in the molar absorption coefficients of *cis*- and *all-trans*-canthaxanthin, we calculated that these preparations contained approximately 28% *cis*- and 72% *all-trans*-canthaxanthin. If peaks 1 and 4 are also cis isomers of canthaxanthin, then the percentage of canthaxanthin in the all-trans form is overestimated. Also, since *all-trans*-canthaxanthin is more stable than *cis*-canthaxanthin, the percentage of canthaxanthin as the all-trans form may be an overestimation due to selective loss of the cis form during sample preparation.

The presence of these compounds in extracts of canthaxanthin beadlets, tablets, and capsules may have clinical relevance, particularly if they are absorbable. Therefore, we fed canthaxanthin beadlets to chicks, which are an appropriate animal model as they absorb a wide spectrum of intact carotenoids, as do humans (Klaui and Bauernfeind, 1981). Liver and membrane extracts from chicks consuming canthaxanthin beadlets showed the presence of *cis*- and *all-trans*-canthaxanthin and the two unknown compounds (Figure 2B,C). None of the four peaks were found in hepatic extracts from placebo beadlet fed chicks. The presence of the four peaks in liver extracts from canthaxanthin beadlet fed chicks implies that the chicks were absorbing and accumulating the four compounds. The carotenoid profile of the tissues differed slightly from that of the beadlets, with the tissues having a greater relative amount of peak 4 and peak 3. This could be due to either a differential absorption of the compounds, preferential storage or uptake of cis isomers by this tissue, or biostereoisomerization of the compounds in the gut as reported by Deuel et al. (1951). It is unlikely that saponification played a role in the observed tissue enrichment in peak 4, as the unsaponified membrane extracts (Figure 2C) also showed enrichment relative to the beadlets, and others have reported that cis–trans isomerization did not occur during moderate saponification of β-carotene (Schwartz and Patroni-Killam, 1985). It is unlikely that peaks 1 and 4 and *cis*-canthaxanthin were formed during storage for three reasons: (i) Tissues analyzed after 3 weeks of storage showed proportions of peaks 1, 3, and 4 relative to peak 2 similar to those of tissue reanalyzed at a much later date (data not shown). (ii) Figure 4 suggests that conversion or degradation of *all-trans*-canthaxanthin to peaks 3 and 4 occurs at a relatively slow rate at −20 °C and the tissues were stored at −70 °C. (iii) Peak 1 was not detected in a solution of crystalline canthaxanthin after storage (Figure 4) but was present in the beadlet and tissue extracts.

The presence of *cis*-canthaxanthin and carotenoid-like compounds of unknown structure in a variety of canthaxanthin preparations indicates the importance of employing appropriate analytical methodology to assess carotenoid preparation purity and sample composition, regardless of the carotenoid employed. Cis isomers of carotenoids have been reported to occur naturally in fruits and vegetables (Bushway, 1986; Schwartz and Patroni-Killam, 1985) and to form during heat processing (Schwartz and Patroni-Killam, 1985). The proximity of absorption maxima of cis and all-trans carotenoids precludes the analysis of all-trans carotenoid content by simple spectrophotometric measurement of sample extract absorbance at 460 nm. Likewise, certain HPLC methods may not be appropriate; canthaxanthin beadlet extracts analyzed by HPLC at 460 nm using a mobile phase of acetonitrile–chloroform–water (78:20:2, v/v/v), more typical of mobile phases used for carotenoid analyses, yielded essentially one peak and appeared to be greater than 97% pure (retention time 2 min at a flow rate of 1.5 mL/min). Inappropriate methodology may thus overestimate the all-trans carotenoid content or underestimate total carotenoid content since cis isomers have substantially lower molar absorptives than their all-trans counterparts (Zechmeister, 1962).

We have shown that canthaxanthin isomers have different chemical stabilities, and they may also have different biological effects. Cis–trans bonds in part determine molecular spatial configuration, which may be important for biological activity. For example, 11-*cis*-retinal will combine with opsin to form rhodopsin, but *all-trans*-retinal and 13-*cis*-retinal will not (Zechmeister, 1962). Cis isomers of provitamin A carotenoids also show different vitamin A potencies relative to their all-trans counterparts as reviewed by Zechmeister (1962).

It has been reported (Boudreault et al., 1983; Rousseau, 1983; Ros et al., 1985) that individuals using oral canthaxanthin preparations for prolonged periods for skin-coloring purposes and canthaxanthin in combination with β-carotene for the treatment of photosensitivity disorders may develop golden crystalline deposits of unknown composition in the inner layers of the retina. These deposits have been associated with minor visual field defects in some patients and may be irreversible (Ros et al., 1985). These deposits do not occur in individuals consuming β-carotene for photosensitivity disorders (Poh-Fitzpatrick and Barbera, 1984). Since cis isomers and other carotenoid-like substances appear to be consistently associated with oral canthaxanthin preparations, but not β-carotene preparations, their potential involvement in the etiology of the crystalline deposition must be considered.

ACKNOWLEDGMENT

We thank James Conboy for technical assistance with the photodiode array detector, Dr. Daphne Roe for technical assistance with the irradiation experiments, and Dr. Gerald F. Combs, Jr., for providing the chicks and their housing.

Registry No. Canthaxanthin, 514-78-3; *cis*-canthaxanthin, 111058-45-8.

LITERATURE CITED

Alam, S. Q.; Alam, B. S. "Lipid Peroxide, alpha-Tocopherol and Retinoid Levels in Plasma and Liver of Rats Fed Diets Containing beta-Carotene and 13-cis Retinoic Acid". *J. Nutr.* **1983**, *113*, 2608–2614..

Bendich, A.; Shapiro, S. S. "Effect of beta-Carotene and Canthaxanthin on the Immune Responses of the Rat". *J. Nutr.* **1986**, *116*, 2254–2262.

Boudreault, G.; Cortin, P.; Corriveau, L. A.; Rousseau, A. P.; Tardif, Y.; Malenfant, M. "La Rètinopathie a la Canthaxanthine: 1. Etude Clinique de 51 Consummateurs". *Can. J. Ophthalmol.* **1983**, *18*, 325–328.

Bushway, R. J. "Determination of α- and β-Carotene in Some Raw Fruits and Vegetables by High-Performance Liquid Chromatography". *J. Agric. Food Chem.* **1986**, *34*, 409–412.

Deuel, H. J., Jr.; Ganguly, J.; Koe, B. K.; Zechmeister, L. "Stereoisomerization of the Polycis Compounds, Pro-gamma-carotene and Prolycopene in Chickens and Hens". *Arch. Biochem. Biophys.* **1951**, *33*, 143–149.

Epstein, J. H. "Effects of beta-Carotene on Ultraviolet Induced Cancer Formation in the Hairless Mouse Skin". *Photochem. Photobiol.* **1977**, *25*, 211–213.

Gupta, A. K.; Haberman, H. F.; Pawlowski, D.; Shulman, G.; Menon, I. A. "Canthaxanthin". *Int. J. Dermatol.* **1985**, *24*, 528–532.

Heywood, R.; Palmer, A. K.; Gregson, R. L.; Hummler, H. "The Toxicity of beta-Carotene". *Toxicology* **1985**, *36*, 91–100.

Klaui, H.; Bauernfeind, J. C. "Carotenoids as Food Color". In *Carotenoids as Colorants and Vitamin A Precursors*; Bauernfeind, J. C., Ed.; Academic: New York, 1981; pp 47–317.

Mathews-Roth, M. M. "Antitumor Activity of beta-Carotene, Canthaxanthin and Phytoene". *Oncology* **1982**, *39*, 33–37.

Mathews-Roth, M. M.; Krinsky, N. I. "Effect of Dietary Fat Level on UV-B Induced Skin Tumors". *Photochem. Photobiol.* **1984**, *40*, 671–673.

Mathews-Roth, M. M.; Krinsky, N. I. "Carotenoid Dose Level and Protection against UV-B Induced Skin Tumors". *Photochem. Photobiol.* **1985**, *42*, 35–38.

Mathews-Roth, M. M.; Hummel, D.; Crean, C. "The Carotenoid Content of Various Organs of Animals Administered Large Amounts of beta-Carotene". *Nutr. Rep. Int.* **1977**, *16*, 419–423.

Mercurio, S. D.; Combs, G. F., Jr. "Drug-Induced Changes in Selenium-Dependent Glutathione Peroxidase Activity in the Chick". *J. Nutr.* **1985**, *115*, 1459-1470.

Nelis, H. J. C. F.; Lavens, P.; Moens, L.; Sorgeloos, P.; Jonckheere, J. A.; Criel, G. R.; De Leenheer, A. P. "Cis-Canthaxanthins: Unusual Carotenoids in the Eggs and the Reproductive System of Female Brine Shrimp *Artemia*". *J. Biol. Chem.* **1984**, *259*, 6063–6066.

Nelis, H. J. C. F.; Van Steenberge, M. M. Z.; Lefevere, M. F.; De Leenheer, A. P. "Selectivity Effects in Non-aqueous Reversed-Phase and Normal Phase Chromatography of Geometric Canthaxanthin Isomers". *J. Chromatogr.* **1986**, *353*, 295–302.

Parker, R. S. "Influence of Dietary beta-Carotene on the Stability of Mitochrondrial Retinol, alpha-Tocopherol, and Fatty Acids during NADPH-Induced Peroxidation". *Fed. Proc.* **1985**, *44*, 543.

Poh-Fitzpatrick M. B.; Barbera, L. G. "Absence of Crystalline Retinopathy after Long Term Therapy with beta-Carotene". *J. Am. Acad. Dermatol.* **1984**, *11*, 111–113.

Ros, A. M.; Leyon, H.; Wennersten, G. "Crystalline Retinopathy in Patients Taking an Oral Drug Containing Canthaxanthine". *Photodermatology* **1985**, *2*, 183–185.

Rousseau, A. "Canthaxanthine Deposits in the Eye". *J. Am. Acad. Dermatol.* **1983**, *8*, 123–124.

Schwartz, S. J.; Patroni-Killam, M. "Detection of Cis–Trans Carotene Isomers by Two-Dimensional Thin-Layer and High-Performance Liquid Chromatography". *J. Agric. Food Chem.* **1985**, *33*, 1160–1163.

Tyczkowski, J. K.; Hamilton, P. B. "Canthaxanthin as a Model for the Study of Utilization of Oxycarotenoids by Chickens". *Poultry Sci.* **1986**, *65*, 1350–1356.

Wamer, W.; Giles, A., Jr.; Kornhauser, A. "Accumulation of Dietary beta-Carotene in the Rat". *Nutr. Rep. Int.* **1985**, *32*, 295–301.

Zechmeister, L. *Cis-Trans Isomeric Carotenoids, Vitamins A and Arylpolyenes*; Academic: New York, 1962.

Received for review May 11, 1987. Revised manuscript received December 28, 1987. Accepted January 9, 1988.