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Confirmation of the Structure of Papaya β-Cryptoxanthin Monoepoxide

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ABSTRACT

β-Cryptoxanthin monoepoxide has been widely found in food, but the position of the epoxy group has not been confirmed. In terms of nutrition, this confirmation is important because, depending on the location of the epoxy substituent, this carotenoid may or may not be a provitamin A. In papaya cultivar Formosa it is present in amounts high enough to affect the vitamin A value substantially. Mass spectrometry and ^{1}*H-NMR spectroscopy have unequivocally proved the structure to be 5,6-epoxy-5,6-dihydro-β,β-caroten-3-ol; thus, it is vitamin A active.*

INTRODUCTION

The occurrence of a β-cryptoxanthin monoepoxide and/or the furanoid oxide derivative (cryptoflavin) has been reported in many foods (Curl & Bailey, 1957, 1961; Curl, 1959, 1960, 1962, 1963; Purcell, 1962; Yamamoto, 1964; Subbarayan & Cama, 1964; Yokoyama & White, 1967; Yokoyama & Vandercook, 1967; Schaller & Elbe, 1971; Gross *et al.*, 1971, 1972; Molnar & Szabolcs, 1980; Rahman & Buckle, 1980; Noga & Lenz, 1983). However, the location of the epoxy group has not been confirmed in any of these cases. It has been generally assumed that the epoxy group is in the 5,6-position (5,8-position in cryptoflavin) so that the carotenoid would be a provitamin A, but the alternative possibility that the epoxy substituent is in the 5′,6′-position (or 5′,8′-position in cryptoflavin) has been raised (Straub, 1971; Davies, 1976). In *Averrhoa carambola*, the structure of cryptoflavin was determined by MS as 5,8-epoxy-5,8-dihydro-β,β-caroten-3-ol (Gross *et al.*, 1983).

In papaya, the presence of β-cryptoxanthin monoepoxide was first reported by Yamamoto (1964). Subbarayan and Cama (1964) found the probable artefact, cryptoflavin, instead.

From the nutritional point of view, the location of the single epoxy group is important because it determines whether β-cryptoxanthin monoepoxide is or is not a provitamin. This confirmation is especially needed in foods such as papaya, where the amount of this carotenoid is sufficient to have a substantial quantitative effect on the vitamin A value.

MATERIALS AND METHODS

Determination of the provitamin A content of papaya

Papayas of the cultivar Formosa were purchased from local markets or groceries in Brazil. To determine the provitamin A content, three samples were analysed. For each sample, three fruits were quartered, seeds and peel were removed from two opposite sections of each fruit, and the pulp was bulked and homogenised in a blender. Aliquots of 20 g were taken for analysis.

The analytical method was based on procedures described in detail previously (Rodriguez *et al.*, 1976). Briefly, the carotenoids were extracted with cold acetone, transferred to light petroleum, saponified with an equal volume of 10% KOH in methanol at room temperature overnight, washed, concentrated and separated on a MgO:HyfloSupercel (1:1) column. The adsorbent was activated for 2 h at 100°C prior to use and the column was developed with increasing concentrations of acetone (1–15%) in light petroleum. Only the vitamin A active carotenoids (β-carotene, β-cryptoxanthin, β-cryptoxanthin monoepoxide) were collected and quantified. The other pigments of papaya Formosa are the inactive carotenoids ζ-carotene, antheraxanthin and lycopene. The vitamin A value was calculated according to NAS-NRC (1980).

Confirmation of the structure of β-cryptoxanthin monoepoxide

The fraction eluted from the MgO:HyfloSupercel column with 10% acetone in light petroleum was rechromatographed on another column of the same adsorbent and then purified by thin-layer chromatography on silica gel with diethyl ether–light petroleum (1:1) as developing solvent. Further purification on a small column (Pasteur pipette) of neutral alumina (grade III, deactivated with 8% water), with ether as eluting solvent, was undertaken immediately before MS and NMR analysis, and the purity of the sample was confirmed by both adsorption phase (silica) and reversed phase (ODS) HPLC.

The electron impact mass spectrum was determined with a VG Micromass 70/70 F instrument. The direct insertion probe was used at an ionizing voltage of 70 eV and ion source temperature of 200–220°C.

RESULTS AND DISCUSSION

Contribution of β-cryptoxanthin monoepoxide to vitamin A value

In some papaya cultivars, including the cultivar Formosa, the β-cryptoxanthin monoepoxide is present in amounts sufficient to influence the vitamin A value substantially (Table 1). Exclusion of the epoxide from the calculation of the vitamin A value if it were active or its inclusion if it were inactive would mean an underestimation or overestimation, respectively, of 33%.

Structure of the β-cryptoxanthin monoepoxide

The possible structures of the β-cryptoxanthin monoepoxide are shown in Fig. 1. In order to have provitamin A activity, a carotenoid must have an

TABLE 1
Provitamin A Content of Papaya Cultivar Formosa

Provitamin	*Concentration*[a] (*μg/g*)	*Contribution to vitamin A value*[a] (*RE/100 g*)
β-Carotene	1·23 ± 0·07	21 ± 8
β-Cryptoxanthin	4·62 ± 0·25	39 ± 5
β-Cryptoxanthin monoepoxide	3·43 ± 0·22	29 ± 6
Total vitamin A value, considering the epoxide active		88 ± 20
Total vitamin A value, considering the epoxide inactive		59 ± 14

[a] Means and standard deviations of three determinations of composite samples.

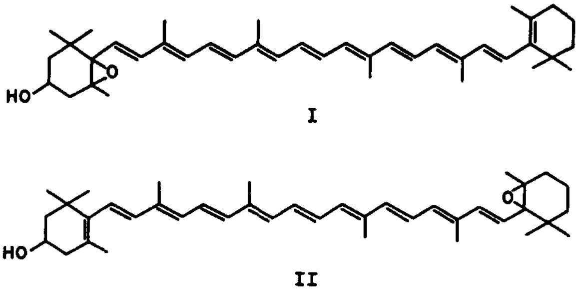

Fig. 1. Possible structures of β-cryptoxanthin monoepoxide.

unsubstituted β-ionone ring with an 11-carbon polyene side chain. Hence, structure I would have about 50% of the activity of β-carotene, whilst structure II would be inactive.

The β-cryptoxanthin monoepoxide, obtained from papaya, exhibited maximum absorbances at (418), 443, 470 nm in light petroleum and at (424), 447, 476 nm in ethanol, consistent with the presence of a 5,6- or 5′,6′-epoxy group. On addition of a drop of dilute HCl, a shift of 20 nm to lower wavelengths was observed, indicative of its conversion into the corresponding furanoid oxide. The pigment formed an acetate with acetic anhydride but was not methylated with acidified methanol, demonstrating the presence of a hydroxyl group in a non-allylic position.

The location of the epoxide group was verified by mass spectrometry. The mass spectrum had the molecular ion M^+ at *m/z* 568 ($C_{40}H_{56}O_2$) and the main fragment ions at *m/z*: 552 [M-16 (O)]—indicative of an epoxide; 550 [M-18 (H_2O)]—low intensity, confirming the presence of a non-allylic hydroxy group; 476 [M-92 (toluene)] and 462 [M-106 (*m*-xylene)]—corresponding to in-chain fragmentations; 448 [M-80]—diagnostic of a 5,6-epoxy-β-ring.

Structure I was proved by the presence of an intense fragment ion at *m/z* 221, which is diagnostic for a 3-hydroxy-5,6-epoxy-β-ring or the corresponding 3-hydroxy-5,8-epoxy-β-ring. For structure II, containing an unsubstituted 5,6-epoxy-β-ring, the corresponding fragmentation would have given a fragment at *m/z* 205 (Vetter *et al.*, 1971). Structure I (5,6-epoxy-5,6-dihydro-β,β-caroten-3-ol) is therefore proved unequivocally. The papaya β-cryptoxanthin monoepoxide is thus a provitamin.

The ^{1}H-NMR spectrum at 400 MHz confirmed the structure. In particular, methyl group signals were observed at 1·04 ppm (6 protons) and 1·78 ppm (3 protons) due to the C-16, 17 and C-18 methyl groups, respectively, of an unsubstituted β-ring, and at 0·98, 1·16 ppm (C-16, C-17) and 1·19 ppm (C-18) of a 3-hydroxy-5,6-epoxy-5,6-dihydro-β-ring (Fig. 2). No signals were observed at 1·07 ppm (C-16, C-17 of a 3-hydroxy-β-ring) or

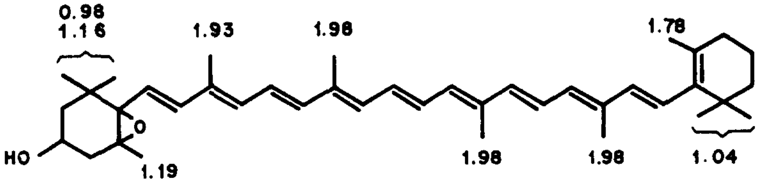

Fig. 2. ¹H-NMR chemical shift (δ) values for the methyl group protons of β-cryptoxanthin-5,6-epoxide.

at 0·94, 1·10 ppm (C-16, C-17 of a 5,6-epoxy-5,6-dihydro-β-ring) thus confirming the absence of the alternative 3-hydroxy-5′,6′-epoxy-5′,6′-dihydro-β,β-carotene structure. Minor signals in the spectrum at 1·34, 1·20, 1·17, 1·68, 1·62, 1·81, 1·72 ppm could be attributed to the presence of small amounts of 3-hydroxy-5,8-epoxy-dihydro-β,β-carotene, presumably produced in small amounts by acid isomerization and detectable by the absorption spectrum of the sample at the conclusion of the NMR analysis.

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