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β-CITRAURIN EPOXIDE, A NEW CAROTENOID FROM VALENCIA ORANGE PEEL

PÉTER MOLNÁR and JÓZSEF SZABOLCS

University Medical School of Pécs, H-7643 Pécs, Hungary

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Key Word Index—Valencia orange; *Citrus*; β-citraurin epoxide; C_{30}-, C_{27}- and C_{25}-epoxy-apo-carotenoids; di*cis*-violaxanthin; citrus carotenoids; distribution of carotenoids.

Abstract—A new minor carotenoid, β-citraurin epoxide (3-hydroxy-5,6-epoxy-5,6-dihydro-8′-apo-β-caroten-8′-al) and several isomers of violaxanthin (5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-caroten-3,3′-diol) have been identified in Valencia orange peel. The previously reported occurrence of apo-10′-violaxanthal (3-hydroxy-5,6-epoxy-5,6-dihydro-10′-apo-β-caroten-10′-al) and apo-12′-violaxanthal (3-hydroxy-5,6-epoxy-5,6-dihydro-12′-apo-β-caroten-12′-al) has been confirmed, and their syntheses are described. The quantitative determination of the carotenoids has also been performed.

INTRODUCTION

It is well known that a number of carbonyl carotenoids, many of epoxy character, are found in orange peel [1–8]. In several cases, however, the first-discovered pigments were not isolated in a crystalline state and only probable structures were proposed [1, 2]. We now report conclusive identification of most of the carotenoids in orange peel using authentic samples for comparison if crystallization failed for lack of material.

Since it was shown that some carbonyl carotenoids found in orange peel might be artefacts, reinvestigation of the distribution of carotenoids also seemed warranted.

RESULTS AND DISCUSSION

The carotenoid content and additional information on the isolation and identification of the pigments are presented in Table 1. The results show that the carotenoid mixture is very complex. Many carotenoids, mainly *cis*-isomers, occur in such small amounts that attempts to identify them conclusively failed.

Major carotenoids

The principal carotenoid was found to be violeoxanthin (9-*cis*-violaxanthin; 61%; $\lambda_{max}^{C_6H_6}$ nm: 478, 488 and 422; $\varepsilon_{cis\text{-peak}} = 8250$). Di*cis*-violaxanthin (12%; $\lambda_{max}^{C_6H_6}$ nm: 470, 441 and 419; $Q = 3.2$) and all-*trans*-violaxanthin were although present in smaller quantities were still major constituents. Both violeoxanthin and di*cis*-violaxanthin were converted by iodine stereomutation into all-*trans*-violaxanthin of 3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*S* absolute configuration [9]. The total amount of the violaxanthin series, including its 5,8-epoxy derivatives, reached 87%, of which 12% was all-*trans*-violaxanthin (13-*cis*-violaxanthin occurs only in negligible traces).

Minor carotenoids

(a) C_{40}-*carotenoids*. Two monoepoxy C_{40}-phytoxanthins, antheraxanthin (5,6-epoxy-5,6-dihydro-β,β-caroten-3,3′-diol) and its peripherial 9(9′)-mono*cis* isomer(s) ($\lambda_{max}^{C_6H_6}$ nm: 483, 453 and 430; $\varepsilon_{cis\text{-peak}} = 9700$) [10] were present. On treatment with acid, both yielded furanoid oxide derivatives. All-*trans*-antheraxanthin was inseparable from an authentic sample on a mixed chromatogram.

Of the constituents, the epimers of luteoxanthin(s) (5,6,5′,8′-diepoxy-5,6,5′8′-tetrahydro-β,β-caroten-3,3′-diol) and cryptoflavin (5′,8′-epoxy-5′,8′-dihydro-β,β-caroten-3-ol) were certainly artefacts formed via epoxide–furanoid rearrangement during work-up. On treatment with acid, luteoxanthin was converted to auroxanthin. Although cryptoflavin did not seperate from an authentic sample, its identification was tentative. Identification by MS failed because of sterol contamination.

Lutein (β,ε-caroten-3,3′-diol) and β-cryptoxanthin (β,β-caroten-3-ol), isolated in crystalline state, showed the expected properties.

The hydrocarbon fraction consisted of β,ε-carotene and phytofluene. It was rather surprising that attempts to detect all-*trans*-β,β-carotene failed. During the chromatographic separation special attention should be paid to this fraction contaminated with colourless impurities.

(b) *Apo-carotenoids*. All these compounds with or without epoxy and hydroxyl groups are of aldehydic character. The two simplest are 8′-apo-β-caroten-8′-al and its 3-hydroxy derivative, β-citraurin. On reduction with $LiAlH_4$ they were converted into C_{30}-alcohols with a hypsochromic shift of *ca* 30 nm

Table 1. Quantitative composition of carotenoids in Valencia orange peel

Carotenoid (Fraction)	Percentage of total carotenoids	Absolute maxima (nm) C_6H_6	HCl–C_6H_6*	$LiAlH_4$–C_6H_6†
Hypophasic pigments				
13-*cis*-Violaxanthin (I, II)	t	476 447 425	433 406 384	
9-*cis*-Violaxanthin‡ (I, II, III)	61	478 449 425	436 409 387	
cis-Luteoxanthin (II)	t	455 427 404	436 410 387	
9,9′-*cis*-Antheraxanthin (II, III)	t	483 453 430	464 436 412	
Luteoxanthin epimer (II)	1	460 432 407	435 409 387	
Luteoxanthin epimer (II)	1	459 431 406	435 408 386	
Di*cis*-violaxanthin‡ (I, II, III)	12	470 441 419	434 408 386	
Violaxanthin‡ (I, II)	12	483 452 426	436 409 386	
Di*cis*-antheraxanthin (II)	t	475 444 (418)	460 434 410	
Antheraxanthin (I, II)	1.8	488 458 433	464 437 413	
Apo-8′-violaxanthin (II)	0.02	488 461 (438)	466 440 (415)	458 431 406
Apo-10′-violaxanthal (I, II)	0.5	(466) 440	(441) 419	429 403 382
Apo-12′-violaxanthal (II)	0.07	(439) 415	(410) 392	400 377 358
Lutein (I, II, III)	2.5	487 457 432		
β-Citraurin‡ (I, II, III)	1	(496) 466		462 453 413
Epiphasic pigments				
β-Cryptochrom§ (IV)	0.2	439 411 385		
β-Cryptoflavin§ (III, IV)	1.5	464 436 413		

Table 1.—*Continued*

Carotenoid (Fraction)	Percentage of total carotenoids	Absolute maxima (nm) C_6H_6	HCl-C_6H_6*	$LiAlH_4$-C_6H_6†
β-Cryptoxanthin‡ (IV)	2.5	491 461 (435)		
OH-α-Carotene§ (IV)	0.4	487 458 431		
8′-Apo-β--caroten-8′-al§ (IV)	0.1	(504) 470		460 436 410
α-Carotene‖ (IV)	0.5	475 445 421		
Phytofluene‖ (IV)	0.5	367 346 331		
Unidentified carotenoids (mainly *cis* isomers)	1			

Carotenoids in order of decreasing adsorption. t = trace.

*Benzene solution containing 1.35% of acetic acid and 0.15% of hydrochloric acid.

†After $LiAlH_4$ reduction.

§Tentative identification.

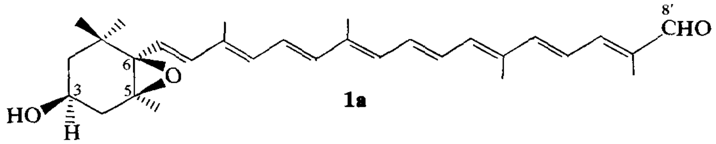
‡Isolated in solid state.

‖λ_{max} in petrol.

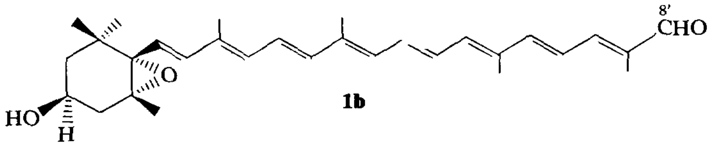
Cochromatography with authentic samples also proved their identity.

The presence of apo-10′-violaxanthal (**2**) and apo-12′-violaxanthal (**3**) is in agreement with Curl's finding [1, 2]. Apo-10′-violaxanthal (**2**) isolated in our laboratory ($\lambda_{max}^{C_6H_6}$ nm: 452, 427 and 405), however, exhibited maxima in hexane at wavelengths 4 nm longer than those of Curl's sample. Therefore, we believe that our sample represents the all-*trans* form, while the other corresponds to 9-*cis*-apo-10′-violaxanthal. Both apo-10′-violaxanthal (**2**) and apo-12′-violaxanthal (**3**) were converted into diols by $LiAlH_4$, and into furanoid oxide derivative by acid treatment (Table 1). For conclusive identification, authentic apo-10′-violaxanthal (**2**) and apo-12′-violaxanthal (**3**) were prepared by controlled oxidative degredation of violaxanthin [11, 12]. Comparison of the synthetic and natural violaxanthals confirmed their identity.

It was interesting to note that according to data in



1a



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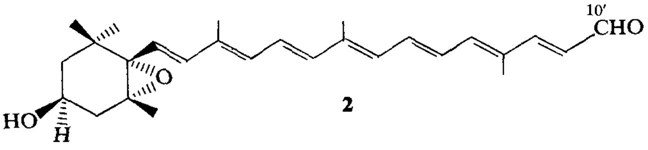

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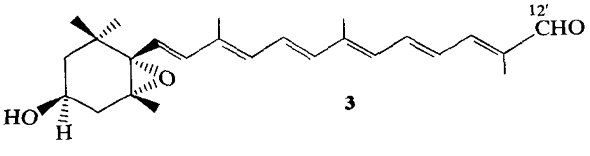

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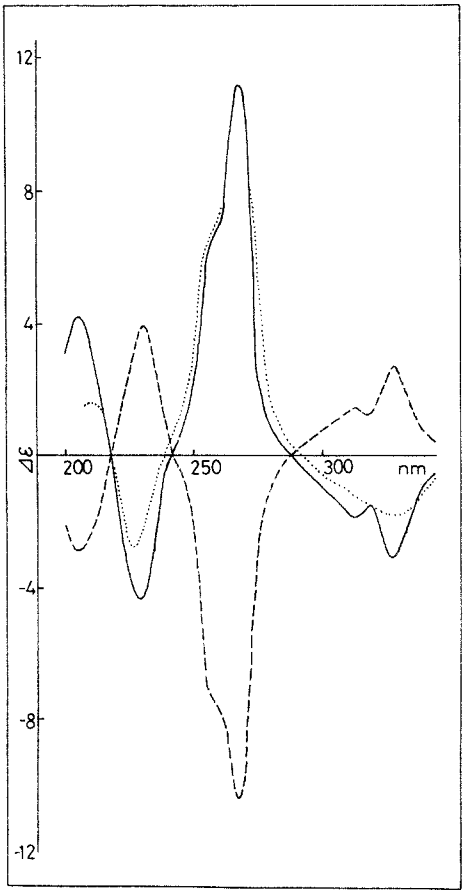

Fig. 1. CD curves for (3*S*, 5*S*, 6*R*)-β-citraurin epoxide (.....), 1/2 (3*S*, 5*S*, 6*R*, 3′*S*, 5′*S*, 6′*R*)-violaxanthin (——) and 1/2 (3*S*, 5*R*, 6*S*, 3′*S*, 5′*R*, 6′*S*)-violaxanthin (– – – –) in methanol. The qualitative CD curve of (3*S*, 5*R*, 6*S*)-β-citraurin epoxide is similar to that of 1/2 (3*S*, 5*R*, 6*S*, 3′*S*, 5′*R*, 6′*S*)-violaxanthin.

the literature β-citraurin had already been found in orange peel [3], but apo-8′-violaxanthal had not. We have now succeeded in isolating apo-8′-violaxanthal (**1**), which is the 5,6-epoxide derivative of β-citraurin. Before the present work we had detected this new compound among the products of oxidative degradation of antheraxanthin but the yield was very poor [12]. Therefore we prepared authentic β-citraurin epoxide via epoxidation [13] of β-citraurin acetate. The resulting two diastereomeric β-citraurin epoxides (**1a**, **1b**) were separated by column chromatography, and as expected, the anti (3*S*, 5*R*, 6*S*)-diastereomer (**1b**) proved to be identical with the natural β-citraurin epoxide present in orange peel, which was checked by CD correlation (Fig. 1) and cochromatography. The MS of **1a** and **1b** established the molecular formula $C_{30}H_{40}O_3$ (m/e 448) and the same fragmentation patterns indicated the presence of an aldehyde (M-29) and of a 5,6-epoxy-5,6-dihydro-cyclohexyl group (m/e 205; M-80) in the molecules. The light absorption properties of **1a** and **1b** were identical (see Experimental).

On acid treatment **1a** and **1b** underwent an epoxide-furanoid oxide rearrangement giving 3-hydroxy-5,8-epoxy-5,8-dihydro-8′-apo-β-caroten-8′-al ($\lambda_{max}^{C_6H_6}$ nm: 466, 440 and 415) which was already found by Gross *et al.* in *Citrus* [7].

EXPERIMENTAL

Biological material. Valencia orange was obtained commercially on 8 April 1978. A total of 19.5 kg of ripe fruit yielded 360 g dry peel.

Methods. The chromatographic methods were those normally employed in our laboratory [14]. λ_{max} shifts after acid treatment (HCl–benzene) and after $LiAlH_4$ reduction (after $LiAlH_4$ reduction) were used on a spectroscopic scale for confirmation of the presence of 5,6-epoxy and aldehyde groups in the chromophore. Other methods of identification are described separately in the text. Quantitative determination of all carotenoids was carried out spectrophotometrically.

Pigment isolation. To avoid loss of minor carotenoids, especially the highly sensitive epoxy-apo-aldehydes, great care was taken during the isolation procedures. The subsequent operations were as follows: cutting the material in small pieces, dehydration with MeOH, grinding, extraction with Et_2O, removal of colourless impurities by overnight precipitation from Et_2O at −25°, alkali saponification, a second overnight precipitation of the colourless impurities, two successive precipitations of hypophasic carotenoids from C_6H_6 with petrol (fractions I and II), partition (petrol–90% aq. MeOH) of the mother liquor to separate hypophasic (fraction III) and epiphasic (fraction IV) pigments, and finally separation of the fractions by CC. The total yield of crude carotenoids was 300 mg (0.08% of dry wt). Fractions I, II and III were subjected to chromatography on $CaCo_3$ (Biogal, Hungary) with C_6H_6 or a mixture of C_6H_6–petrol (1:1 and 1:3). The distribution of carotenoids in the various fractions and the data used for identification are summarized in Table 1. As described above, the epiphasic pigments in the mother liquor of fracton II formed fraction IV. A considerable portion of the colourless impurities was removed by overnight precipitation from petrol at −20°, then it was chromatographed on MgO–Celite (1:1) with C_6H_6–petrol. It was noticed that the contaminating orange oil was concd mainly in this fraction, which made chromatography more difficult.

Violaxanthin (mp 170°), 9-*cis*-violaxanthin (mp 105°), lutein (mp 175°) β-cryptoxanthin (mp 162°) and β-citraurin (mp 146°) from Valencia orange peels were crystallized in the usual way, and the crystalline pigments showed the expected properties. Di*cis*-violaxanthin (mp 64°) was gained in amorphous state by precipitation; iodine stereomutation gave all-*trans* violaxanthin.

Reference compounds. *3-Hydroxy-5,6-epoxy-5,6-dihydro-8′-apo-β-caroten-8′-al* (**1a**, **1b**). 67 mg β-citraurin acetate (mp 118°; $\lambda_{max}^{C_6H_6}$ nm: 497, 467), prepared by standard acetylation of (3*R*)-β-citraurin, was allowed to stand in the presence of perphthalic acid (9 mg O_2) in darkness for 44 hr [13]. After saponification, the products were separated on $CaCO_3$ (Biogal, Hungary) with C_6H_6–petrol (1:3) and crystallized from C_6H_6 by the addition of petrol. The more polar zone resulted in 4.2 mg **1a** (mp 158°; λ_{max}^{Hexane} nm: 477, 448 and 424; $\varepsilon_{max}^{Hexane}$ 104 900, 119 400 and 84 500; IR ν_{max}^{KBr} cm^{-1}: 1675) and the less polar zone gave 1.7 mg of **1b** (λ_{max} and ε_{max} as **1a**). **1a** and **1b** had m/e 448 (M^+) and the predicted fragmentation pattern [15, 16]. 3-*Hydroxy*-5,6-*epoxy*-5,6-*dihydro*-10′-*apo*-*β*-*caroten*-10′-*al* (**2**) was prepared by controlled permanganate oxidation [12] of violaxanthin (mp 115°; λ_{max}^{Hexane} nm: 453, 427 and 405; IR ν_{max}^{KBr} cm^{-1}: 1670). 3-*Hydroxy*-5,6-*epoxy*-5,6-*dihydro*-12′-*apo*-*β*-*caroten*-12′-*al* (**3**) was prepared by published procedure [12] (mp 87°; λ_{max}^{Hexane} nm; 425, 401 and 383; IR ν_{max}^{KBr} cm^{-1}: 1670).

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