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THE CONSTITUTION AND ABSOLUTE STEREOCHEMISTRY OF PERSICAXANTHIN

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Key Word Index—*Prunus persica*; Rosaceae; C_{25}-epoxy-apo-carotenoids; apo-carotenols; persicaxanthin; persicachrome.

Abstract—Persicaxanthin, isolated from the fruits of peach, has been obtained in crystalline form. By spectroscopic (MS, ^{1}H NMR and CD) methods, its constitution and absolute stereochemistry have been determined as (3*S*,5*R*,6*S*)-5,6-epoxy-3-hydroxy-5,6-dihydro-12′-apo-β-carotene-3,12′-diol. The structural conclusions have received full support by comparison with semi-synthetic persicaxanthin obtained from natural violaxanthin.

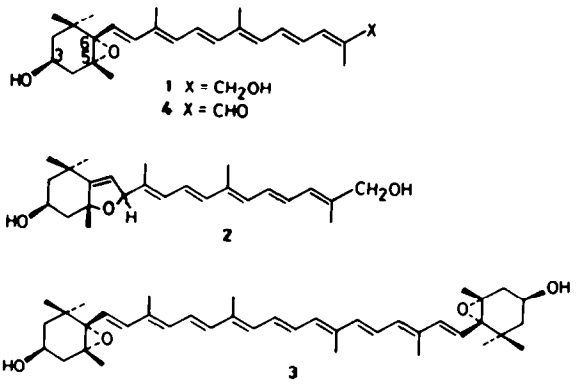
INTRODUCTION

Persicaxanthin (**1**), one of the numerous apo-carotenoids occurring in nature, was first detected by Curl in cling peaches [1]. Subsequently, **1** has been identified in many other fruits, always accompanied by its furanoid oxide derivative, persicachrome (**2**) [2–4]. The probable C_{25}-epoxy-diol structure of **1** was originally proposed by Curl [1–3, 5, 6] and supported later by Gross and Eckhardt on the basis of mass spectral data and routine analytical tests [7]. Native **1**, however, has never been obtained in sizeable amounts in a fully characterizable, crystalline form; nor have its real constitution and stereochemistry been established in a conclusive manner. Consequently, the proposed biological formation of **1** via Glover–Readfearn degradation of violaxanthin (**3**) has remained unverified.

As part of our studies of natural and semi-synthetic apo-carotenoids (many of which feature an epoxy function) [8–11], we now report the structure and absolute stereochemistry of **1** as inferred from high-field proton NMR and CD spectral studies.

RESULTS AND DISCUSSION

The isolated natural pigment **1** crystallized from a mixture of benzene and petrol in yellow plates (mp 92°). Its EIMS (direct inlet, 70 eV) *m/z* (rel. int.) 384 (60; M), 366 (28; [M–H_2O]), 353 (5; [M – CH_2OH]), 304 (100; [M – 80]), 221 (18), 181 (18) confirmed the elemental composition $C_{25}H_{36}O_3$ and indicated the presence of one epoxy and two hydroxy functions in the molecule. The UV-vis spectrum exhibited a well-defined fine structure characteristic of the hexaene chromophore $\lambda_{max}^{C_6H_6}$ (ε_{max}) nm: 401 (69 000), 378 (71 000), 358 (45 000) and, as expected, the IR spectrum attested to the absence of CO group in **1**. In response to the usual acidic treatment, **1** underwent epoxide–furanoid-oxide rearrangement leading to persicachrome (**2**), readily identified through its UV-vis spectrum (see Fig. 1). In accordance with the dihydroxy nature of persicaxanthin, acetylation afforded the diacetate of **1**.



The constitution of persicaxanthin displayed in **1** followed from the analysis of its 400 MHz proton NMR spectrum. The assignment of the resonance signals in terms of proton–proton connectivities was conveniently performed by two-dimensional (2D) correlation spectroscopic (COSY) techniques [12]. As usual, evaluation of the chemical shift coordinates of the correlation peaks in the double-transformed 2D data matrix afforded a consistent labelling of sequentially spin–spin coupled protons which, in turn, led to a straightforward assignment of the resonances to the individual proton sites of the molecule. In addition to the more intense correlation peaks due to vicinal *J*-couplings, correlations mediated by longer range (^4J, ^6J) spin–spin interactions were also observed which facilitated the establishment of proton–proton connectivities across quaternary carbon atoms. NOE difference spectra [13] were also recorded for the identification of the head-group methyl signals. The fully assigned spectral parameters are summarized in Table 1.

The absolute stereochemistry of **1** followed from comparative CD spectral analysis of persicaxanthin with a single chiral end group of violaxanthin type and its related homodichiral carotenoid, natural violaxanthin (**3**) [14]. As seen from Fig. 2 there was reasonable qualitative

Table 1. ^{1}H NMR data of persicaxanthin (1)*

H-2_{ax}	1.584	$^2J_{2ax,2eq}$ − 12.6; $^3J_{2ax,3}$ 10.1
H-2_{eq}	1.913	$^3J_{2eq,3}$ 3.4; $^4J_{2eq,4}$ 1.7
H-3	4.253	$^3J_{3,4ax}$ 8.6; $^3J_{3,4eq}$ 5.0
H-4_{ax}	2.006	$^2J_{4ax,4eq}$ − 14.3
H-4_{eq}	2.641	
H-7	6.138	$^3J_{7,8}$ 15.45
H-8	6.652	$^4J_{8,10}$ 0.6
H-10	6.339	$^3J_{10,11}$ 11.23; $^4J_{10,12}$ 0.5; $^4J_{10,19}$ 1.1
H-11	6.808	$^3J_{11,12}$ 15.14
H-12	6.583	$^4J_{12,20}$ 0.4; $^6J_{12,19}$ 0.2; $^4J_{12,14}$ 0.8
H-14	6.437	$^3J_{14,15}$ 10.36; $^4J_{10,20}$ 0.6
H-15	6.897	$^3J_{15,15'}$ 14.28
H-15'	6.755	$^3J_{14',15'}$ 10.62
H-14'	6.674	$^4J_{14',20'}$ 1.1; $^4J_{12',14'}$ 1.2
H_2-12'	4.376	
H_3-16	1.261	
H_3-17	1.148	
H_3-18	1.285	
H_3-19	2.011	
H_3-20	1.961	
H_3-20'	1.924	
12'-OH	5.90	
3-OH	6.45	

* In pyridine soln at 26°. Chemical shifts are relative to internal TMS, coupling constants in Hz. Coupling values less than 1 Hz were estimated on the basis of cross peak intensity in COSY experiment. Mutual couplings are given only once, at their first occurrence in table.

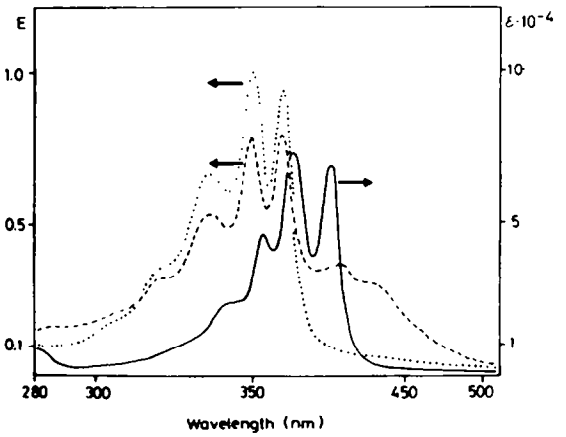


Fig. 1. UV and visible light absorption spectra of persicaxanthin (——), persicachrome (– – –) and persicachrome diacetate (· · · · · · ·) in benzene.

agreement, which settled the absolute configuration of the chiral centres of persicaxanthin (1) as 3*S*,5*R*,6*S*.

The relationship between 1 and 3 implied in the above results received full support by chemical transformations. Controlled oxidative degradation of natural violaxanthin (3) [8–10] gave apo-12'-violaxanthal (3*S*,5*R*,6*S*)-5,6-epoxy-3-hydroxy-5,6-dihydro-12'-apo-β-carotene-12'-al (4) which upon treatment with $NaBH_4$ afforded a crystalline product (mp 90°) that in every respect proved identical with persicaxanthin (1).

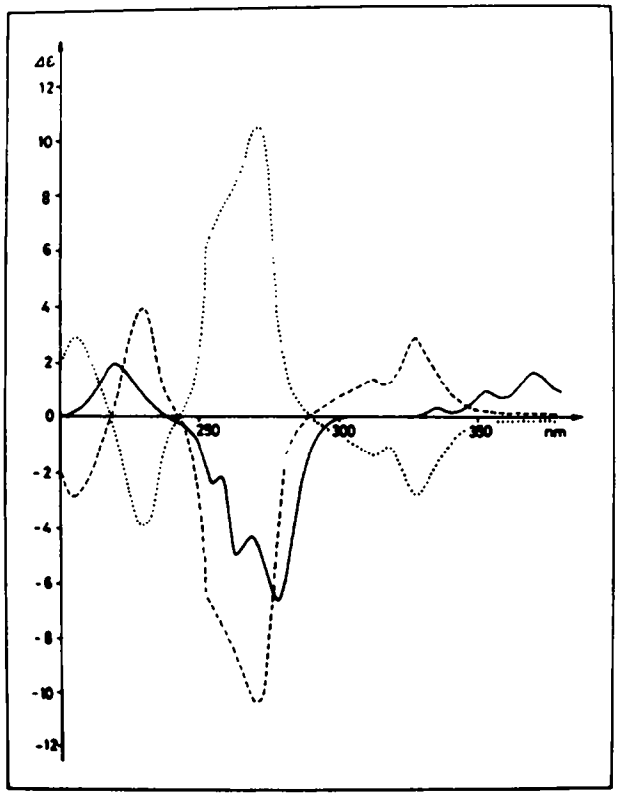


Fig. 2. CD curves for (3*S*,5*R*,6*S*)-persicaxanthin (——), 1/2 (3*S*,5*R*,6*S*,3'*S*,5'*R*,6'*S*)-violaxanthin (– – – – –) and 1/2 (3*S*,5*S*,6*R*,3'*S*,5'*S*,6'*R*)-violaxanthin (· · · · · ·) in methanol.

EXPERIMENTAL

Biological material and methods. Peaches (*Prunus persica*, cv Elberta) were obtained in Pécs (southern Hungary) in September 1985. General handling methods, including routine tests and column chromatography, have been described elsewhere [10]. All operations were performed in darkness. Persicaxanthin and persicachrome showed strong fluorescence at 366 nm ('Fluotest Forte', Hanau).

Mps were determined with a Boetius hot stage apparatus and are uncorrected. UV-vis spectra were recorded with a Perkin–Elmer 402 instrument. The CD spectra were taken in MeOH solns at room temp. on a Roussel-Juan Dichrographe III (Jobin Yvon), in quartz cells. Conventional (1D) and homonuclear (^{1}H) chemical shift correlation 2D spectra were obtained on a Varian XL-400 instrument. Time-domain data matrix size in the COSY experiment was 2048* 512, this was transformed after 'pseudo-echo' multiplication in both dimensions to give a symmetrized, absolute value correlation map of digital resolution 2 Hz/data point. Homonuclear selective NOE data were obtained by the difference method [15] using the frequency cycling technique [13] for selective pre-irradiation. In order to prevent cleavage of the highly sensitive epoxide rings during measurements, all NMR spectra were obtained in pyridine-d_5 solns (1.2 mg in 0.5 ml) at ambient temp., using TMS as the internal reference.

Carotenoid analysis. The Elberta cv was chosen on the basis of carotenoid analyses of different subspecies and because it was commercially available. The analysis consisted of dehydration, extraction, saponification and column chromatography of the fruit. The pigments of the subspecies of *Prunus persica* (20 g fr. wt) were identified in soln by λ_{max}, furanoid oxide test, a partition test and mixed chromatography. Full experimental details are given below. From the extract of the *Elberta* subspecies, the following zones were obtained: band 1 (a mixture of 9-*cis*-, 13-*cis*-violaxanthin and epimers of luteoxanthin), band 2 (persicaxanthin contaminated with violaxanthin), band 3 (violaxanthin), band 4 (antheraxanthin), band 5 (lutein), band 6 (zeaxanthin-like pigment), band 7 (β-cryptoxanthin epoxide?) and band 8 (β,β-carotene contaminated with phytofluene). It should be noted that the pigments only occurring in traces in very thin zones were not identified. The following cvs were analysed (the presence of persicaxanthin is marked with (+): Napsugár (+), Ford (−), Regina (−), T8 (+), Jerseyland (+), Suncrest (+), and Elberta (+).

Pigment isolation. The fruits (40 kg fr. wt) were halved, peeled and pitted (24.6 kg). The halves, yellow in colour, were blended in the presence of about 1% $CaCO_3$. The blendate, in portions of 5 kg, was allowed to stand in 48 l. MeOH at room temp. for dehydration. After 24 hr the mixture was gently squeezed through a fine cloth and the MeOH soln was discarded. The operation was repeated, using 30 l. MeOH. Then the mixture was filtered off by suction and the dry residue kept in 16 l. MeOH at room temp. for 60 hr. After suction the two MeOH extracts were combined, concentrated *in vacuo* to about 20 l. and the pigments transferred into Et_2O. The extraction of the dry fruit was completed with 6 l. Et_2O. Then the Et_2O solns were combined, washed free of MeOH, dried (Na_2SO_4), evapd to about 4 l. and saponified in two portions with 30% KOH–MeOH at room temp. for 15 hr. After saponification, the Et_2O solns were combined, washed free of alkali, evapd to dryness *in vacuo*, dissolved in C_6H_6 and precipitated with an excess of petrol at 2–4° (1.18 g). The mother liquor was evapd *in vacuo* to dryness and the residue was partitioned between petrol and 90% MeOH. The xanthophylls were transferred to Et_2O, dried (Na_2SO_4), evapd to dryness, dissolved in C_6H_6 and precipitated with petrol at 2–4° (16 mg). After evapn the epiphasic pigments were taken up in C_6H_6 and diluted with a large amount of MeOH at 2–4° for precipitation (1.22 g).

The xanthophylls (1.18 + 0.016 g) contaminated with colourless impurities were dissolved in C_6H_6 and chromatographed on $CaCO_3$ (Biogal, Hungary) with a mixture (1:3) of C_6H_6 and petrol. After the usual work-up, the main zone (a mixture of persicaxanthin and violaxanthin) was subjected to re-chromatography on $CaCO_3$ (Biogal, Hungary) with a mixture (1:9) of C_6H_6–petrol containing increasing amounts of Me_2CO up to 1.2%. Excluding several thin zones at the top and at the bottom of the column, two main zones developed: persicaxanthin (**1**) and violaxanthin (**3**). After the routine work-up, the C_6H_6 soln of persicaxanthin (**1**) was evapd *in vacuo* at 30°, and the residue was crystallized from C_6H_6 by addition of petrol at −20°. Plates, pale yellow in colour (3.8 mg) were obtained, mp 92°. VIS $\lambda_{max}^{C_6H_6}$ nm (log ε): 401 (4.84), 378 (4.85) and 358 (4.66); λ_{max}^{petrol} nm: 392, 369 and 351; λ_{max}^{EtOH} nm: 392, 371 and 353; $\lambda_{max}^{C_6H_6}$ nm (after acid treatment): 372, 352 and 337. The UV-vis spectra are presented in Fig. 1. On partition between petrol and 90% MeOH persicaxanthin (**1**) was entirely hypophasic.

Preparation of persicaxanthin (**1**) *by degradation of natural violaxanthin* (**3**) (*ex* Viola tricolor). Ten milligrams of 3-hydroxy-5,6-epoxy-5,6-dihydro-12′-apo-β-carotene-12′-al (**4**) obtained by controlled permanganate oxidation of (3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*S*)-violaxanthin diacetate [10] was stirred in 30 ml C_6H_6–96% EtOH with $NaBH_4$ at room temp. for 30 min. After the usual work-up the products were separated on $CaCO_3$ ('Chemapol', Prague) with C_6H_6–petrol (1:1). The following zones were obtained: band 1 (silvery), band 2 (ochre), band 3 (silvery), band 4 (pale ochre) and band 5 (persicaxanthin). Re-chromatography of persicaxanthin contaminated with an unidentified impurity on $CaCO_3$ ('Chemapol', Prague) with C_6H_6–petrol (2:3) as eluant gave pure persicaxanthin (**1**) (1 mg). The physical and chemical properties of 'semi-synthetic' persicaxanthin were identical with those of the compound from peach.

Persicaxanthin diacetate. Persicaxanthin (**1**) (0.5 mg) was acetylated [10] and the product was purified by column chromatography on $CaCO_3$ ('Biogal', Hungary) with C_6H_6–petrol (1:7). On partition between petrol and 95% MeOH persicaxanthin diacetate [$\lambda_{max}^{C_6H_6}$ nm: 401, 378 and 359; λ_{max}^{hexane} nm: 392, 370 and 351; λ_{max}^{petrol} nm: 392, 370 and 351; $\lambda_{max}^{C_6H_6}$ nm (after acid treatment): 372, 352 and 337] exhibited entirely epiphasic properties. It is interesting to note that the UV-vis spectra of persicachrome (**2**) exhibited a broad plateau between 390 and 510 nm whereas that of persicachrome diacetate showed a well-defined UV-vis spectrum of fine structure (Fig. 1).

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