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STRUCTURES OF PERSICAXANTHIN, PERSICACHROME AND OTHER APOCAROTENOLS OF VARIOUS FRUITS

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Abstract—The structure of persicaxanthin and persicachrome, two UV-fluorescent pigments found in French plum of the Sagiv cv, was elucidated by chemical tests and mass spectroscopy. They are C_{25}-epoxyapocarotenols, their respective structures being 5,6-epoxy-5,6-dihydro-12′-apo-β-carotene-3,12′-diol and 5,8-epoxy-5,8-dihydro-12′-apo-β-carotene-3,12′-diol. The former is obtained by reduction of apo-12′-violaxanthal, which was also detected in the fruit in small amounts. Its proposed structure, 5,6-epoxy-3-hydroxy-5,6-dihydro-12′-apo-β-caroten-12′-al, was confirmed. The number of natural apocarotenols of known structure has thus increased to five, including two other C_{27}-epoxyapocarotenols and a C_{30}-apocarotenol, which were also isolated from various fruits.

INTRODUCTION

While investigating the carotenoids of French plum (*Prunus domestica*, the Sagiv cv) it was observed that the absorption spectrum of the total extract in ethanol, with λ_{max} at 373, 393, 420, 440, 468 nm, had two unusual peaks in the near-UV region. Separating the pigments by TLC (on silica gel developed with 30% acetone in petrol) according to the new rapid method of Gross [1], a strong yellow fluorescence in the UV was observed between the polar fractions of violaxanthin and neoxanthin. By rechromatography this fluorescent zone could be resolved further into a less polar pigment with maxima at 330, 348, 367 nm in EtOH showing a blue fluorescence in the UV and a more polar pigment with maxima at 353, 372, 393 nm showing a strong yellow fluorescence. Both had a sharp fine structure typical for a short in-chain chromophore, a conjugated pentaene and a conjugated hexaene respectively. These spectrophotometric and chromatographic properties were described for the two pigments persicaxanthin and persicachrome, which were isolated for the first time from cling peaches (*Prunus persica*) cv Halford by Curl [2], who named them accordingly. Later the same author found them also in apricots [3] and in Italian plums [4]. Their structures are still unknown since in the latest carotenoid monographs [5, 6] they are still mentioned under their trivial names.

The amount of persicaxanthin and persicachrome was about 8% of the total carotenoid content in the ripe Sagiv plum. The plum also contained small amounts of an apocarotenal with the properties of 'apo-12′-violaxanthal' isolated also by Curl from clementine and Dancy tangerine peels [7]. This pigment was detected by Curl also in other fruits, cling peaches [2], apricots [3], Italian plums [4] and cranberries [8]. The structure proposed by Curl was not then confirmed.

In the present study the structure of persicaxanthin and persicachrome was established and confirmed by mass spectroscopy. The structure of apo-12′-violaxanthal was also confirmed by mass spectroscopy. The relationship between the three pigments was also established.

RESULTS AND DISCUSSION

The apocarotenols persicaxanthin and persicachrome are discussed first as the apocarotenal could be identified precisely because through reduction and subsequent acidification it could be transformed into the known persicaxanthin and persicachrome.

Persicaxanthin

Persicaxanthin, the pigment with yellow fluorescence found on silica gel plates between violaxanthin and neoxanthin, had a sharp fine structure with maxima at 353, 372, 393 nm, revealing a conjugated hexaene chromophore. It could be separated from violaxanthin by multiple TLC on MgO–Kieselgur plates developed with 20% acetone in petrol–EtOH (99:1). In the epoxide test it gave an intense blue-violet colour with a hypsochromic shift of 24 nm. The 5,8-epoxide with maxima at 330, 348, 368 nm was obtained. It was identical with the natural persicachrome. The structure of persicaxanthin is 5,6-epoxy-5,6-dihydro-12′-apo-β-carotene-3,12′-diol, a C_{25}-epoxycarotenol (**2**). Its MS is as follows: *m/z* 384 ($C_{25}H_{36}O_3$, $M^{+\cdot}$, 60%); 382 ($[M-2H]^{+\cdot}$, 5%); 366 ($[M-H_2O]^{+\cdot}$, 7%); 353 ($[M-CH_2OH]^{+}$, 3%); epoxide fragments: 304 [$(M-C_6H_8]^{+\cdot}$, 100%); 299 ($C_{20}H_{27}O_2$, 6%); 287 ($C_{19}H_{27}O_2$, 6%); 273 ($C_{18}H_{25}O_2$, 6%) 247 ($C_{16}H_{23}O_2$, 8%); 234 ($C_{15}H_{22}O_2$, 6%); 221 ($C_{14}H_{21}O_2$, 23%); 208 ($C_{13}H_{20}O_2$, 13%); 181 ($C_{11}H_{17}O_2$, 21%). The MS of the diacetate is in good agreement with the proposed structure: *m/z* 468 ($C_{29}H_{40}O_5$, $M^{+\cdot}$); 408 ($[M-MeCOOH]^{+\cdot}$); 388 ($[M-C_6H_8]^{+\cdot}$).

Persicachrome

Persicachrome, the pigment with a blue fluorescence in the UV, was found in small amounts in the fruit. Its maxima are at 330, 348, 368 nm, indicative of a conjugated pentaene chromophore. On MgO–Kieselgur the polarity towards persicaxanthin is inversed. For MS determination it was prepared through the acidification of persicaxanthin. Its structure (**3**) is 5,8-epoxy-5,8-dihydro-12′-apo-β-carotene-3,12′-diol. This structure is confirmed by MS: *m/z* 384 ($C_{25}H_{36}O_3$, $M^{+\cdot}$, 23%); 382 ($[M-2H]^{+\cdot}$, 3%); 366 ($[M-H_2O]^{+\cdot}$, 5%); 353 ($[M-CH_2OH]^+$, 2%); epoxide fragments: 304 ($[M-C_6H_8]^{+\cdot}$, 100%); 299 ($C_{20}H_{27}O_2$, 5%); 287 ($C_{19}H_{27}O_2$, 5%); 273 ($C_{18}H_{25}O_2$, 6%); 247 ($C_{16}H_{23}O_2$, 5%); 234 ($C_{15}H_{22}O_2$, 5%); 221 ($C_{14}H_{21}O_2$, 15%); 208 ($C_{13}H_{20}O_2$, 11%); 181 ($C_{11}H_{17}O_2$, 13%).

Apo-12′-violaxanthal

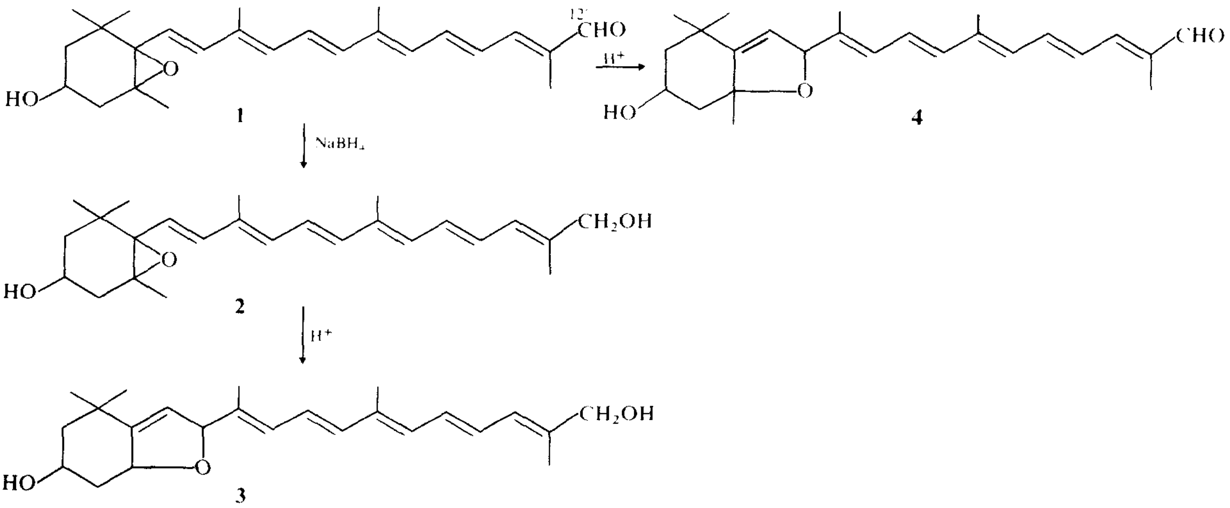
Apo-12′-violaxanthal was found in the lutein fraction that separated on silica gel developed with 30% acetone–petrol. After rechromatography on MgO–Kieselgur (1:1) developed with the same solvent system, this pigment was the least polar in the fraction which contained mainly lutein and small amounts of zeaxanthin. Its absorption spectrum, devoid of fine structure had a maximum at 412 nm in EtOH. The spectrum of violaxanthal reduced with $NaBH_4$ was identical with that of persicaxanthin. On acidification it gave persicachrome. Both these pigments could not be separated from natural persicaxanthin and persicachrome by cochromatography. The acidified apo-12′-violaxanthal gave a hypsochromic shift of 19 nm as the 5,8-epoxide was formed, with a maximum at 393 nm in EtOH (**4**). The structure of apo-12′-violaxanthal (**1**) is 5,6-epoxy-3-hydroxy-5,6-dihydro-12′-apo-β-caroten-12′-al. The MS confirmed this structure which was initially proposed by Curl [7]. The MS showed: *m/z* 382 ($C_{25}H_{34}O_3$, $M^{+\cdot}$, 100%); 364 ($[M-H_2O]^{+\cdot}$, 10%); epoxide fragments: 302 ($[M-C_6H_8]^{+\cdot}$, 42%); 287 ($C_{19}H_{27}O_2$, 20%); 247 ($C_{16}H_{23}O_2$, 6%); 234 ($C_{15}H_{22}O_2$, 7%); 221 ($C_{14}H_{21}O_2$, 27%); 208 ($C_{13}H_{20}O_2$, 19%); 181 ($C_{11}H_{17}O_2$, 21%).

The two natural C_{25}-epoxyapocarotenols with UV fluorescence increase the number of natural apocarotenols found in fruits to five. From the avocado fruit pulp the C_{27}-homologue of persicachrome was isolated, the 5,8-epoxy-5,8-dihydro-10′-apo-β-carotene-3,10′-diol [9]. In the peel of ripe Golden Delicious apple the C_{27}-homologue of persicaxanthin, the 5,6-epoxy-5,6-dihydro-10′-apo-β-carotene-3,10′-diol was identified [10]. From a citrus hybrid β-citraurol, a C_{30}-apocarotenol, 8′-apo-β-caroten-3,8′-diol was isolated and identified [11]. All these pigments can be obtained *in vitro*. Curl reduced and acidified the natural C_{25}- and C_{27}-apocarotenals in order to determine their structure [7, 12]. They were also obtained by alkaline permanganate oxidation of C_{40}-carotenoids [13].

In fruits the metabolites may be formed through Glover–Redfearn degradation of the most abundant C_{40}-pigment present, followed by an enzymatic reduction. As shown in a recent investigation [14] when the parent C_{40}-carotenoid is an epoxycarotenoid (mono- or di-), the cleavage at carbons 9–10, 9′–10′ and 11–12, 11′–12′ is favoured so that mainly C_{25}- and C_{27}-epoxyaldehydes are formed. This must be valid too for the enzymatic oxidation in the fruit. Evidence that the enzymatic reduction occurs subsequently must still be produced, since carotenogenesis involves mainly dehydrogenation and oxidation reactions. Only in the violaxanthin cycle, a component of the thylakoid, both de-epoxidation and epoxidation of violaxanthin proceed through reductive mechanisms [15]. But this may be a particular mechanism related to the carotenoids which are functionally involved in light reactions.

The relationship between the apocarotenoids, shown in Scheme 1, was clearly in evidence in the Sagiv plum in which all the three pigments were present. Curl also found in various fruits the apocarotenal along with its reduction and 5,8-isomerization products [3, 4, 8].

So far, the C_{25}- and C_{27}-epoxyapocarotenoids were detected only in ripe fruits. In apple, where carotenoid changes were followed during maturation and storage, the C_{27}-apocarotenol appeared only in the fruit ripened on the tree or during storage [16].



Scheme 1.

Table 1. Allylic apocarotenols

Semisystematic name	Trivial name	Formula	λ_{max} (nm in EtOH)	Occurrence in fruits	Reference
5,6-Epoxy-5,6-dihydro-12'-apo-β-carotene-3,12'-diol	Persicaxanthin Valenciaxanthin	$C_{25}H_{36}O_3$	353,372,393 351,369,390	Cling peach (*Prunus persica*)	[2]
				Apricot (*Prunus armeniaca*)	[3]
				Italian plum (*Prunus domestica*)	[4]
				French plum	This paper
5,8-Epoxy-5,8-dihydro-12'-apo-β-carotene-3,12'-diol	Persicachrome Valenciachrome	$C_{25}H_{36}O_3$	330,348,367 333,348,368	Valencia orange	[7][9]
				Navel orange (*Citrus sinensis*)	[8]
				Cranberry (*Vaccinium macrocarpon*)	[8]
				Fig (*Ficus carica*)	
5,6-Epoxy-5,6-dihydro-10'-apo-β-carotene-3,10'-diol	Sinensiaxanthin Apple apocarotenol	$C_{27}H_{38}O_3$	370,392,414 372,394,418	Golden Delicious apple (*Malus*)	[10]
5,8-Epoxy-5,8-dihydro-10'-apo-β-carotene-3,10'-diol	Sinensiachrome	$C_{27}H_{38}O_3$	353,371,396	Avocado (*Persea americana*)	[16]

It is obvious that many UV-fluorescent pigments with short in-chain chromophores, detected by Curl, belong to the group of allylic apocarotenols. Their trivial names are valenciaxanthin, valenciachrome, sinensiaxanthin and sinensiachrome, as can be seen in Table 1. The slight differences in absorption spectra may be due to the fact that they are different stereoisomers [13]. According to the existing data, the C_{25}-epoxycarotenols are prevalent in Rosaceae, whereas in Rutaceae (citrus) both C_{25}- and C_{27}-derivatives are found. Their presence is also related to the fruit cultivar studied. Persicaxanthin and persicachrome, present in the peach Halford cv, were not detected in two others, Redhaven [17] and Earliglo [18]. They were also absent from another French plum, d'Ente cv [19].

EXPERIMENTAL

The analytical methods were as reported previously [20]. Column chromatography was omitted, the pigment being separated directly by successive TLC on two adsorbents according to the method of Gross [1]. The first chromatogram developed with the solvent system Me_2CO–petrol (3:7) gives a preliminary fractionation into groups of different polarity. A further separation of each group into individual carotenoids is obtained by rechromatography on MgO–Kieselgur (1:1). The same solvent system is used, the Me_2CO percentage being increased according to the polarity of each group (from 4% to 30%). For the purification of persicaxanthin the solvent system 20% in Me_2CO in petrol–EtOH (99:1) was also used.

All mass spectra are high resolution spectra. They were run on a Kratos MS-50 mass spectrometer linked with a Kratos DS-50 data system at a dynamic resolution of 15000. The samples were directly introduced (source temp. 180°) and ionized at 70 eV. The elementary composition of the ions is given in brackets together with their relative intensities which were obtained by normalization on the most intense peak above m/z 75.

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REFERENCES

1. Gross, J. (1980) *Chromatographia* **13**, 572.
2. Curl, A. L. (1959) *Food Res.* **24**, 413.
3. Curl, A. L. (1960) *Food Res.* **25**, 190.
4. Curl, A. L. (1963) *Food Res.* **28**, 623.
5. Straub, O. (1976) in *Key to Carotenoids: Lists of Natural Carotenoids*, p. 103, Birkhäuser, Basel.
6. Goodwin, T. W. (1980) in *The Biochemistry of the Carotenoids*, 2nd edn, Vol. 1, p. 159. Chapman & Hall, London.
7. Curl, A. L. (1965) *J. Food Sci.* **30**, 13.
8. Curl, A. L. (1964) *J. Food Sci.* **29**, 241.
9. Gross, J., Gabai, M., Lifshitz, A. and Sklarz, B. (1974) *Phytochemistry* **13**, 1917.
10. Gross, J. and Eckhardt, G. (1978) *Phytochemistry* **17**, 1803.
11. Leuenberger, U., Stewart, I. and King, R. W. (1976) *J. Org. Chem.* **41**, 891.
12. Curl, A. L. (1967) *J. Food Sci.* **32**, 141.
13. Szabolcs, J. (1976) *Pure Appl. Chem.* **47**, 147.
14. Molnár, P. and Szabolcs, J. (1979) *Acta Chim. Acad. Sci. Hung.* **99**, 155.
15. Yamamoto, H. Y. (1979) *Pure Appl. Chem.* **51**, 639.
16. Gross, J., Zahariae, A., Lenz, F. and Eckhardt, G. (1978) *Z. Pflanzenphysiol.* **89**, 321.
17. Gross, J. (1979) *Z. Pflanzenphysiol.* **94**, 461.
18. Lessertois, D. and R. Monéger (1978) *Phytochemistry* **17**, 411.
19. Moutounet, M. (1976) *Ann. Technol. Agric.* **25**, 73.
20. Gross, J., Gabai, M., Lifshitz, A. and Sklarz, B. (1973) *Phytochemistry* **12**, 1775.