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CAROTENOIDS IN PULP, PEEL AND LEAVES OF *PERSEA AMERICANA*

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Abstract—A complex pattern of chloroplast pigments was found to be common to pulp, peel and leaves of avocado. This is explained by the presence of chlorophyll in the ripe fruit and its peel. Two chromoplast-specific pigments were found in ripe pulp and are tentatively identified as α-citraurin and mimulaxanthin. The identity of the latter and of neochrome, was established *inter alia* by the MS. The xanthophylls occur esterified to a major extent in pulp but entirely free in peel. The coexistence of chloroplast and chromoplast pigments in avocado fruit make this a suitable object for the ultrastructural studies of the origin of the chromoplasts.

INTRODUCTION

IN A previous study on the qualitative and quantitative distribution of carotenoids in the avocado fruit (*Persea americana*), Nabal variety,[1] the major discernible pattern was found to consist of: β-carotene, cryptoxanthin and unusually large amounts of lutein and chrysanthemaxanthin, as well as large amounts of isolutein and polyols. This pigment pattern is closer to that of chloroplasts than to the other main patterns found in fruits.[2] This fact can be explained by the presence of relatively large amounts of chlorophyll in the avocado fruit. Therefore, it was of interest to analyze other chloroplasts-containing tissues like the leaves, and the green peel of the ripe fruit.

RESULTS

Table 1 gives the carotenoid distribution in pulp, peel and leaves of avocado. The pigments are presented in order of increasing adsorption affinity (in general) on the several adsorbents used for column and TLC. Broadly, the major discernible pattern is common to all three tissues investigated. In the pulp (mesocarp), the carotenoid mixture is more complex owing to the appearance of minor pigments and of various *cis*-isomers. Thus, ζ-carotene was detected only in the pulp and γ-carotene only in the fully ripe fruit.

In the monol fraction, three unknown pigments with some unusual properties were separated. A new carbonyl pigment was tentatively identified as α-citraurin. In the diol fraction,

[1] GROSS, J., GABAI, M. and LIFSHITZ, A. (1972) *J. Food Sci.* **37**, 589.
[2] GOODWIN, T. W. and GOAD, L. J. (1970) in *The Biochemistry of Fruits and Their Products* (HULME, A. C., ed.), Vol. I, p. 305, Academic Press, New York.

cis-isolutein and two isomers of chrysanthemaxanthin appeared only in the pulp. A more polar carbonyl pigment 446, and an unknown pigment with the main absorption maximum at 370 nm, were also characteristic of the pulp. In the most polar fraction, the pigment previously designated trollichrome (398, 420, 448 nm) has now been identified as neochrome. It has two acetylatable hydroxyl groups. It was inseparable on TLC from the pigment obtained through the epoxy-furanoxy isomerization of neoxanthin with acid. The MS revealed 600 (7%, M^+); 582 (10% [M-18]); 564 (7% [M-2 × 18]); 548 (3%); 520 (2% [M-80]); 508 (7%); 502 (18%); 490 (10%); 484 (3%); 476 (3%); 456 (100%); 444 (12%); 221; 219; 181 a.m.u. and resembled closely the spectrum reproduced by Budzikiewicz *et al.*[3] with some additional peaks at 456 a.m.u. and below, (later recognized as an impurity).

Another polyol pulp-specific pigment, unknown 437 (418, 437, 467 nm), with the same chromophore as neoxanthin, occurred in two forms and gave a negative epoxide test. The main form was investigated further. The structure previously suggested, 3,3′,5′-trihydroxy-6′,7′-dehydro-α-carotene, was not borne out by the MS: 602 (30%, M^+); 584 (100% [M-18]); 566 (30% [M-2 × 18]); 510 (12% [M-92]); 508 (15%); 502 (12%); 492 (18% [M-18-92]) a.m.u. As the pigment has the same chromophore as neoxanthin and contains no epoxide function, the MW (602) suggested that the epoxide ring of neoxanthin has undergone reductive opening. The new hydroxyl group could be located at C_5 or C_6. The acetyl derivative of the pigment gave a MS with peaks at 686 (4%, M^+); 626 (100% [M-60]); 608 (4% [M-60-18]); 592 (2%); 584 (3% [M-60-42]); 566 (4% [M-60-60]); 564 (4%); 534 (8%); 520 (16%). Thus, the derivative is a diacetate. The entry of only two acetyl groups confirms that the new hydroxyl in the nonallenic portion is tertiary.

The chromatographic and spectrophotometric properties of our pigment resemble those of minulaxanthin recently described by Nitsche.[4] The MS we have obtained are in agreement with the structures proposed by him. We conclude that our pigment is probably mimulaxanthin, 6,7-Didehydro-5,6,5′,6′-tetrahydro-β,β-carotene-3,5,3′,5′-tetrol. The configurations at C_3 and C_5 have not been established but are likely to be the same as in neoxanthin (3*S*, 5*R*).[5]

In the peel the carotenoid pattern was nearly the same as in leaves. The leaf pigments do not differ from the expected pattern of Angiosperms[6] and belong to the α-carotene-containing category. Besides the main pattern, zeaxanthin, antheraxanthin, isolutein, chrysanthemaxanthin and neochrome were also found. Pigments which are found in leaves but not in peel are γ-carotene, zeaxanthin and antheraxanthin.

The degree of esterification of the carotenoids in the pulp, peel and leaves was determined. The pulp xanthophylls, which constitute about 95% of the total carotenoids, are 55–60% in esterified forms, whereas the carotenoids in the peel and leaves exist only in the free form.

The quantitative carotenoid composition of pulp, peel and leaves of avocado is given in Table 2. The total carotenoid content was about 10–14 μg/g fresh matter in pulp (mesocarp), 40 μg/g fresh matter of peel (exocarp) and 300 μg/g fresh matter of leaves. The pigment distribution in peel is very similar to that in leaves. The α- to β-carotene ratio is about the same in pulp and peel, but inverted in leaves where unusually large amounts of α-carotene are present. Lutein appears in pulp in half the concentration found in peel and leaves.

[3] BUDZIKIEWICZ, H., BRZEZINKA, H. and JOHANNES, B. (1970) *Monatsh. Chem.* **101**, 579.
[4] NITSCHE, H. (1972) *Phytochemistry* **11**, 401.
[5] BARTLETT, L., KLYNE, W., MOSE, W. P., SCOPES, P. M., GALASKO, G., MALLAMS, A. L., WEEDON, B. C. L., SZABOLCS, J. and TOTH, GY. (1969) *J. Chem. Soc.* **C**, 2527.
[6] STRAIN, H. H. (1966) *Biochemistry of Chloroplasts* (GOODWIN, T. W., ed.), Vol. I, p. 387, Academic Press, London.

TABLE 1. QUALITATIVE CAROTENOID DISTRIBUTION IN PULP, PEEL AND LEAVES OF *Persea americana*

Carotenoid	Pulp (mesocarp)	Peel (exocarp)	Leaves	Carotenoid	Pulp (mesocarp)	Peel (exocarp)	Leaves
α-Carotene	+	+	+	Chrysanthemaxanthin b	+	+	−
β-Carotene	+	+	+	Chrysanthemaxanthin c	+	−	+
ζ-Carotene	+	−	−	Zeaxanthin	−	−	+
γ-Carotene	+	−	+	Antheraxanthin	−	−	+
OH-α-carotene	+	+	+	Luteoxanthin	+	+	+
Cryptoxanthin	+	+	+	Unknown 370	+	−	−
Unknown 388	+	−	−	Auroxanthin	−	−	+
Unknown 420	+	−	−	Neochrome	+	+	+
Unknown 390	+	−	−	Neoxanthin a	+	+	+
α-Citraurin 442	+	−	−	Unknown 437 a (Mimulaxanthin?)	+	+	−
Lutein	+	+	+	Neoxanthin b	+	−	−
cis-Lutein	+	+	+	Unknown 437 b	+	−	−
Isolutein	+	+	+				
cis-Isolutein	+	−	−				
Violaxanthin	+	+	+				
Carbonyl 446	+	−	−				
Chrysanthemaxanthin a	+	+	−				

Chrysanthemaxanthin is found in pulp in a very high percentage. Neochrome and the new allenic xanthophyll are major pigments in the pulp. In peel and pulp neochrome appears in small quantities and the new pigment is absent.

TABLE 2. QUANTITATIVE CAROTENOID DISTRIBUTION IN PULP, PEEL AND LEAVES OF *Persea americana*

Carotenoid	% of total carotenoids* Pulp	Peel	Leaves	Carotenoid	% of total carotenoids* Pulp	Peel	Leaves
α-Carotene	0·9	6·1	16·0	Zeaxanthin	—	—	1·6
β-Carotene	4·0	10·8	11·3	Antheraxanthin	—	—	0·3
ζ-Carotene	0·5	—	—	Luteoxanthin	2·1	1·5	1·6
γ-Carotene	—	—	0·5	Neochrome	9·2	0·9	0·5
OH-α-Carotene	1·2	0·4	0·5	Neoxanthin a	7·3	9·8	7·5
Cryptoxanthin	5·2	1·4	0·5	Unknown 437 a (Mimulaxanthin?)	8·1	—	—
α-Citraurin	0·7	—	—	Neoxanthin b	0·5	—	—
Lutein	25·0	55·8	51·0	Unknown 437 b	0·5	—	—
Isolutein	9·0	6·4	1·7				
Violaxanthin	4·0	3·1	5·0				
Chrysanthemaxanthin	20·4	4·2	2·0				

* Expressed as β-carotene. Isomeric forms of the same pigment were determined together. Minor pigments were not recorded.

DISCUSSION

The basic chloroplast pigment pattern is similar in all three tissue groups, but refined analytical methods used by us, and by other investigators,[7-11] have revealed additional complexity among the chloroplast pigments. Thus, in avocado leaves γ-carotene appears among the carotenes and hydroxy-α-carotene accompanies cryptoxanthin. Similarly, isolutein, and chrysanthemaxanthin are found together with lutein, and neochrome occurs together with neoxanthin; zeaxanthin and antheraxanthin occur only in leaves. This may be due to their formation under the influence of sunlight.[12] The absence of zeaxanthin and antheraxanthin in peel may be due to the shading of the fruit by the leaves.

Ripe fruits (green peppers,[2] grape,[2] certain tomato varieties[13]) containing chlorophyll may contain unique carotenoid pigments, together with the chloroplast pigments. In certain green apple varieties such, unique pigments are absent and the chloroplast pigments undergo esterification.[14] These are indications that the chloroplast–chromoplast transformation occurs without the complete disappearance of chlorophyll. In ripe avocado fruit α-citraurin and the new allenic xanthophyll (mimulaxanthin) can be considered unique, but occur in low concentrations. The appearance of 60% of the xanthophylls in esterified form is a clear indication of chromoplast formation in pulp. Thus, in ripe avocado pulp, chloroplasts coexist with chromoplasts. The latter may arise from transformed chloroplasts[15] or they may be mainly developed directly from proplastids.[16]

Avocado peel remains green in the ripe fruit. Only chloroplast carotenoids are found and the xanthophylls are not esterified. We conclude that there is no chromoplast formation in peel. The unusual relationships between chloroplast and chromoplast pigments in avocado make this fruit an interesting subject for ultrastructural studies. The relative concentrations of the major carotenoids are of biosynthetic and taxonomic interest. The high ratio of α- to β-carotene in leaves is noteworthy.

The high percentage of lutein in all three tissues is of considerable interest. In peel and leaves it constitutes more than half of the pigment. In pulp, half of the lutein seems to be transformed via isolutein, to the lutein-furanoxide, chrysanthemaxanthin, the second main pigment of the pulp. However, some chrysanthemaxanthin is also found in peel and leaves.

EXPERIMENTAL

Methods. The analytical methods have been described previously. Extraction, saponification, and removal of sterols and acetylenic and olefinic alcohols were carried out as described.[1] Chromatography, identification of pigments and chemical tests, including the acetylation test, were carried out as described elsewhere.[17,18] MS were recorded under the same conditions as previously, using perfluoroalkane as external or internal standard. As relative abundances varied with time, they are quoted as a per cent of the strongest peak in the high mass region to which principal attention was given.

[7] CURL, A. L. and BAILEY, G. F. (1957) *J. Food Res.* **22,** 323.
[8] EICHENBERGER, W. and GROB, E. (1962) *Helv. Chim. Acta* **45,** 1556.
[9] COSTES, C. (1965) *Ann. Physiol. Vég.* **7,** 105.
[10] HAGER A. and MEYER-BERTHENRATH, T. (1966) *Planta* **69,** 198.
[11] HAGER, A. and MEYER-BERTHENRATH, T. (1967) *Planta* **76,** 149.
[12] HORVATH, G., KISSIMON, J. and FALUDI-DÁNIEL, A. (1972) *Phytochemistry* **11,** 183.
[13] LAVAL-MARTIN, D., QUENNEMENT, J. et MONÉGER, M. R. (1972) *Compt. Rend.* **274,** 2879.
[14] KNEE, M. (1972) *J. Exp. Botany* **23,** 184.
[15] BEN-SHAUL, J. and NAFTALI, Y. (1969) *Protoplasma* **67,** 333.
[16] EGGER, K. (1964) *Ber. Dtsch. Bot. Ges.* **77,** 145.
[17] GROSS, J., GABAI, M. and LIFSHITZ, A. (1971) *J. Food Sci.* **36,** 466.
[18] GROSS, J., GABAI, M., LIFSHITZ, A. and SKLARZ, B. (1973) *Phytochemistry* **12,** 1775.

For the degree of saponification of pulp carotenoids, a special method was elaborated because the unusually high oil content (between 10 and 30%) made normal chromatography unsatisfactory. Prior removal of oils was achieved according to the method of Fox.[19]

The lipid residue obtained after the evaporation of the acetone extract in vacuum (about 30 g oil) was taken up in 200 ml light petroleum (L.P.), and about 200 g alumina was added while swirling. The slightly colored supernatant was analyzed separately. It contained about half of the total quantity of oil. The pigments were eluted from the adsorbent with Et_2O–EtOH. The solvent mixture was evaporated, the residue taken up in L.P. and chromatographed on a MgO-Hyflo-Super-Cel column. The first eluate obtained from the column, with 5–10% acetone in L.P. contained the remaining oil and a part of the less polar pigments. The column was further developed with 10% acetone in L.P. and EtOH (99:1). Thus, three other zones were obtained in the column which was mechanically cut and eluted with Et_2O–EtOH. Each fraction, including the supernatant were analyzed separately on TLC. On the same plate, the unsaponified extract was compared with the respective saponified pigment extract, using as standards cryptoxanthin acetate and lutein diacetate. The appropriate adsorbents and solvent systems were used for esters—mainly, silica gel G developed with 10% acetone in L.P. The pigments were scraped from the plates, eluted and determined. For peel and leaves the extract was directly chromatographed on thin layer plates.

[19] Fox, D. L. and Hopkins, Th. S. (1966) *Comp. Biochem. Physiol.* **17**, 841.