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CAROTENOIDS OF THE FRUIT OF *AVERRHOA CARAMBOLA*

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Abstract—The carotenoids of the tropical fruit *Averrhoa carambola* were investigated and from most of them mass spectra were taken. The total carotenoid content was 22 μg/g fr. wt. The carotenoid pattern was uncommon, the main pigments were phytofluene (17%), ζ-carotene (25%), β-cryptoflavin (34%) and mutatoxanthin (14%). Additionally, β-carotene, β-apo-8'-carotenal, cryptoxanthin, cryptochrome and lutein were present in small amounts.

INTRODUCTION

The carotenoids of carambola, or star fruit (*Averrhoa carambola*), which are mainly responsible for its colour have not been previously investigated and are not mentioned in the latest carotenoid monograph [1]. Carambola is a tropical fruit which originates in southeast Asia, where it is widely cultivated. In Israel it is grown sporadically. The investigated fruits were the acid form 'Golden Star' cv which contains *ca* 1% oxalic acid [2]. The fruit is *ca* 12 cm long with a yellow waxy rind and yellow flesh and resembles a five-pointed star in cross-section.

RESULTS AND DISCUSSION

Table 1 shows the quantitative distribution of the carotenoids of the carambola fruit at two ripening stages. The pigments are listed in order of increasing polarity. The identifications were based on the UV characteristics and on cochromatography with authentic pigments. Additional evidence for identification was obtained from the mass spectra of the principal and more uncommon pigments.

ζ-Carotene *m/z* (rel. int.): 540 ($C_{40}H_{60}$) $[M]^+$ (100), 403 ($C_{30}H_{43}$) (21). Cryptoflavin *m/z* (rel. int.): 568 ($C_{40}H_{56}O_2$) $[M]^+$ (78), 566 $[M-2H]^+$ (18), 550 $[M-H_2O]^+$ (3), epoxide fragments: 488 $[M-C_6H_8]^+$ (69), 476 $[M-C_7H_8]^+$ (11), 462 $[M-C_8H_{10}]^+$ (2); 422 $[M-C_{11}H_{14}]^+$ (17), 396 $[M-C_{13}H_{16}]^+$ (9), 365 ($C_{25}H_{33}O_2$) (12), 352 ($C_{24}H_{32}O_2$) (43), 325 ($C_{22}H_{29}O_2$) (9), 312 ($C_{21}H_{28}O_2$) (4), 299 ($C_{20}H_{27}O_2$) (15), 287 ($C_{19}H_{27}O_2$) (19), 274 ($C_{18}H_{26}O_2$) (8), 247 ($C_{16}H_{23}O_2$) (19), 234 ($C_{15}H_{22}O_2$) (18), 221 ($C_{14}H_{21}O_2$) (100), 208 ($C_{13}H_{20}O_2$) (17), 181 ($C_{11}H_{17}O_2$) (55). Epoxide test: blue colour, no hypsochromic shift.

Table 1. Characterization and quantitative distribution of carotenoids of the carambola fruit (*Averrhoa carambola*) cv 'Golden Star'

Carotenoid pattern	λ_{max}^{EtOH} nm	% of total carotenoids	
		Unripe	Ripe
Phytofluene	331, 348, 367	8.0	16.7
β-Carotene	425, 450, 478	0.8	0.6
ξ-Carotene	380, 400, 424	14.9	25.3
Neurosporene	413, 438, 465	—	0.2
β-Apo-8'-carotenal	454	trace	1.0
β-Apo-8'-carotenol	402, 425, 450	—	—
β-Cryptoxanthin	425, 450, 478	1.5	1.3
β-Cryptoflavin	404, 427, 453	37.0	34.2
β-Cryptochrome	380, 402, 428	2.7	2.8
Mixture (unidentified)*	—	5.3	—
Lutein	420, 445, 473	2.4	1.3
Mutatoxanthin	404, 427, 453	21.1	13.9
Mixture (unidentified)*	—	6.3	2.7
Total carotenoids (μg/g fr. wt)	—	15.0	22.0

*Mixture of 10–15 pigments in trace amounts characterized only by electronic spectra.

Cryptoflavin acetate *m/z* (rel. int.): 610 ($C_{42}H_{58}O_3$) $[M]^+$ (62), epoxide fragments: 530 $[M-C_6H_8]^+$ (48), 518 $[M-C_7H_8]^+$ (20), 464 $[M-C_{11}H_{14}]^+$ (7), 438 $[M-C_{13}H_{16}]^+$ (14), 394 ($C_{26}H_{34}O_3$) (22), 341 ($C_{22}H_{29}O_3$) (18), 329 ($C_{21}H_{29}O_3$) (19), 289 ($C_{18}H_{25}O_3$) (8), 276 ($C_{17}H_{24}O_3$) (9), 263 ($C_{16}H_{23}O_3$) (55), 250 ($C_{15}H_{22}O_3$) (12), 223 ($C_{13}H_{19}O_3$) (32), 203 ($C_{14}H_{19}O$) $[263-HOAc]^+$ (100).

A slightly more polar fraction was a mixture of cryptoflavin and the 5',8'-epoxide since the mass spectrum showed additional peaks of medium intensity at *m/z* 205 ($C_{14}H_{21}O$) and 165 ($C_{11}H_{17}O$) that were not shifted to higher masses after acetylation.

Cryptochrome *m/z* (rel. int.): 584 ($C_{40}H_{56}O_3$) $[M]^+$ (34), 582 $[M-2H]^+$ (11), 504 $[M-C_6H_8]^+$ (14), 492 $[M-C_7H_8]^+$ (3), 438 $[M-C_{11}H_{14}]^+$ (2). The fragment 424 $[M-2C_6H_8]^+$ (19) proved the presence of two epoxy groups. The end-group which contains the hydroxyl function gave the fragments: 419 ($C_{29}H_{39}O_2$) (6), 392 ($C_{27}H_{36}O_2$) (2), 379 ($C_{26}H_{35}O_2$) (3), 365 ($C_{25}H_{33}O_2$) (3), 352 ($C_{24}H_{32}O_2$) (6), 325 ($C_{22}H_{29}O_2$) (5), 313 ($C_{21}H_{29}O_2$) (5), 299 ($C_{20}H_{27}O_2$) (8), 287 ($C_{19}H_{27}O_2$) (8), 274 ($C_{18}H_{26}O_2$) (10), 247 ($C_{16}H_{23}O_2$) (8), 234 ($C_{15}H_{22}O_2$) (9), 221 ($C_{14}H_{21}O_2$) (45), 208 ($C_{13}H_{20}O_2$) (7), 181 ($C_{11}H_{17}O_2$) (27), the hydroxyl-free end group gave the ions 403 ($C_{29}H_{39}O$) (10), 376 ($C_{27}H_{36}O$) (3), 363 ($C_{26}H_{35}O$) (4), 349 ($C_{25}H_{33}O$) (5), 336 ($C_{24}H_{32}O$) (13), 309 ($C_{22}H_{29}O$) (7), 297 ($C_{21}H_{29}O$) (11), 283 ($C_{20}H_{27}O$) (15), 271 ($C_{19}H_{27}O$) (20), 258 ($C_{18}H_{26}O$) (17), 231 ($C_{16}H_{23}O$) (17), 218 ($C_{15}H_{22}O$) (24), 205 ($C_{14}H_{21}O$) (100), 192 ($C_{13}H_{20}O$) (17), 165 ($C_{11}H_{17}O$) (61). Epoxide test: blue colour, no hypsochromic shift.

Mutatoxanthin *m/z* (rel. int.): 584 ($C_{40}H_{56}O_3$) $[M]^+$ (51), 582 $[M-2H]^+$ (7), 566 $[M-H_2O]^+$ (4), epoxide fragments: 504 $[M-C_6H_8]^+$ (48), 492 $[M-C_7H_8]^+$ (12), 478 $[M-C_8H_{10}]^+$ (2), 438 $[M-C_{11}H_{14}]^+$ (10), 412 $[M-C_{13}H_{16}]^+$ (7), 365 ($C_{25}H_{33}O_2$) (11), 352 ($C_{24}H_{32}O_2$) (32), 325 ($C_{22}H_{29}O_2$) (8), 312 ($C_{21}H_{28}O_2$) (7), 299 ($C_{20}H_{27}O_2$) (13), 287 ($C_{19}H_{27}O_2$) (22), 274 ($C_{18}H_{26}O_2$) (10), 247 ($C_{16}H_{23}O_2$) (17), 234 ($C_{15}H_{22}O_2$) (20), 221 ($C_{14}H_{21}O_2$) (100), 208 ($C_{13}H_{20}O_2$) (15), 181 ($C_{11}H_{17}O_2$) (56). Epoxide test: blue colour, no hypsochromic shift.

Mutatoxanthin diacetate *m/z* (rel. int.): 668 ($C_{44}H_{60}O_5$) $[M]^+$ (80), 608 $[M-HOAc]^+$ (11), epoxide fragments: 588 $[M-C_6H_8]^+$ (74), 576 $[M-C_7H_8]^+$ (11), 522 $[M-C_{11}H_{14}]^+$ (11), 496 $[M-C_{13}H_{16}]^+$ (3), 407 ($C_{27}H_{35}O_3$) (10), 394 ($C_{26}H_{34}O_3$) (38), 341 ($C_{22}H_{29}O_3$) (16), 329 ($C_{21}H_{29}O_3$) (28), 289 ($C_{18}H_{25}O_3$) (19), 276 ($C_{17}H_{24}O_3$) (26), 263 ($C_{16}H_{23}O_3$) (100), 250 ($C_{15}H_{22}O_3$) (13), 223 ($C_{13}H_{19}O_3$) (45), 203 ($C_{14}H_{19}O$) (95).

The total carotenoid content increased from 15 μg in the less ripe fruit, which still contained small amounts of chlorophyll (3.4 μg/g fr. wt), to 22 μg/g in the fully ripe fruit. The absorption spectra of the total carotenoid extract had the same maxima at both stages (380), 402, 426, 452 nm with the main peak at 426 nm due to the predominating 5,8-epoxides.

The carotenoid pattern was very uncommon and did not match any of the eight patterns of fruit carotenoids classified by Goodwin [1]. It consisted of two partly saturated acyclic polyenes and two carotenoid 5,8-epoxides: phytofluene (17%), ζ-carotene (25%), β-cryptoflavin (5,8-epoxy-5,8-dihydro-β,β-caroten-3-ol) (34%) and mutatoxanthin (5,8-epoxy-5,8-dihydro-β,β-carotene-3,3'-diol) (14%). Three common carotenoids, β-carotene, β-cryptoxanthin and lutein were present in small quantities only. Neurosporene and β-apo-8'-carotenal, which are less commonly found, were present in trace amounts and β-cryptochrome, the 5,8,5',8'-diepoxide of cryptoxanthin, an unusual carotenoid, constituted *ca* 3% of the carotenoid content.

Additionally, 10–15 pigments in trace amounts were characterized only by their electronic spectra.

ζ-Carotene occurs mainly in fruits which synthesize lycopene, such as tomatoes which contain also the related more saturated acyclic precursors. However, it accumulates at the ripe stages in many *Citrus* species reaching, in lemon peels, a level as high as 17% [3, 4]. Cryptoxanthin is found in many fruits which synthesize it along with β-carotene and zeaxanthin. It may be accompanied by almost all of its possible epoxides, especially cryptoflavin, its 5,8-epoxide, which is almost always present in various *Citrus* species but never exceeding the cryptoxanthin level [3]. However, cryptochrome, the 5,8,5',8'-diepoxide of cryptoxanthin, is quite rare and has been detected to date only in lemon peel [4] and in the juice of the Valencia orange [5].

Noteworthy is the presence of β-apo-8'-carotenal a C_{30}-carotenoid usually occurring in *Citrus* [3]. Mutatoxanthin occurs as a minor pigment in many fruits and is present in substantial quantities (13–15%) in the juice of three orange varieties (*Citrus sinensis*) [5]. The carotenoid pattern of carambola is similar to a certain extent to that of the carotenoids of the fruit juice of *Passiflora edulis*. This juice contains almost the same carotenoids as carambola excepting the epoxides of cryptoxanthin. The extent of this similarity is not clear since only the three main pigments phytofluene, β-carotene and ζ-carotene were determined quantitatively for *Passiflora edulis*. Of these, β-carotene was one of the principal pigments, unlike the case for carambola [6].

The carotenoid pattern of the fully ripe carambola fruits, in which the chlorophylls were totally disintegrated, differed from that of the less ripe fruit in the relative percentages of the pigments and the presence of neurosporene.

Generally, in a ripe fruit, as the turnover rate slows down, the more saturated carotenoid precursors accumulate. Thus, in the ripe carambola, phytofluene and ζ-carotene, the carotenoids of the earlier biosynthetic steps, were found at higher levels than in the unripe fruit. Even traces of neurosporene were detected. The other two main carotenoids, the 5,8-epoxides cryptoflavin and mutatoxanthin, were found at lower levels in the ripe fruit presumably because of the cessation of the later reactions of carotenoid biosynthesis involving insertion of hydroxyl and epoxide groups. In any case, since fruits were cut under buffer solution these pigments could not be artefacts resulting from the isomerization of the 5,6-epoxides in the presence of trace amounts of acid. Not excluded is the possibility that the oxalic acid present in the fruit, the molecule of which is smaller than that of other acids usually found in fruits (malic and citric acid), may penetrate the chromoplast membrane causing the isomerization *in situ*.

EXPERIMENTAL

The whole fruit, rind and flesh together, were analysed at two ripening stages. The unripe fruit was light yellow and green at the

edges, whereas the fully ripe fruit had a deeper yellow colour with an orange tint.

Pigment extraction. Preliminary analysis showed that the main pigments were carotenoid 5,8-epoxides, which may be artefacts formed by the isomerization of the corresponding 5,6-epoxides during the isolation procedure especially in such acidic material with pH 2–3. To prevent this, the fruit was cut under a soln of Tris buffer [(tris-hydroxymethyl)aminomethane]. Subsequently, it was extracted with Me_2CO in an Ultra Turrax homogenizer adding sufficient buffer to adjust the pH to *ca* 8 and BHT (butylated hydroxytoluene) as antioxidant, cooling exteriorly with ice.

The saponification time was 3 hr at room temp. Sterols were removed as previously described [7]. The acetylation with Ac_2O in pyridine was performed as described in ref. [8].

The pigments were separated according to a rapid TLC method worked out by Gross [9].

The MS are high resolution spectra. The samples were directly introduced (source temp. 210°) and ionized at 40 eV.

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REFERENCES

1. Goodwin, T. W. (1980) in *The Biochemistry of the Carotenoids* 2nd edn, Vol. 1, p. 159. Chapman & Hall, London.
2. Samson, J. A. (1980) *Tropical Fruits*. Longman, London.
3. Gross, J. (1977) in *Citrus Science and Technology* Vol. 1, p. 302. AVI Westport, Connecticut.
4. Yokoyama, H. and Vandercook, C. E. (1967) *J. Food Sci.* **32**, 42.
5. Gross, J., Gabai, M. and Lifshitz, A. (1972) *Phytochemistry* **11**, 303.
6. Leuenberger, F. J. and Thommen, H. (1972) *Z. Lebensm. Unters. Forsch.* **149**, 279.
7. Gross, J., Zahariae, A., Lenz, F. and Eckhardt, G. (1978) *Z. Pflanzenphysiol.* **89**, 321.
8. Gross, J., Gabai, M. and Lifshitz, A. (1973) *Phytochemistry* **12**, 1975.
9. Gross, J. (1980) *Chromatographia* **13**, 572.