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STRUCTURES OF SOME CAROTENOIDS FROM THE PULP OF *PERSEA AMERICANA*

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Abstract—The identity of chrysanthemaxanthin as a major pigment in avocado pulp has been confirmed by MS and the identity of neoxanthin similarly established. A carbonyl pigment was identified as 3-hydroxy-sintaxanthin. Two new UV fluorescent apocarotenoids were isolated. On the basis of spectrum, behaviour in acid and MS, one of these is assigned the structure 5,8-epoxy-5,8-dihydro-10′-apo-β-caroten-3,10′-diol. The other has an acid labile pentaene chromophore, and structure (**1**) has been tentatively assigned on similar evidence. These are the first natural allyic apocarotenols whose structures have been established.

INTRODUCTION

THE CAROTENOIDS of the avocado fruit (*Persea americana*), Nabal variety were characterized in a previous study.[1] While comparing the carotenoids of pulp, peel and leaves of avocado,[2] a more accurate structure elucidation was possible using MS. Thus the pigment previously designated trollichrome, (398, 420, 448 nm) was found to be neochrome. Further, the unknown pigment 437, was shown to be identical with the new allenic xanthophyll mimulaxanthin (6′,7′-didehydro-5,6,5′,6′-tetrahydro-β,β-carotene-3,5,3′,5′,-tetrol), isolated from the petals of *Mimulus guttatus*.[3] In the present study the identity of some known pigments from avocado pulp was confirmed, through MS and the structures of three minor pigments were established.

RESULTS AND DISCUSSION

20 kg of avocado pulp (mesocarp) were extracted as described previously.[1] Since the pattern of carotenoids varies with season, the fruit of the 1973 harvest was found to differ slightly in the relative content of minor pigments from those found earlier. The pigments are discussed in order of their increasing adsorption affinity on the MgO-Hyflo-Super-Cel column (see Table 1 in the earlier paper[1]).

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[1] GROSS, J., GABAI, M. and LIFSHITZ, A. (1972) *J. Food Sci.* **37**, 589.

[2] GROSS, J., GABAI, M., LIFSHITZ, A. and SKLARZ, B. (1973) *Phytochemistry* **12**, 2259.

[3] NITSCHE, H. (1972) *Phytochemistry* **11**, 401.

Carbonyl 449

In the diol–polyol fraction, "carbonyl 446" which follows violaxanthin was examined further. The maximum of its broad spectrum was at 449 nm, and its reduction spectrum had fine structure with λ_{max} 400, 420, 445 nm. These properties resemble those of β-citraurin as well as of 3-hydroxysintaxanthin. The MS showed a molecular ion (446 a.m.u.) and a weak M-18 peak. On cochromatography, the pigment did not separate from 3-hydroxysintaxanthin (MW 446) of citrus origin, but proved to be more polar than β-citraurin (MW 432). Despite the absence of (M-43)[4] from the weak MS, we tentatively consider this carbonyl pigment to be 3-hydroxysintaxanthin, i.e. 3-hydroxy-7′8′-dihydro-7′-apo-β-caroten-8′-one. This is the first report of this pigment outside the Rutaceae.[5]

Chrysanthemaxanthin

After lutein, this is the major pigment of the diol–polyol fraction. The other isomeric forms of chrysanthemaxanthin *a* and *c*, differing slightly in absorption spectra, were not observed in the present batch of fruit. The epoxide test produced the blue colour but no hypsochromic shift, indicating a 5,8-epoxy group. The MS accorded well with the structure 5,8-epoxy-5,8-dihydro-β,ε-carotene-3,3′-diol and contained the following prominent ions: 584 (5% M^+); 566 (55%, [M-18]); 564 (13%[M-18-2]); 551 (5%[M-18-15]); 504 (9%[M-80]); 492 (5%[M-92]); 486 (40%[M-80-18]); 474 (10%[M-92-18]); 221 (75%); 189 (100%); 181 (28%). The loss of 80 a.m.u. is characteristic of 5,8-epoxides, the prominent ions at 181 and 221 indicating a 3-hydroxyl group.[6]

The pigment afforded a mono- and a diacetate. The MS of the former contained the following peaks: 626 (3%,M^+); 608 (8%[M-18]); 566 (100%[M-60]); 551 (3%[M-60-15]); 546 (4%[M-80]); 528 (5%[M-80-18]); 486 (44%[M-60-80]); 473 (9%); 420 (5%); 394 (7%); 221 (100%); 181 (50%). A metastable ion at 417 arises from the transition 566 → 486 a.m.u. The prominent peaks at 221, 181 a.m.u. derive from the 5,8-epoxide ring with free 3-hydroxyl group. The 3′-hydroxyl is therefore acetylated in this derivative. The diacetate failed to give a molecular ion. The major ions were 608 (M-60); 528 (M-60-80); 518; 396; 263; 223; 203; 163. There were no prominent peaks at 221, 181. The last four masses correspond to the ions formed before and after elimination of acetic acid.[6]

Fluorescent allylic apocarotenols

A fluorescent pigment "unknown 370" which appeared between luteoxanthin and neochrome, was reported in our earlier work.[1] By repeated column chromatography on MgO-Hyflo-Super-Cel (solvent 10%A/PE-Et from 99:1 to 97:3) two pigments were separated. The less polar, F348, after several further chromatographic purifications (particularly from luteoxanthin), showed a bright green fluorescence similar to phytofluene in UV-light, and a spectrum with λ_{max} 332, 348, 368 nm, suggesting a pentaene chromophore as in phytofluene. The pigment gave a positive Carr–Price reaction. The pigment is extraordinarily sensitive to acids as revealed during tests for epoxide and allylic hydroxyl. The reaction was followed kinetically at intervals up to 30 min (see Fig. 1).

The blue colour characteristic for 5,6- and 5,8 epoxides did not appear, but the colourless solution became yellow. Two new pigments were formed in poor yield: a carbonyl

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[4] FRANCIS, G. W. (1969) *Acta. Chem. Scand.* **23**, 2916.

[5] GOAD, L. J. and GOODWIN, T. W. (1970) In *The Biochemistry of Fruits and their Products* (HULME, A. C., ed.), Vol. I, p. 305, Academic Press, New York.

[6] CHOLNOKY, L., GYÖRGYFY, K., RONAY, A., SZABOLCS, J., TOTH, G., GALASKO, G., MALLAMS, A., WAIGHT, E. and WEEDON, B. L. C. (1969) *J. Chem. Soc.* C 1256.

compound, λ_{max} 365 nm and a more polar yellow pigment λ_{max} 397, 423, 452 n.m. The latter spectrum resembles that of reduced β-apo-10′-carotenal with a conjugated octaene chromophore. This behaviour with acids suggests the presence in the fluorescent pigment of an interrupted chromophore. The MS gave the following peaks; 412 (26%M^+); 410 (10%); 397 (34%[M-15]); 332 (100%[M-80]); 232 (48%); 221 (20%); 181 (18%). The last two peaks, though not large, were locally prominent. Together with the transition 412 → 332 confirmed by a metastable ion at 267.5 a.m.u. they indicate the presence of a 3-hydroxy-5,6 or 5,8-epoxide or a 5,8-diol as in isofucoxanthol.[7] In the light of the behaviour with acid (see above), the latter group is indicated.

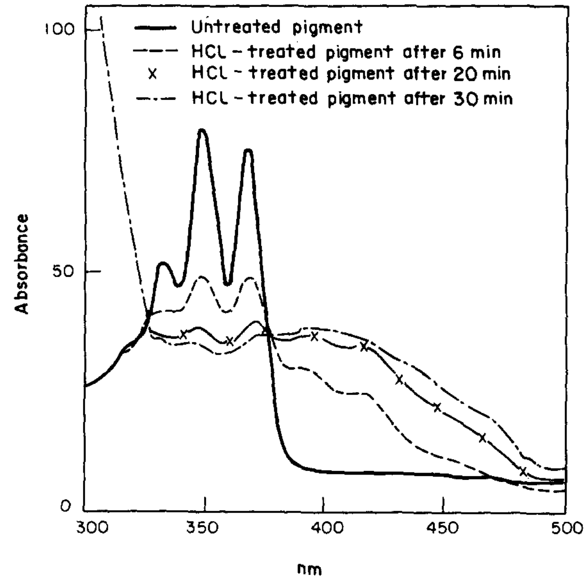

FIG. 1. THE SPECTRUM OF CAROTENOID F348 RECORDED AT INTERVALS DURING TREATMENT WITH HCL.

Acetylation of the pigment yielded a diacetate and a monoacetate. The former is characterized by its MS with 496 (25%,M^+); 494 (5%); 416 (100%[M-80]); 356 (12%[M-80-60]); 274 (42%); 264 (35%); 256 (21%); 236 (30%); 223 (18%); 222 (17%); 221 (17%). A metastable ion at 349 a.m.u. indicates the transition 496 → 416. The evidence for a 3-acetoxy group (peaks at 223 and 263) is not clear cut.

The monoacetate was contaminated by some diacetate as revealed by the mass spectrum: 496 (25%[M^+ diacetate]); 454 (20%[M^+ monacetate]); 416 (100%[M^+, diacetate-80]); 374 (75%[M^+, monoacetate-80]); 274 (65%); 263 (10%); 223 (10%), 221 (15%); 181 (20%). The last two ions indicate that in the monoacetate the 3-hydroxyl group is free. Metastable ions at 349 and 208 correspond to the loss of 80 a.m.u. from each of the molecular ions. Under the above acid conditions (see Fig. 1) the chromophores of both acetates were stable. We tentatively suggest structure (**1**), with an unusual terminal methylene group, for this pigment. On treatment with acid, a protonation of the bisallylic 8-OH group followed by conjugated elimination would lead to an octaene, a reaction prevented by acetylation of the 8-OH group.

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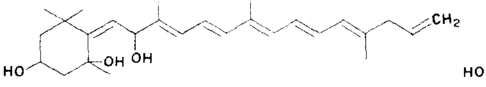
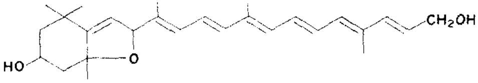
[7] BONNETT, R., MALLAMS, A. K., SPARK, A. A., TEE, J. L., WEEDON, B. C. L. and MCCORMICK, A. (1969) *J. Chem. Soc.* C 429.

The more polar fluorescent pigment, F371, displayed a yellow fluorescence in UV. The spectrum with sharp fine structure max. 353, 371, 396 nm suggests a hexaene chromophore. The response to the Carr–Price test was similar to that shown by the previous pigment. In the epoxide test an intense blue colour, without shift, indicated a 5,8-epoxide. On allylic etherification a new less polar compound was formed indicating an allylic hydroxyl group.[8] This appears to be primary since on treatment with HCl-chloroform,[9] no extension of the chromophore occurred.[10] Structures (**2**), 5,8-epoxy-5,8-dihydro-10′-apo-β-caroten-3,10′-diol, includes these features.



(1)



(2)

This structure is borne out by MS. Ions appeared at 410 (100%,M$^+$), 392 (15%[M-18]); 330 (100%[M-80]); 221 (40%); 181 (26%). The intensity and prominence of the past peak varied. However, the intense loss of 80 a.m.u. confirms the furanoxide structure. The pigment again yields two acetates. The MS of the monoacetate shows 452 (100% M$^+$); 410 (25%[M-42]); 392 (40%[M-60]); 372 (80%[M-80]); 264 (65%); 236 (100%); 221 (50%); 181 (60%). The last two peaks are the most intense of their kind. From the absence of significant peaks at 263 and 223 the 3-hydroxyl group is free and the terminal allylic hydroxyl is acetylated. Ketene (42 a.m.u.) would be eliminated from such a position (giving 410) along with acetic acid (giving 392). The MS of the diacetate accords well with the proposed structure 494 (86%,M$^+$); 452 (10%[M-42]); 434 (40%[M-60]); 414 (100%,[M-80]); 368 (30%); 355 (25%); 354 (25%); 341 (34%); 281 (45%); 272 (40%); 263 (50%); 223 (40%); This pigment is presumably a metabolite of chrysanthemaxanthin, one of the main pigments of the avocado fruit. The chemical oxidation of violaxanthin and chrysanthemaxanthin is now under investigation. The pigment is related to the natural apo-10′-violaxanthal (5,6-epoxy-3-hydroxy-5,6-dihydro-10′-apo-β-caroten-10′-al) found by Curl in Valencia orange peels.[11] This compound (λ_{max} 440) was reduced to the corresponding alcohol λ_{max} 370, 391, 414 nm. On treatment with HCl the maxima shifted to 352, 371, 394 nm through 5,8-isomerization of the 5,6-epoxide.

Curl's "*in vitro*" product appears to be identical with the natural fluorescent pigment whose structure (**2**) we have established. This affords confirmation for the structure of apo-10′-violaxanthal. Our pigment is the first naturally occurring allylic apocarotenol whose structure has been established. The occurrence of β-apo-2′-carotenol (alcohol C$_{37}$) was briefly reported without discussion.[12]

In various fruits, Curl has repeatedly found polar UV fluorescent pigments with sharp fine structure typical of short in-chain chromophores: valenciaxanthin in Valencia orange peel;[13] valenciachromes *a* and *b* in Navel oranges[14] (green fluorescence in UV) and a phytofluoenol-like pigment as well as pigment 378 in Meyer lemons.[14] Similar UV fluorescent

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[8] AITZETMÜLLER, K., STRAIN, H. H., SVEC, W. A., GRANDOLFO, M. and KATZ, J. J. (1969) *Phytochemistry* **8**, 1761.
[9] HERTZBERG, S. and LIAAEN-JENSEN, S. (1969) *Phytochemistry* **8**, 1259.
[10] ARPIN, N. and LIAAEN-JENSEN, S. (1967) *Compt. Rend. Ser D* **265**, 1083.
[11] CURL, A. L. (1967) *J. Food Sci.* **32**, 141.
[12] BONALY, R. and MALENGE, J. P. (1968) *Biochem. Biophys. Acta* **164**, 366.
[13] CURL, A. L. and BAILEY, G. F. (1954) *J. Agr. Food Chem.* **2**, 685.
[14] CURL, A. L. and BAILEY, G. F. (1961) *J. Food. Sci.* **26**, 422.
[15] CURL, A. L. (1962) *J. Food Sci.* **27**, 171.

pigments (persicaxanthin and persicachrome) were found in Rosaceae (peaches,[16] apricots,[17] prunes[18]) and in other fruits with low carotenoid content (figs, blackberries, grapes, etc.).[19] On reinvestigating the polar carotenoids of citrus (Valencia orange juice) we were able to identify the same two fluorescent pigments reported above. This new class of apocarotenols must be metabolites formed through the degradative transformation postulated by Glover and Redfearn. The absence of apocarotenoic acids in plants,[20] can be explained by the enzymatic reduction of apocarotenals to apocarotenols. The two fluorescent pigments with MW differing by only two mass units are probably related biogenetically. As the structure for one of them (F348) is tentative, speculation as to this relationship is premature.

Neoxanthin

The avocado neoxanthin appeared in two isomeric forms. The minor more polar pigment had the same R_f as spinach neoxanthin. The MS of the major neoxanthin showed peaks at 600(54%,M^+); 582(77%[M-18]) 564(46%[M-2x18]); 520(23%[M-80]); 502 (100%[M-80-18]); 484 (15%[M-80-2x18]). These peaks correspond to those reproduced by Budzickiewicz.[21] Peaks at 181 and 221 a.m.u. were absent, as found by Cholnoky[5] for 5,6-epoxides including neoxanthin from maple leaves. These peaks in Budzikiewicz's spectrum may arise by thermal epoxy–furanoxy rearrangement.[6] Our spectrum also resembles that of neoxanthin from *Eriobotrya japonica*,[22] and is not complicated by internal standard. Similar major fragment ions were recorded for neoxanthins from other plant sources by Nitsche.[23]

Recently, H. Nitsche (personal communication) has found that mimulaxanthin, the pigment isolated from *Mimulus guttatus*, is a diallenic pigment so that the pigment 437 from avocado-with the structure 6′,7′-didehydro-5,6,5′,6′-tetrahydro-β,β-carotene-3,5,3′,5′,-tetrol is a new allenic pigment, so far only known from avocado.

EXPERIMENTAL

The analytical methods were carried out as reported elsewhere.[1] Acetylation was as reported previously.[23] Allylic hydroxyl tests: Preparation of methyl ethers was carried out as described in Ref. 8, but on microscale. The products were separated on TLC (silica gel G developed with 10–20% A/PE). The dehydration test with acidic chloroform was carried out in the usual way.[9] MS were recorded as before,[2,23] using perfluoroalkane as external standard only.

Acknowledgement—Thanks are due to Mrs. Sima Alfi for recording the MS.

[16] CURL, A. L. (1959) *Food Res.* **24**, 413.

[17] CURL, A. L. (1960) *Food Res.* **25**, 190.

[18] CURL, A. L. (1963) *Food Res.* **28**, 623.

[19] CURL, A. L. (1963) *J. Food Sci.* **29**, 241.

[20] THOMMEN, H. (1971) In: *Carotenoids* (ISLER, O., ed), p. 639, Birkhäuser, Basel.

[21] BUDZIKIEWICZ, H., BRZEZINKA, H. and JOHANNES, B. (1970) *Monatsh. Chem.* **101**, 579.

[22] GROSS, J., GABAI, M., LIFSHITZ, A. and SKLARZ, B. (1973) *Phytochemistry* **12**, 1775.

[23] NITSCHE, H. and PLEUGEL, C. (1972) *Phytochemistry* **11**, 3383.