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STRUCTURAL ELUCIDATION OF SOME ORANGE JUICE CAROTENOIDS

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Key Word Index—*Citrus sinensis*; Rutaceae; Valencia orange juice; carotenoids; apocarotenoids; carotene-3,3′,5,6-tetrols.

Abstract—The structure of some carotenoids of Valencia orange juice were elucidated by chemical tests and MS of the free pigments and their derivatives. A new apocarotenal was shown to be 3-hydroxy-5,8-epoxy-5,8-dihydro-8′-apo-β-caroten-8′-al. Two UV-fluorescent apocarotenols found recently in avocado were also present. For the pigments previously designated trollixanthin and trollichrome, the new structures 5,6-dihydro-β,β-carotene-3-3′,5,6-tetrol and 5,8-epoxy-5,8,5′,6′-tetrahydro-β,β-carotene-3,3′,5′,6′-tetrol are assigned, both containing a trihydroxylated ring as in heteroxanthin.

INTRODUCTION

The carotenoids of three varieties of *Citrus sinensis* grown in Israel, were characterised in previous studies [1,2]. The orange fruit, pulp and peel revealed a very complex carotenoid pattern in which the diol–polyol fraction predominates, comprising about 70% of the total. The study of citrus carotenoids was initiated by Curl as early as 1954 with an investigation of the polyoxygen carotenoids of Valencia orange juice [3]. Since then, the citrus carotenoids have been the subject of many investigations. However the structures of several highly oxidised pigments remain unsolved. In this study, chemical and MS evidence is presented for the structure of some of these compounds.

RESULTS AND DISCUSSION

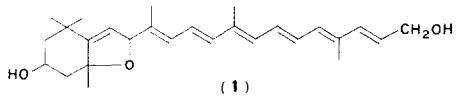
About 20 l. of Valencia juice were extracted, separated and purified as previously described [1]. The differences found in the qualitative and quantitative distribution must be ascribed to seasonal variation. The fractions designated in this paper refer to those described in Table 1 in refs. 1 and 2.

The zeaxanthin fraction (3B), which in the previous season contained four pigments with a single broad absorption maxima at 450 nm and the spectrum of the $NaBH_4$ reduction product at 400, 422 and 445 nm, was in the present season dominated by only one carbonyl pigment. The MS* showed *m/e* 432 (22%, M^+); 326 (35%, [M^+-106]); 308 (8%, [M^+-106-18]); 236 (50%); 43 (100%). Correspondingly, reduction with $NaBH_4$ gave a product with MS* *m/e* 434 (44%, M^+); 416 (40%, [M^+-18]); 408 (15%); 398 (2%, [M^+-2 × 18]); 397 (2%); 390 (10%); 368 (6%); 342 (3%, [M^+-92]); 324 (5%, [M^+-92-18]); 43 (100%). From the MS the pigment was identified as β-citraurin, which, according to electronic spectra, chemical and chromatographic properties is very similar to 3-hydroxysintaxanthin. The expected M^+-28 and M^+-29 ions were not observed, but were absent also in a spectrum of authentic 8′-apocarotenal (kindly supplied by Hoffmann-La Roche, Basle).

In the most polar fraction (3C), the fluorescent UV zone between auroxanthin and trollichrome could be resolved into a less polar substance, λ_{max} 332, 348, 368 nm, with a green fluorescence, and a more polar one, λ_{max} 353, 371, 396 nm, with yellow fluorescence. On cochromatography, they were inseparable from the two fluorescent avocado pig-

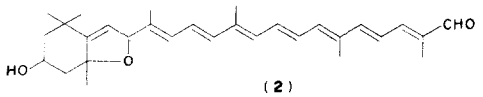
* The base peak was saturated; hence relative intensities are significant only for comparison of neighbouring peaks.

ments described earlier [4]. Fluorescent metabolites seem to occur in the fruits of many different families [4]. In particular the more polar compound was shown to be the novel 5,8-epoxy-5,8-dihydro-10′-apo-β-caroten-3,10′-diol, (**1**).



(**1**)

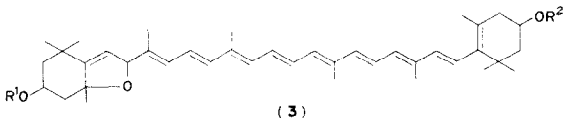
In fraction 3C, only one of the carbonyl pigments at the top of the column, was present in amounts sufficient for characterisation. It absorbed at λ_{max} 430 nm in EtOH, λ_{max} 445 nm in $CHCl_3$ and with fine structure, at λ_{max} 406, 428, 452 nm in hexane. The spectrum of the reduced compound at λ_{max} 379, 394 and 418 nm in EtOH indicated a heptaene chromophore. The HCl-test for epoxide was positive without a spectral shift, indicating a 5,8-epoxide and the MS showed *m/e* 448 (10%, M^+); 368 (5%, [M^+-80]); 356 (1·2%, [M^+-92]); 339 (1·3%, [M^+-80-29]); 221 (9·5%); 181 (5%); 43 (100%). The large (M^+-80) peak and the significant peaks at *m/e* 221 and 181, together with the M^+, support the 5,8-oxide structure (**2**)



(**2**)

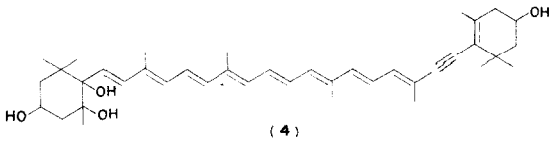
which we assign to this new pigment, 3-hydroxy-5,8-epoxy-5,8-dihydro-8′-apo-β-caroten-8′-al. It is thus the second of the novel group of apocarotenoid furanoxides which we have detected (c.f.I) and also poses an interesting biogenetic problem. It could arise by the Glover–Redfearn degradation mechanism from a furanoid carotenoid, or by the epoxidation of β-citraurin. The intermediate 5,6-epoxycitraurin could not be detected.

In an investigation of the most polar pigment of fraction 3C ('trollixanthins' and 'trollichromes' [1,2]) a trollixanthin-like pigment was isolated, with λ_{max} 424, 445 and 474 nm. However, the MW (602), did not correspond to that of trollixanthin (600). The latter had been isolated for the first time by Karrer *et al.* [5,6] from the flower *Trollius europaeus* and subjected since then to multiple investigations without giving a definite result [7–12]. The MS of the citrus 'trollixanthin', its diacetate and the products of acid treatment were recorded. The MS of the free pigments exhibited *m/e* 602 (1%, M^+); 584 (0·15%, [M^+-18]); 566 (0·02%, [M^+-2 × 18]); 551 (0·03%); 538 (0·06%); 510 (0·23%, [M^+-92]); 496 (0·03%, [M^+-106]); 492 (0·06%, [M^+-92-18]); 472 (0·08%); 368 (2·8%); 43 (100%). The pigment yielded a diacetate with the appropriate M^+ at *m/e* 686 (0·64%) and major fragments at *m/e* 668 (0·13%, [M^+-18]); 626 (0·32%, [M^+-60]); 594 (0·13%, [M^+-92]); 568 (0·16%); 534 (0·16%, [M^+-92-60]); 43 (100%). These MS show that the compound is not a 5,6 or 5,8-oxide. Nevertheless, on treatment with NHCl a hypsochromic shift of 20 nm and a blue colouration were observed after several hr. The main purified product (after acid treatment overnight) showed in the MS ion at *m/e* 584 (2%, M^+); 544 (1·2%); 504 (1·4%, [M^+-80]); 492 (1·2%, M^+-92); 443 (1·3%); 368 (8%); 43 (100%). Peaks at *m/e* 221 and 181 were not prominent, but the prominent (M^+-80) ion is a good indication that a 5,8-epoxide had been formed with the acid, as is also suggested by the blue colouration. This product was spectroscopically identical with mutatoxanthin (5,8-epoxy-5,8-dihydro-β,β-carotene-3,3′-diol (**3**, R^1 = R^2 = H), (λ_{max} 404, 425, 450 nm) and inseparable from it on cochromatography, the identification being in full accord with the MS.

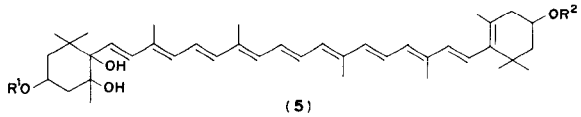


(**3**)

The slow acid-catalysed elimination of water to produce a 5,8-oxide ring recalls Nitsche's [13] observation that heteroxanthin (from *Euglena gracilis*) on acid treatment slowly loses water giving isomers of diadinochrome. His assignment of a 5,6-dihydroxy structure (**4**) was supported by an M^+-143 ion in the MS. We did not find this fragmentation in our pigment, but on the basis of the slow catalysed formation of (**3**) assign to it the new structure (**5**, R^1 = R^2 = H), 5,6-dihydro-β,β-carotene-3,3′,5,6-tetrol. A negative test for allylic OH confirmed the β-structure.



(**4**)



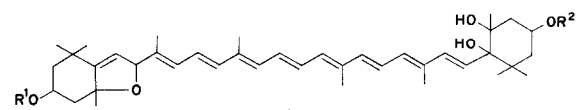
(5)

The diacetate has the corresponding structure (V, $R^1 = R^2 =$ Ac). However, it failed to undergo silylation and on prolonged treatment with HCl, the diacetate underwent furanoxide ring-closure and partial hydrolysis. The one product, the less polar on TLC, had in the MS *m/e* 668 (0·2%, M_1^+); 626 (1·8%, M_2^+); 608 (0·23%, $[M_1^+-60]$); 588 (0·23%, $[M_1^+-80]$); 546 (2·3%, $[M_2^+-80]$); 534 (0·9%, $[M_2^+-92]$); 394 (2·4%); 368 (4%); 263 (6%); 223 (4%); 43 (100%). The dominant peaks are due to a monoacetate ($M_2^+ = 626$) which contains an oxide ring. The locally prominent peaks at *m/e* 263 and 223 and the peak at 394 [14] suggest the furanoxide ring system had remained acetylated (**5**, $R^1 =$ Ac, $R^2 =$ H). A second more polar product, consisted mainly of mutatoxanthin (**3**, $R^1 = R^2 =$ H; $M_2^+ =$ 584), and some of its monoacetate ($M_1^+ = 626$). The MS showed *m/e* 626 (0·67% M_1^+); 584 (13%, M_2^+); 568 (1·3%); 566 (1·1%, $[M_1^+-60]$); 546 (0·8%, $[M_1^+-80]$); 504 (15%, $[M_2^+-80]$); 492 (4%, $[M_2^+-92]$); 368 (5%); 352 (9%); 221 (15%); 181 (8%); 43 (100%). A metastable ion at *m/e* 436 arises from the transition 584 → 504. The peaks at *m/e* 221 and 181 were prominent and, with *m/e* 352 [14] are in good agreement with the 3-hydroxy-5,8-oxide system of mutatoxanthin.

Trollichrome-like pigments

The tetrol ($V_1 R^1 = R^2 =$ H) was preceded on TLC (Silicagel G, petrol.–Me_2CO, 3:2) by a pigment whose λ_{max} at 396, 420 and 448 nm indicated an octaene chromophore. MS of the free pigment revealed a mixture of compounds with MWs of 626, 618 and 542. The dominant peak *m/e* 542 in the spectra may be due to an oxygenated apocarotenol with an octaene chromophore analogous to the fluorescent pigments and overlapping chromatographically with the trollichrome-like pigments. Further purifications are needed to establish the structures of these compounds but the extreme reactivity of the pigment towards acids makes investigation difficult.

After TLC purification the tetrol V ($R^1 = R^2 =$ H) still had four absorption maxima at 396, 422, 447, 470 nm. On repurification on a MgO column, a trollichrome-like pigment remained at the top and showed λ_{max} 396, 422, 448 nm characteristic of an octaene chromophore and gave the colour test for furanoxides. The MS showed *m/e* 618 (4·7%, M^+); 616 (1%, $[M^+-2]$); 602 (1·5, M_2^+); 600 (20%, $[M_1^+-18]$); 584 (0·6%, $[M_2^+-18]$); 582 (0·8%, $[M^+-2 \times 18]$); 542 (1·2%); 538 (6%, $[M_1^+-80]$); 526 (4·0%, $[M^+-92]$); 368 (2%); 352 (3·6%); 221 (18%); 181 (12%); 43 (100%). The prominent ions at *m/e* (M_1^+-80), 352, 221 and 181 support the furanoxide structure. The ion at *m/e* 602 (M_2^+) is ascribed to the presence of some of the tetrol (V, $R^1 = R^2 =$ H). Acid treatment of the compound gave a product spectroscopically and chromatographically identical with auroxanthin. The MS contained peaks at *m/e* 600 (12%, M^+); 598 (4%, $[M^+-2]$); 584 (4%, M_1^+); 582 (1·5%); 566 (1·2%, $[M_1^+-18]$); 520 (2·7%, $[M^+-80]$); 508 (1·8%, $[M^+-92]$); 504 (2·0% $[M_1^+-80]$); 492 (1·2% $[M^+-80-18]$); 487 (2·0%); 457 (1·3%); 443 (7%); 440 (2%, $[M^+-2 \times 80]$); 368 (7%); 352 (2%); 221 (9%); 181 (8%); 43 (100%). The product (MW 600) was thus a bisfuranoxide (M^+-80) and ($M^+-2 \times 80$), as shown also by prominent peaks at *m/e* 352, 221 and 181; the MS of auroxanthin (MW 600) [15] showed all these features. The acidification product however, contains some mutatoxanthin (peaks *m/e* 584 and 504) arising from cyclisation of the tetrol still present. The new furanoxide pigment thus contained one oxygen atom more than the tetrol (**5**, $R^1 = R^2 =$ H). Like the latter it slowly lost a molecule of water in NHCl, to give the bisfuranoxide auroxanthin. Hence, we assign to it the structure (**6**) 5,8-epoxy-5,8,5′,6′-tetrahydro-β,β-carotene-3,3′,5′,6′-tetrol.

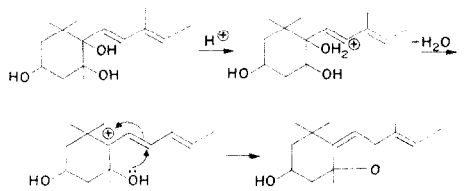


(6)

The new pigment also yielded a diacetate MS *m/e* 702 (100%, M^+); 684 (20%, $[M^+-18]$); 660 (7·5%, M^+ for monoacetate); 642 (26%, $[M^+-60]$); 622 (70%, $[M^+-80]$); 610 (50%, $[M^+-92]$); 592 (15%, $[M^+-92-18]$); 394 (50%). The loss of 80 a.m.u. and the peak at *m/e* 394 confirm the acetylated structure (**6**, $R^1 = R^2 =$ Ac). The relatively small peak at *m/e* 660 is due to the monoacetate. This was also prepared but was not entirely free of diacetate as shown by the MS *m/e* 702 (0·5%, M_1^+); 660 (1·3%, M_2^+); 642 (0·5%, M_1^+-60); 584 (0·8%); 580 (2·0%,

[M_2^+-80]); 568 (1·1%, [M^+-92]); 542 (1·0%); 462 (0·7%); 394 (1·1%); 352 (1·8%); 263 (5·3%); 239 (7·8%); 221 (12%); 181 (9%); 43 (100%). The spectrum is consistent with the general structure (**6**) but acetylation may have occurred at either end.

The slow acid-catalysed ring closure found for heteroxanthin and for pigments **5** and **6** may be reasonably formulated as follows:



The very similar behavior of trollixanthin from *Trollius europaeus* in acid, described by Eugster and Karrer [16], may in the light of the proposed scheme, be ascribed to a similar structure.

EXPERIMENTAL

The full analytical methods have been described previously [1,2,17]. For convenience, the chromatographic procedures involved in the present work are resummarized. The first fractionation into hydrocarbons, monols, diols and polyols was carried out on a column of MgO–Hyflo Super Cel (1:1, w/w). The column was eluted stepwise with increasing amounts of Me_2CO in petrol. (60–80°). The hydrocarbons were eluted with 1·5% Me_2CO in petrol. and with 10% Me_2CO two monol subfractions (2A and 2B) were separated. The third Fraction of diols and polyols was eluted with EtOH–petrol. (1:1) and rechromatographed on the same adsorbent. On development with 10% Me_2CO in petrol.–EtOH (99:1) and 10% Me_2CO in petrol.–EtOH (97:3), three Fractions were obtained: 3A (less polar diols), 3B (more polar diols) and 3C (polyols). Fractions 3A, B and C were rechromatographed by silica gel G TLC using 20%, 30% and 40% Me_2CO in petrol. as solvents. Other solvent systems used were: petrol.–EtOAc–*iso*PrOH (19:2:1) or CH_2Cl_2–EtOAc (4:1). Individual pigments were repurified by multiple development TLC. The fluorescent pigments were separated by repeated column chromatography as described above for Fraction 3. The acetates were separated on silica gel G developed with Me_2CO–petrol. (1:4). The large R_f differences between mono and diesters allowed a good separation.

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