Algal carotenoids. Part 64. Structure and chemistry of 4-keto-19\'-hexanoyloxyfucoxanthin with a novel carotenoid end group

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The structural elucidation of a new carotenoid 4-keto-19\'-hexanoyloxyfucoxanthin 5 from Emiliania huxleyi is documented by chromatographic (HPLC, TLC), spectroscopic (VIS, EIMS, FABMS, FABMSMS, 2D 1H NMR) and chemical evidence. The novel carotenoid end group exhibits particular spectroscopic and chemical properties. In particular the reactions with base and acid are investigated.

Due to a very weak molecular ion upon electron impact and facile cleavage to paracentrone 20 related fragments, the new carotenoid was previously misidentified as 19\'-hexanoyloxyparacentrone 3-acetate 8, also found in other prymnesiophytes (haptophytes).

This novel carotenoid readily undergoes cleavage to a C\textsubscript{31}-skeletal paracentrone 20 related product upon storage, preferably in methanol solution.

The new end group represents a plausible precursor for C\textsubscript{31}-skeletal methyl ketone apocarotenoid metabolites in animals, and differs from the previously suggested precursor.

Introduction

Whereas carotenoids with few oxygen functions readily undergo predictable chemical reactions, xanthophylls with several functional groups such as peridinin 1 and fucoxanthin 2 offer interesting chemistry.\textsuperscript{2–5}

Detailed accounts on the structure elucidation of fucoxanthin 2 and of paracentrone 20 by Weedon’s school were published in this journal in 1969.\textsuperscript{3,6} The present paper deals with a novel, naturally occurring fucoxanthin related carotenoid, crowded with oxygen functions, which has revealed unexpected chemistry including exceptional instability with cleavage in neutral solvents to C\textsubscript{19}-skeletal paracentrone 20 related products.

Early work\textsuperscript{7,8} on the carotenoid composition of the microalga Emiliania huxleyi (Coccolithus huxleyi, Prymnesiophyceae
(Haptophyceae) has recently been reviewed, and also includes subsequent HPLC studies.

By improved HPLC techniques Garrido and Zapata have recently isolated and partly characterised a new fucoxanthin related carotenoid from Emiliania huxleyi. The new carotenoid had polarity similar to that of fucoxanthin and had VIS properties close to those of and the major carotenoid 19′-hexanoyloxyfucoxanthin, and had spectral fine-structure between that of and . A molecular weight of 786 was established by FABMS; loss of 116 mass units, compatible with the loss of hexanoic acid, indicated further a structural relationship to (M = 772).

The structural elucidation of this new carotenoid is reported here.

**Results and discussion**

The new carotenoid represents around 19% of the total carotenoids of Emiliania huxleyi, and extensive chromatographic purification was required for separation from the major carotenoid 3 for isolation of 1 mg of the new carotenoid.

Further spectroscopic evidence (2D 1H NMR, EIMS and FABMS) and chemical derivatisation are compatible with the structure of product 2 and the major carotenoid 19′-hexanoyloxyfucoxanthin and had spectral fine-structure. A molecular weight of 786 was established by FABMS; loss of 116 mass units, compatible with the loss of hexanoic acid, indicated further a structural relationship to (M = 772).

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The driving force of this retroaldol type reaction, following a weak enolisation of 5, may be the formation of the hypothetical, conjugated cyclohexenedione 14.

Upon treatment of the new carotenoid 5 with added base two of the three products, Products 1 16 and 3 (tentatively 19), observed are also based on enolisation of the non-conjugated α-ketol function, Scheme 4.

The reaction with base was monitored by HPLC, thereby revealing the conversion of Product 2 17 to Product 3 19. The latter transformation (Scheme 4) serves to explain why thermodynamically controlled hemiketal products, cf. conversion of 2 via 9 to 10 (Scheme 2) did not occur due to competing enolisation of Product 2 17 and subsequent dehydration to provide a conjugated system.

The evidence for the structures of Products 1 16, 2 17 and 3 19 will now be discussed. The spectroscopic properties of...
Product 1 (19'-hydroxyparacentrone) were compared with those of the synthetic methyl ketone apocarotenoid paracentrone \(20\).\(^{19}\) VIS and MS data for Product 1 were compatible with the structure assigned, supported by the comparative \(^1\)H NMR assignments given in Fig. 2.

Product 2, more polar than Product 1, had VIS absorption bathochromically shifted relative to that of 5, resembling the VIS spectrum of isofucoxanthinol \(9\). Comparative \(^1\)H NMR assignments\(^4\) are given in Fig. 3. Of diagnostic importance is the H-7 proton singlet at \(\delta 6.31\) and three methyl singlets (\(\delta 1.11, 1.41\) and 1.69) belonging to the unprimed end group. Upon electron impact no molecular ion for 17 could be observed, but an \(M+\) ion compatible with the \(C_{40}H_{34}O_7\) structure was observed.

Product 3 (Scheme 4) had VIS absorption slightly more bathochromically shifted than Product 2, and was less polar than Product 2 and Product 1, which is compatible with the tentative structure assigned including the hydrogen bonded enolised \(\alpha\)-diketone in the unprimed end group, cf. ref. 16. No NMR data were obtained.

Diosphenol formation, as for astaxanthin \(11\) to astacene \(12\),\(^{15}\) was not observed upon base treatment of 5. According to previous experience astaxanthin \(11\) with two \(\alpha\)-ketol end groups readily form astacene \(12\),\(^{15}\) whereas carotenoids like flexi-xanthin with one \(\alpha\)-ketol end group are more resistant towards diosphenol formation under alkaline conditions in the presence of traces of \(O_2\).\(^{13}\) Presumably lack of conjugation of the keto function in 5 and 17 reduces the tendency to direct diosphenol formation.

It has previously been proposed\(^{18}\) that paracentrone \(20\) in sea urchins originated from dietary fucoxanthin \(2\) via a hypothetical 3-keto derivative of 2, and an \textit{in vitro} conversion of fucoxanthin \(2\) to paracentrone \(20\) acetate was effected under Oppenauer oxidation conditions.\(^{18}\) The alternative route presented here (Scheme 4) for the retroaldol cleavage of 4-keto-19'-hexanoyloxyfucoxanthin 5 is based on enolization of a 3-hydroxy-4-keto \(\alpha\)-ketol. The new end group represents a plausible precursor to \(C_{31}\)-skeletal methyl ketone apocarotenoid metabolites in animals.

Fucoxanthin 2 is known to produce a dark blue colour upon reaction with strong acids. The reaction has been rationalised by the formation of a blue oxonium ion \(21\), Scheme 5. In parallel experiments with 4-keto-19'-hexanoyloxyfucoxanthin 5 the new carotenoid developed a blue colour only slowly when directly compared to fucoxanthin 2 upon acid treatment. This result is predicted if acid catalysed enolisation of the 4-keto compound 5 to 22 is a competing reaction to oxonium ion formation 23 as shown in Scheme 5. Also the blue oxonium ion 23 formed from 5 was reacted to give a yellow product 24 upon base treatment with nucleophilic attack at C-4, cf. ref. 5.

In conclusion the spectroscopic evidence and chemical behaviour of the new algal carotenoid are consistent with structure 5. The \(R\)-chirality of the allenic end group follows from the chemical shift of the H-8’ proton.\(^{19}\) Furthermore, the relative stereochemistry of the primed end group is compatible with the chemical shifts, whereas the chirality proposed for the unprimed end group is based on biosynthetic analogy, as for fucoxanthin \(2\).\(^{20}\)

The particular instability of the new carotenoid 5, which is readily cleaved upon storage in methanol or acetone solution, is unique in carotenoid context. Obviously fast isolation and
storage in the dry state in the absence of solvents is recommended. Also the requirement of efficient HPLC and TLC systems for the separation of this carotenoid from the 4-deoxo derivative should be emphasized, as well as facile retroaldol type cleavage upon electron impact, providing no or a weak molecular ion by EIMS.

19-Hexanoyloxyparacentrone (as a 3-acetate, 8) was reported as a new carotenoid in 1976. The carotenoid was isolated from Emiliania (Coccolithus) huxleyi clone BT-6. Later, 19-hexanoyloxyparacentrone 3-acetate 8 was identified as a minor carotenoid in Chrysochromulina species. The mass spectra from these analyses are still available. In our present study of E. huxleyi clone CCMP 370, no 19-hexanoyloxyparacentrone 3-acetate 8 could be detected. By comparison of the mass spectra of

Scheme 4
4-keto-19'-hexanoyloxyfucoxanthin 5 obtained in this work with the earlier obtained mass spectra of 19-hexanoyloxy-paracentrone 3-acetate 8,21 it became obvious that the latter represented a misidentification of the new carotenoid 4-keto-19'-hexanoyloxyfucoxanthin 5. The prominent fragment ion at m/z 618 had earlier been erroneously identified as the molecular ion, instead of the proper, very weak molecular ion at m/z 786, observed in the original mass spectrum together with weak fragment ions at m/z 652 (M − 116 − 18) and m/z 634 (M − 116 − 18 − 18).

The question of further occurrence of the new fucoxanthin derivative 5 and other 4-keto analogues will be pursued.

**Experimental**

**Biological material**

For growth conditions of *Emiliania huxleyi* clone CCMP 370 (isolated from Oslofjorden, Norway by E. Paasche in 1959), see ref. 9.

**General methods**

Solvents were of distilled or p.a./HPLC quality. All carotenoid samples were stored in a freezer (−20 °C) under nitrogen. Manipulations were carried out as far as possible in darkness, in the absence of air, acids, alkali and at low temperature.
Instruments
HPLC was carried out on a Hewlett Packard instrument series 1050, detector 1040 A, and a HP 79994 HPLC ChemStation program. 1H NMR spectra were recorded on a Bruker 400 MHz instrument with CDC13 as solvent. El mass spectra were recorded on an AE1 MS 902 spectrometer with a direct inlet to the ion source. FAB mass spectra were obtained with a VG Quattro spectrometer, using 3-nitrobenzyl alcohol as sample matrix. Visible light (VIS) spectra were recorded on a Perkin-Elmer 552 spectrophotometer.

Extraction
The frozen cell concentrate was treated repeatedly with cold (−10 °C) 30% MeOH in Me2CO on a glass sinter filter until the filtrate was colourless.

Chromatography and spectroscopy
Systems for analytical TLC and HPLC and preparative separation by TLC were as specified elsewhere. In addition the HPLC system of Rodriguez et al.24 was employed for the stability studies of 5. Spectral fine-structure of the VIS absorption spectra is defined as 3%III/42 and 5. Wavelengths given in parentheses denote shoulders. Only diagnostically useful peaks with m/z >100 are reported for the mass spectra. 1H NMR coupling constants J are given in Hz.

Reactions
Alkali treatment was performed with 5% KOH in MeOH. After 30 to 60 min, H2O was added and the mixture extracted with EtOAc. Acetylation was carried out in dry pyridine with Ac2O. The mixture was extracted after 2 h with EtOAc upon dilution with H2O.

The colour test for 8-keto-5,6-epoxycarotenoids was performed in parallel experiments for fucoxanthin 2 and 4-keto-19’-hexanoyloxyfucoxanthin 5 (20 µg carotenoid in 5 ml MeOH). The carotenoids were reacted with concentrated HCl (1 drop). Upon shaking fucoxanthin 2 developed immediately a blue colour, cf. ref. 5, whereas the 4-keto derivative 5 after 4 h had attained only a green colour. VIS spectroscopy demonstrated that a blue product (λmax MeOH 690 nm, compared to λmax MeOH 700 nm for the fucoxanthin product) was present beside carotenoid of unchanged chromophore. Upon addition of KOH in MeOH to the reaction mixture from 5 an immediate colour change to yellow was observed. The VIS spectrum indicated the presence of new product(s) with λmax MeOH ≥420 nm in addition to product(s) of the same chromophore as 5.

4-Keto-19’-hexanoyloxyfucoxanthin 5
Available in total 1 mg; VIS λmax acetone/nm: (420), 443, 467, 476, 100, 200, 250, 290, 330, 360, 380, 420, 430, 440, 470 nm; 1H NMR (400 MHz, CDCl3, δ 1H = 7.25, ppm): 1.28 (3H, t, J = 7.0, H-7a), 1.34 (3H, s, H-17), 1.58 (9H, s, Me-18), 2.00 (1H, d, J = 11.6, H-10a), 3.65 (1H, d, J = 3.5, H-5a), 4.44 (1H, d, H-3, d after addition of D2O), 4.74 (1H, distorted d, H-1, J = 11.7, H-19a), 4.80 (1H, distorted d, J = 11.7, H-19b), 5.36 (1H, Me, H-3), 6.05 (1H, s, H-8), 6.30 (2H, “dd”, H-10’ and H-14’), 6.41 (2H, “dd”, H-14 and H-12), 6.56–6.78 (5H, m, H-11, H-12, H-15, H-11’ and H-15’), see Fig. 1. Acetylation gave one product with less polarity, VIS for 5, MS not informative.

Clearage of 5 upon storage in solution
Two aliquots of 5 (each about 0.8 µg) were kept in air in acetone or ii) methanol solution in darkness at room temperature. The reaction mixture was analysed by HPLC (system 25) after 1, 4 and 7 days. In acetone peak ratios between 5 (tR = 21.08) and the product (B, tR = 21.34) were 6.63, 3.36 and 0.07 respectively. In methanol solution the corresponding peak ratios were 1.22, 0.44 and 0.39 respectively. Prolonged storage in acetone solution in darkness at −20 °C resulted in complete conversion of 5 to 8.

The product 10 had VIS λmax 453 nm (round-shaped) relative to 5 with VIS λmax 446 and 470 nm in the same HPLC solvent.

Alkaline treatment of 5
Conditions for the alkaline treatment are specified in reaction.

19'-Hydroxyproparacetone (Product 1, 16). 19'-Hydroxyproparacetone (Product 1, 16). VIS λmax acetone/nm: 440, 463, 476, 511(II) = 13, D7 = 0.90; Rf = 0.26 (TLC silica Merck 5555, 50% acetone in heptane), tR = 7.55 (system,25 flow rate 2.5 ml min−1); EIMS 70 eV, 220, m/z (rel. int. %): 478 [M]+, 460 [M – 18]+ (100), 442 [M – 18 – 18]+ (44), 426 [M – 18 – 18 – 18]+ (12), 424 [M – 18 – 18 – 18 – 18]+ (11); 1H NMR (400 MHz, CDCl3, including 1H-H COSY), see Fig. 2: δ 1.09 (3H, s, H-17), 1.33 (3H, s, H-16), 1.39 (3H, s, H-18), 1.93 (3H, s, H-19), 1.98 (≈1.5H, s, H-20), 1.99 (3H, d, J = 11.0, H-20b), 2.00 (=1.5H, s, H-20), 2.36 (3H, s, H-7), 6.01 (=1H, s, H-8), 6.21 (1H, d, J = 11.6, H-10), 6.29 (1H, d, J = 11.3, H-14), 6.40 (2H, m, H-12 and H-14’), 6.50 (=0.25H, s, H-8), 6.60 (1H, d, H-11’, 6.65 (3H, m, H-15, H-12’ and H-15’), 6.72 (1H, m, H-11), 7.15 (1H, d, J = 11.4, s, H-10).
H-15 and H-15'), 6.72 (1H, m, H-11'), 7.13 (1H, "dd", J = 10.3, 1=1, H-10).

Product 3. VIS λ\text{max} HPLC eluent/nm: ≈ 475 (round, broad); t_R = 6.5 (system, ^2 flow 1.25 ml min ^-1).

In a separate experiment 5 was treated with 5% KOH in methanol for 30 min. The reaction mixture was examined by HPLC in the system of Rodriguez et al. ^23 Four peaks with t_R = 5.20 (VIS λ\text{max} in eluent 452 nm, rounded, 7% of total), t_R = 6.28 (VIS λ\text{max} 447, 465 nm, 65% of total), t_R = 6.72 (VIS λ\text{max} 460 nm, rounded, 23% of total) and t_R = 8.05 (VIS λ\text{max} 470 nm, 5% of total) were observed, tentatively identified as Product 2 17, 22 (hydrolysed 5), Product 1 16 and Product 3 19 respectively.

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References