View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Digital.CSIC

Algal carotenoids. Part 64.[1] Structure and chemistry of 4-keto-19′-hexanoyloxyfucoxanthin with a novel carotenoid end group

Einar Skarstad Egeland,[a] José Luis Garrido,[b] Manuel Zapata,[c] Miguel Angel Maestro[d] and Synnøve Liaaen-Jensen *[a]

[a] *Organic Chemistry Laboratories, Department of Chemistry, Norwegian University of Science and Technology, Gløshaugen, NO-7491 Trondheim, Norway*
[b] *Instituto de Investigacións Mariñas, C.S.I.C., Av. Eduardo Cabello 6, ES-36208 Vigo, Spain*
[c] *Centro de Investigacións Mariñas, Consellería de Pesca-Xunta de Galicia, Apdo. 13, ES-36620 Vilanova de Arousa, Spain*
[d] *Servicios Xerais de Apoio á Investigación, Universidade da Coruña, Campus da Zapateira, ES-15071 A Coruña, Spain*

Received (in Lund, Sweden) 20th December 1999, Accepted 22nd February 2000
Published on the Web 29th March 2000

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 ^{1}H 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_{31}-skeletal paracentrone **20** related product upon storage, preferably in methanol solution.

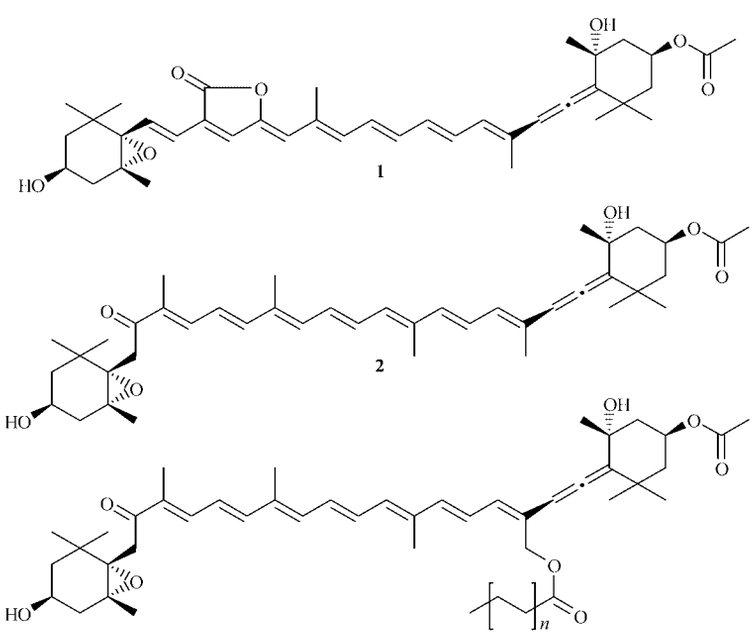
The new end group represents a plausible precursor for C_{31}-skeletal methyl ketone apocarotenoid metabolites in animals, and differs from the previously suggested precursor.

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.[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.[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_{31}-skeletal paracentrone **20** related products.

Early work[7,8] on the carotenoid composition of the microalga *Emiliania huxleyi* [*Coccolithus huxleyi*, Prymnesiophyceae



This journal is © The Royal Society of Chemistry 2000

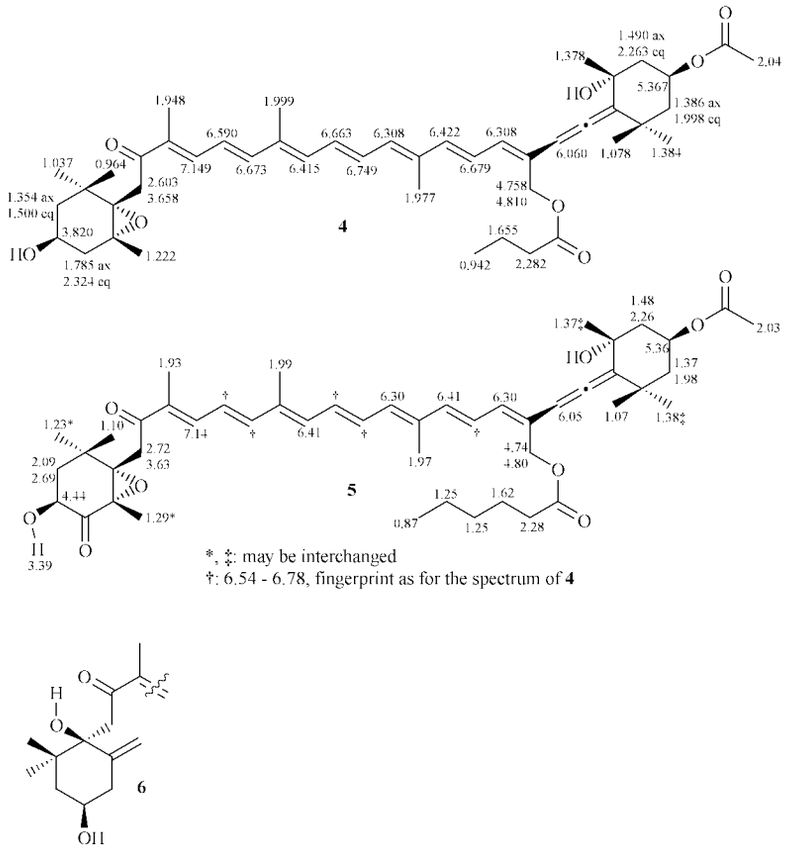


Fig. 1

(Haptophyceae)] has recently been reviewed,[9] and also includes subsequent HPLC studies.[10]

By improved HPLC techniques Garrido and Zapata[9] have recently isolated and partly characterised a new fucoxanthin related carotenoid from *Emiliania huxleyi*. The new carotenoid had polarity similar to that of fucoxanthin **2**,[9] and had VIS properties close to those of **2** and the major carotenoid 19′-hexanoyloxyfucoxanthin **3**,[8] and had spectral fine-structure between that of **2** and **3**. A molecular weight of 786 was established by FABMS;[9] loss of 116 mass units, compatible with the loss of hexanoic acid,[8] indicated further a structural relationship to **3** ($M = 772$).[9]

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 (2 D ^{1}H NMR, EIMS and FABMS) and chemical derivatisation are compatible with structure **5** (4-keto-19′-hexanoyloxyfucoxanthin) for the new carotenoid, thus possessing a novel carotenoid end group, imposing particular ^{1}H NMR and chemical properties.

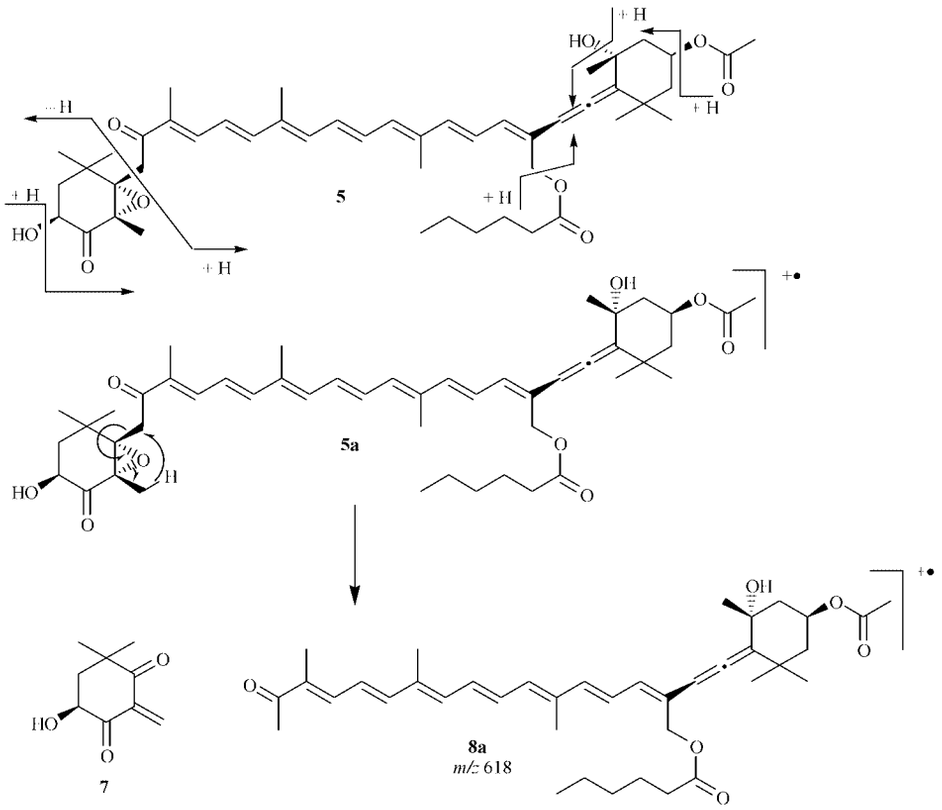
2D ^{1}H NMR (400 MHz, $CDCl_3$)-Assignments are shown in Fig. 1. Available for direct comparison was the fully assigned high field ^{1}H NMR spectrum of 19′-butanoyloxyfucoxanthin **4**.[11] Identification of the structural moiety from the C-8 keto group onwards of the carotenoid skeletons of **5** and **4** was supported by comparison of chemical shifts and coupling patterns, as well as a fingerprint similarity of the crowded 6.3–6.6 ppm olefinic region. Attachment of an acetate function at C-3′ and a fatty acid ester at C-19′ was evident. Assignment of the previously unknown end group was supported by two doublets at δ 2.72 and 3.63 for CH_2-7 and the coupling system for CH_2-2 and CH-3. The interconnectivities were established by the ^{1}H–^{1}H COSY spectrum. Three methyl singlets were compatible with the predicted chemical shifts for CH_3-16, 17, 18. Deuterium exchange of the 3-hydroxy proton was noted. An alternative prasinoxanthin[12] related end group **6** could be ruled out from the lack of a hydrogen bonded hydroxy group at C-6 and of terminal methylene.

Observed mass spectrometric fragments by EIMS including exact mass measurements were compatible with the cleavages indicated in Scheme 1. Arrow heads are pointing towards the charged fragment. The molecular ion was very weak. A prominent peak at *m*/*z* 618 is compatible with α-cleavage of C-6,7 with hydrogen transfer to the polyene moiety. FABMS confirmed the molecular ion ($M = 786$) and FABMSMS showed fragments compatible with combined losses of water, acetic acid and hexanoic acid.

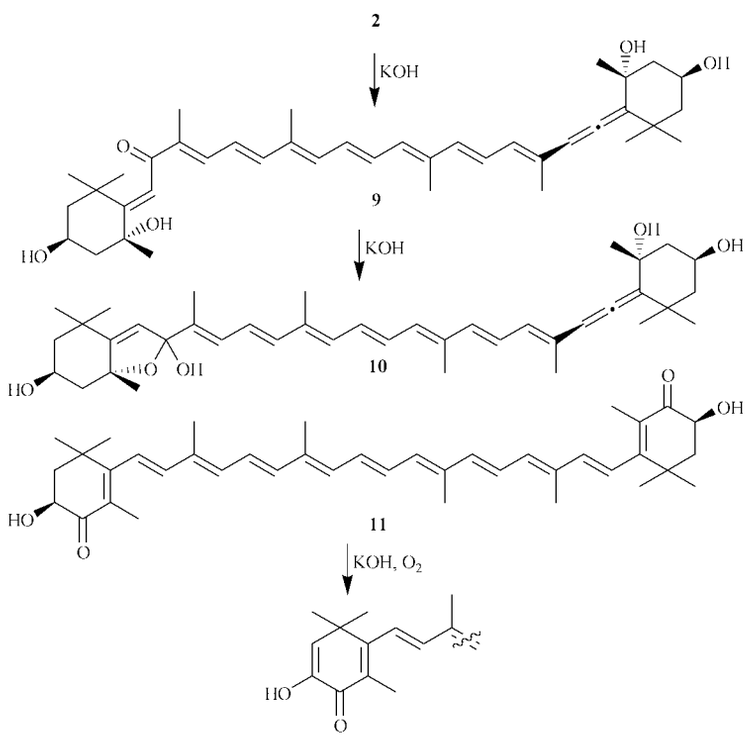
Additional support for structure **5** was sought by chemical derivatisation. Acetylation provided a less polar product of unchanged chromophore, compatible with the introduction of a second acetate function at C-3.

A priori treatment of **5** with base was expected to result in three sets of reactions: i) hydrolysis of the two ester functions, ii) fucoxanthin **2** related chemistry with the formation of kinetically controlled products of isofucoxanthinol **9** type, as well as thermodynamically controlled yellow hemiketal products **10**,[4] Scheme 2, and iii) astaxanthin **11** related chemistry with diosphenol (enolised α-diketone) formation **12**,[13] Scheme 2.

However, the new carotenoid behaved somewhat unexpectedly upon treatment with base. Even in the absence of added base, upon prolonged storage in acetone solution at −20 °C, or shorter storage in darkness at room temperature in acetone or preferably methanol, rearrangement to a new, less polar product **8** was observed. On the basis of structure **5** and the reported partial racemisation of astaxanthin **11** under basic conditions,[14] which must occur *via* enolate formation, the formation and structure of product **8** is rationalised in Scheme 3.



Scheme 1



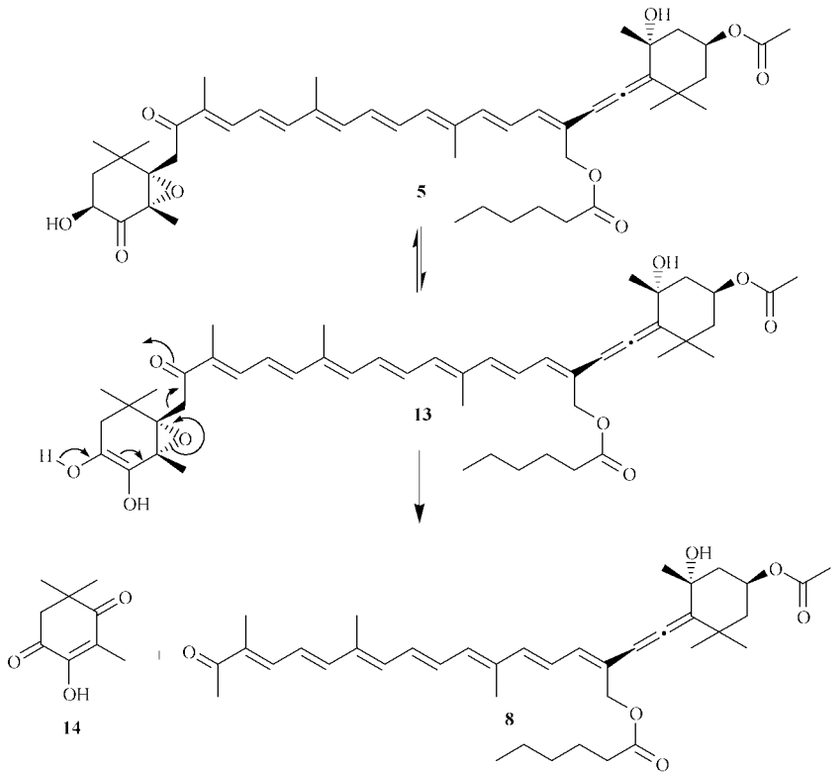
Scheme 2

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



Scheme 3

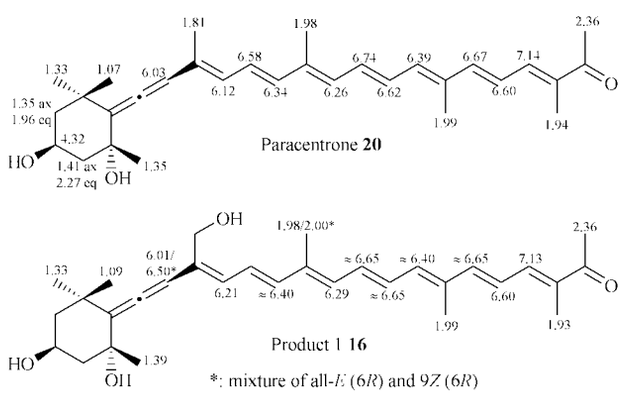


Fig. 2

Product 1 **16** (19′-hydroxyparacentrone) were compared with those of the synthetic methyl ketone apocarotenoid paracentrone **20**.[15] 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 **17**, more polar than Product 1 **16**, 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 δ 6.31 and three methyl singlets (δ 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 $M - 18$ ion compatible with the $C_{40}H_{34}O_7$ structure was observed.

Product 3 **19** (Scheme 4) had VIS absorption slightly more bathochromically shifted than Product 2 **17**, and was less polar than Product 2 **17** and Product 1 **16**, which is compatible with the tentative structure assigned including the hydrogen bonded enolised α-diketone in the unprimed end group, *cf.* ref. 16. No NMR data were obtained.

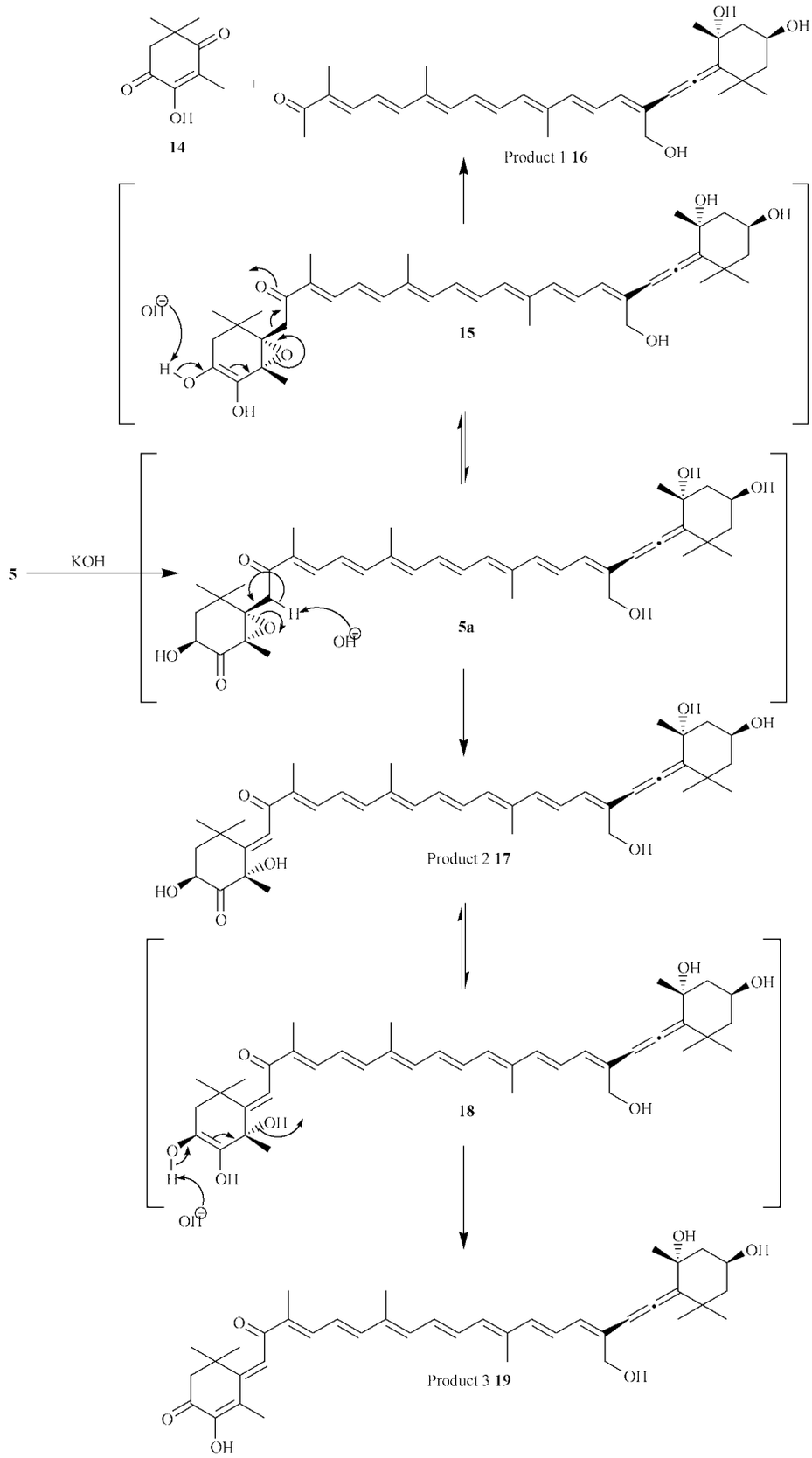
Diosphenol formation, as for astaxanthin **11** to astacene **12**, was not observed upon base treatment of **5**. According to previous experience astaxanthin **11** with two α-ketol end groups readily form astacene **12**,[13] whereas carotenoids like flexixanthin with one α-ketol end group are more resistant towards diosphenol formation under alkaline conditions in the presence of traces of O_2.[17] 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 *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 α-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**,[5] 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]

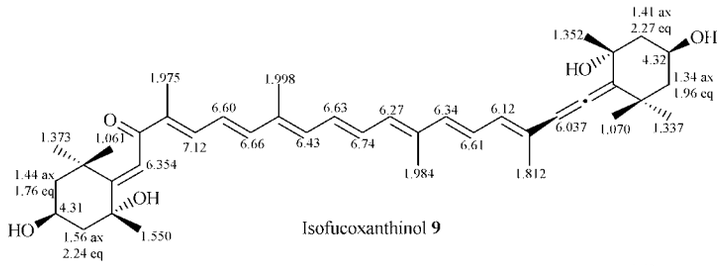
The particular instability of the new carotenoid **5**, which is readily cleaved upon storage in methanol or acetone solution, is unique in carotenoid context. Obviously fast isolation and



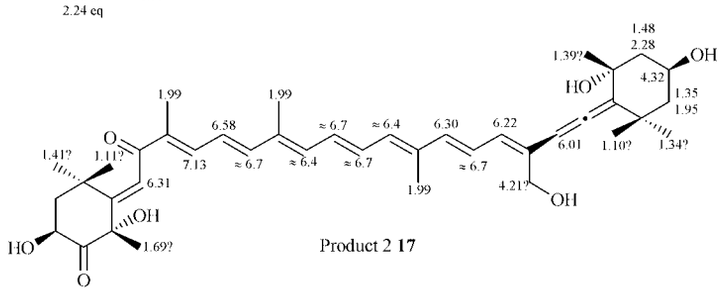
Scheme 4

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.[8] 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.[21] 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

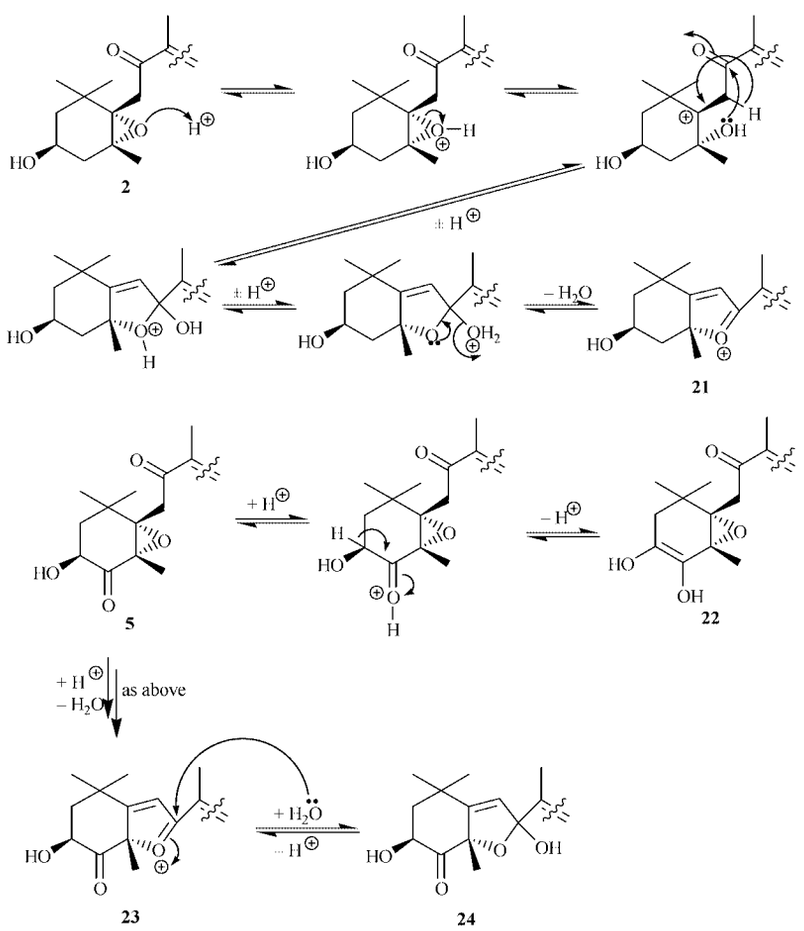


Isofucoxanthinol **9**



Product 2 **17**

Fig. 3



Scheme 5

4-keto-19′-hexanoyloxyfucoxanthin **5** obtained in this work with the earlier obtained mass spectra of 19-hexanoyloxyparacentrone 3-acetate **8**,[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. ^{1}H NMR spectra were recorded on a Bruker 400 MHz instrument with $CDCl_3$ as solvent. EI mass spectra were recorded on an AEI MS 902 spectrometer with a direct inlet to the ion source. FAB mass spectra were obtained with a VG Quattro spectrometer, using 3-nitrobenzoyl 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 Me_2CO 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.[22] In addition the HPLC system of Rodriguez *et al.*[23] was employed for the stability studies of **5**. Spectral fine-structure in the VIS absorption spectra is defined as %III/II[24] and D_v.[25] Wavelengths given in parentheses denote shoulders. Only diagnostically useful peaks with *m*/*z* >100 are reported for the mass spectra. ^{1}H NMR coupling constants *J* are given in Hz.

Reactions

Alkali treatment was performed with 5% KOH in MeOH. After 30 to 60 min, H_2O was added and the mixture extracted with EtOAc. Acetylation was carried out in dry pyridine with Ac_2O. The mixture was extracted after 2 h with EtOAc upon dilution with H_2O.

The colour test for 8-keto-5,6-epoxidic carotenoids was performed in parallel experiments for fucoxanthin **2** and 4-keto-19′-hexanoyloxyfucoxanthin **5** (20 μg carotenoid in 3 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 (λ_{max}MeOH 690 nm, compared to λ_{max}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 λ_{max}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 λ_{max} acetone/nm: (420), 443, 467, %III/II = 38, D_v = 0.88, VIS λ_{max} benzene/nm: (430), 455, 481, %III/II = 9, D_v = 0.86, VIS λ_{max} MeOH/nm: 443, 465, %III/II = 28, D_v = 0.95, addition of dilute HCl caused no change; R_f = 0.55 (TLC silica–calcium carbonate 2:1, 40% Me_2CO in heptane), R_f = 0.56 (TLC silica Merck 5553, Me_2CO–EtOH–$CHCl_3$ 12:1:87), t_R = 17.2 (system,[23] flow 1.25 ml min^{-1}); FABMS,[9] 787.47 $[M + H]^+$, 786.46 $[M]^+$, 769.47 $[M + H - H_2O]^+$; EIMS 70 eV, 220°, *m*/*z* (rel. int.%): 786 $[M]^+$ (0.2), 768 $[M - 18]^+$ (0.3), 750 $[M - 18 - 18]^+$ (0.2), 618 $[M - 168]^+$ (17), 600 $[M - 168 - 18]^+$ (7), 540 $[M - 168 - 60 - 18]^+$ (3), 504 $[M - 282]^+$ (5), 502 $[M - 284]^+$ (4), 486 $[M - 282 - 18]^+$ (4), 484 $[M - 284 - 18]^+$ (3), 424 $[M - 168 - 116 - 60 - 18]^+$ (6), 326 (8), 311 (8), 195 (26), 168 (29), 140 (71), 125 (100), fragment ions observed also at 652 and 634 in another spectrum, corresponding to $[M - 116 - 18]^+$ and $[M - 116 - 18 - 18]^+$.[8] The FABMS showed a prominent peak at *m*/*z* 786 (M^+) and peak at 810 $(M + Na)^+$. FABMSMS performed by collision induced (Ar) dissociation of the molecular ion showed major fragments at 768.4 ($M - 18$), 670.5 ($M - 116$), 652.9 ($M - 18 - 116$) and 610.4 ($M - 60 - 116$) and minor fragments at 706.9, 627.4, 593.4 and 577.4; ^{1}H NMR (400 MHz, $CDCl_3$, including ^{1}H–^{1}H COSY), double primed numbers refer to the fatty acid side chain: δ 0.87 (3H, t, J = 7, H-6″), 1.07 (3H, s, H-17′), 1.10 (3H, s, H-17), 1.23 (3H, s, H-16 or H-18), 1.25 (4H, broad s, H-4‴, H-5″), 1.29 (3H, s, H-16 or H-18), 1.37 (3H, s, H-16′ or H-18′), 1.37 (1H, m, H-2′ax), 1.38 (3H, s, H-16′ or H-18′), 1.48 (1H, m, H-4′ax), 1.62 (2H, quintet, J = 7.5, H-3″), 1.93 (3H, s, H-19), 1.97 (3H, s, H-20′), 1.98 (1H, m, H-2′eq), 1.99 (3H, s, H-20), 2.03 (3H, s, H-acetate), 2.09 (1H, dd, J = 12.2, 14.0, H-2ax), 2.26 (1H, m, H-4′eq), 2.28 (2H, t, J = 7.5, H-2″), 2.69 (1H, dd, J = 7.4, 14.1, H-2eq), 2.72 (1H, d, J = 18.1, H-7a), 3.39 (1H, d, J = 3.2, 3-OH, disappeared after addition of D_2O), 3.63 (1H, d, J = 18.3, H-7b), 4.44 (1H, m, H-3, dd after addition of D_2O), 4.74 (1H, distorted d, J = 11.7, H-19′a), 4.80 (1H, distorted d, J = 11.7, H-19′b), 5.36 (1H, m, 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′), 6.54–6.78 (5H, m, H-11, H-12, H-15, H-11′ and H-15′), see Fig. 1.

Acetylation gave one product with less polarity, VIS as for **5**, MS not informative.

Cleavage of 5 upon storage in solution

Two aliquots of **5** (each about 0.8 μg) were kept in i) acetone or ii) methanol solution in darkness at room temperature. The reaction mixture was analyzed by HPLC (system[23]) after 1, 4 and 7 days. In acetone peak ratios between **5** (t_R = 21.08) and the product (**8**, t_R = 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 λ_{max} 453 nm (round-shaped) relative to **5** with VIS λ_{max} 446 and 470 nm in the same HPLC solvent.

Alkali treatment of 5

Conditions for the alkali treatment are specified under Reactions. Products were isolated by TLC (silica Merck 5553 or 5554, 50% acetone in heptane).

19′-Hydroxyparacentrone (Product 1, 16). VIS λ_{max} acetone/nm: 440, 463, %III/II = 10, D_v = 0.90; R_f = 0.26 (TLC silica Merck 5554, 50% acetone in heptane), t_R = 7.5 (system,[23] flow 1.25 ml min^{-1}); EIMS 70 eV, 220°, *m*/*z* (rel. int.%): 478 $[M]^+$ (11), 460 $[M - 18]^+$ (100), 442 $[M - 18 - 18]^+$ (44), 426 $[M - 18 - 18 - 16]^+$ (12), 424 $[M - 18 - 18 - 18]^+$ (11); ^{1}H NMR (400 MHz, $CDCl_3$, including ^{1}H–^{1}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 ≈ 1, H-20′), 2.00 (≈1.5 H, 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, m, H-11′), ≈6.65 (3H, m, H-15, H-12′ and H-15′), 6.72 (1H, m, H-11), 7.13 (1H, d, J = 11.4, ≈1, H-10′).

4-Keto-19′-hydroxyisofucoxanthinol (Product 2, 17). VIS λ_{max} acetone/nm: 448, 463, %III/II = 13, D_v = 0.99; R_f = 0.63 (TLC silica Merck 5553, 16% EtOH in $CHCl_3$), t_R = 6.7 (system,[23] flow 1.25 ml min^{-1}); EIMS 70 eV, 220°, *m*/*z* (rel. int.%): 626 $[M - 18]^+$ (61), 608 $[M - 18 - 18]^+$ (34), 602 (42), 582 (61), 551 (100); ^{1}H NMR (400 MHz, $CDCl_3$, including ^{1}H–^{1}H COSY), see Fig. 3: δ 1.10 (3H, s, H-17′?), 1.11 (3H, s, H-17?), 1.34 (3H, s, H-16′?), ≈1.35 (1H, m, H-2′), 1.39 (3H, s, H-18′?), 1.41 (3H, s, H-16′?), ≈1.48 (1H, m, H-4′), 1.69 (3H, s, H-18?), ≈1.95 (1H, m, H-2′), ≈2.28 (1H, m, H-4′), 4.32 (1H, m, H-3′), 6.01 (≤1H, s, H-8′), 6.22 (1H, d, J = 11.6, H-10′), 6.30 (1H, d, J = 11.1, H-14′), ≈6.40 (2H, m, H-12′ and H-14), 6.51 (≈0.3H, s, H-8′?), 6.58 (1H, dd, J = 11.1, 15.0, H-11), ≈6.65 (3H, m, H-12,

H-15 and H-15′), 6.72 (1H, m, H-11′), 7.13 (1H, "dd", J = 10.3, ≈1, H-10).

Product 3. VIS λ_{max} HPLC eluent/nm: ≈ 475 (round, broad); t_R = 8.5 (system,[23] 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_{max} in eluent 452 nm, rounded, 7% of total), t_R = 6.28 (VIS λ_{max} 447, 465 nm, 65% of total), t_R = 6.72 (VIS λ_{max} 460 nm, rounded, 23% of total) and t_R = 8.05 (VIS λ_{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.

Acknowledgements

E. S. E. was supported by a research grant from Hoffmann-La Roche, Basel, to S. L.-J.

References

1 Part 63, E. S. Egeland, R. R. L. Guillard and S. Liaaen-Jensen, *Phytochemistry*, 1997, **44**, 1087.
2 H. Kjøsen, S. Norgård, S. Liaaen-Jensen, W. A. Svec, H. H. Strain, P. Wegfahrt, H. Rapoport and F. T. Haxo, *Acta Chem. Scand.*, *Ser. B*, 1976, **30**, 157.
3 R. Bonnett, A. K. Mallams, A. A. Spark, J. L. Tee and B. C. L. Weedon, *J. Chem. Soc. C*, 1969, 429.
4 J. A. Haugan, G. Englert and S. Liaaen-Jensen, *Acta Chem. Scand.*, 1992, **46**, 614.
5 J. A. Haugan and S. Liaaen-Jensen, *Acta Chem. Scand.*, 1994, **48**, 68.
6 G. Galasko, J. Hora, T. P. Toube, B. C. L. Weedon, D. André, M. Barbier, E. Lederer and V. R. Villanueva, *J. Chem. Soc. C*, 1969, 1264.
7 (*a*) S. W. Jeffrey and M. B. Allen, *J. Gen. Microbiol.*, 1964, **36**, 227; (*b*) S. Norgård, W. A. Svec, S. Liaaen-Jensen, A. Jensen and R. R. L. Guillard, *Biochem. Syst. Ecol.*, 1974, **2**, 7; (*c*) S. Hertzberg, T. Mortensen, G. Borch, H. W. Siegelman and S. Liaaen-Jensen, *Phytochemistry*, 1977, **16**, 587.
8 N. Arpin, W. A. Svec and S. Liaaen-Jensen, *Phytochemistry*, 1976, **15**, 529.
9 J. L. Garrido and M. Zapata, *J. Phycol.*, 1998, **34**, 70.
10 (*a*) S. W. Wright and S. W. Jeffrey, *Mar. Ecol. Prog. Ser.*, 1987, **38**, 1987; (*b*) J. R. Nelson and S. G. Wakeham, *J. Phycol.*, 1989, **25**, 761; (*c*) S. W. Wright, S. Jeffrey, R. F. C. Mantoura, C. A. Llewellyn, T. Bjørnland, D. Repeta and N. Welschmeyer, *Mar. Ecol. Prog. Ser.*, 1991, **77**, 1991; (*d*) G. W. Kraay, M. Zapata and M. J. W. Veldhuis, *J. Phycol.*, 1992, **28**, 708; (*e*) S. W. Jeffrey and S. W. Wright, in *The Haptophyte Algae*, eds. J. C. Green and B. S. C. Leadbeater, Clarendon, Oxford, 1994, p. 111.
11 G. Englert, T. Bjørnland and S. Liaaen-Jensen, *Magn. Reson. Chem.*, 1990, **28**, 519.
12 (*a*) P. Foss, R. R. L. Guillard and S. Liaaen-Jensen, *Phytochemistry*, 1984, **23**, 1629; (*b*) P. Foss, T. Skjetne and S. Liaaen-Jensen, *Acta Chem. Scand.*, *Ser. B*, 1986, **40**, 172.
13 R. Kuhn, J. Stene and N. A. Sörensen, *Chem. Ber.*, 1939, **72**, 1688.
14 R. K. Müller, K. Bernhard, H. Meyer, A. Rüttimann and M. Vecchi, *Helv. Chim. Acta*, 1980, **63**, 1654.
15 J. A. Haugan, *J. Chem. Soc.*, *Perkin Trans. 1*, 1997, 2731.
16 (*a*) K. Schiedt, in *Carotenoids, Chemistry and Biology*, ed. N. I. Krinsky, M. M. Mathews-Roth and R. F. Taylor, Plenum, London, 1990, p. 247; (*b*) G. Englert, in *Carotenoids*, Vol. 1B, eds. G. Britton, S. Liaaen-Jensen and H. Pfander, Birkhäuser, Basel, 1995, p. 147.
17 A. J. Aasen and S. L. Jensen, *Acta Chem. Scand.*, 1966, **20**, 1970.
18 J. Hora, T. P. Toube and B. C. L. Weedon, *J. Chem. Soc. C*, 1970, 241.
19 J. A. Haugan and S. Liaaen-Jensen, *Tetrahedron Lett.*, 1994, **35**, 2245.
20 Y. Yamano, C. Tode and M. Ito, *J. Chem. Soc.*, *Perkin Trans. 1*, 1995, 1895.
21 B. Bjerkeng, J. A. Haugan and S. Liaaen-Jensen, *Biol. Oceanogr.*, 1991, **6**, 271.
22 E. S. Egeland, G. Johnsen, W. Eikrem, J. Throndsen and S. Liaaen-Jensen, *J. Phycol.*, 1995, **31**, 554.
23 F. Rodriguez, M. Zapata and J. L. Garrido, *Chromatographia*, 1998, **48**, 677.
24 B. Ke, F. Imsgard, H. Kjøsen and S. Liaaen-Jensen, *Biochim. Biophys. Acta*, 1970, **210**, 139.
25 E. S. Egeland and S. Liaaen-Jensen, *Phytochemistry*, 1995, **40**, 515.