Algal Carotenoids 50.† Alkali Lability of Fucoxanthin – Reactions and Products

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The reaction of fucoxanthin with bases has been examined by modern methods. The reactions studied were (i) fucoxanthin or protected fucoxanthin with NaH as strong base/weak nucleophile in THF followed by CH_3I, (ii) protected fucoxanthin with NaH followed by H_2O, and (iii) fucoxanthin with KOH in methanol in different molar ratios. Kinetic studies are reported.

All coloured products were identified. Novel cyclic carotenoid hemiketal and methyl ketal products were characterized by means of VIS, MS, IR, ^1H NMR and ^{13}C NMR data, including COSY and hetero-COSY. Isofucoxanthin and isofucoxanthinol were identified by means of VIS, CD, MS, IR and detailed ^1H NMR and ^{13}C NMR data, including COSY, hetero-COSY, ROESY and TOCSY, demonstrating the configuration of the cross-conjugated chromophore.

The reactions observed have been rationalized mechanistically. Isofucoxanthinol is the kinetically controlled product upon reaction of fucoxanthin with KOH, and fucoxanthinol hemiketal the thermodynamically controlled product. A high molar ratio of KOH:fucoxanthin increases the rate of production of fucoxanthinol hemiketal.

Dedicated to Professor Lars Skattebøl on the occasion of his 65th birthday.

Fucoxanthin (**1**, scheme 1), the major carotenoid of brown algae,[1] has recently been isolated by an improved procedure[2,3] for further stereochemical[4] and chemical studies.

The lability of **1** towards alkali is a well-known phenomenon that prevents the inclusion of saponification in the isolation procedure of carotenoid mixtures containing **1**.[5] The chromophoric changes that occur upon treatment of **1** with weak base (K_2CO_3) were reported long ago.[6] However, no products have been identified.

In this paper further studies on the reaction of **1** with base are described, including characterization and identification of new cyclic carotenoid hemiketals and ketals. Preliminary results have been reported.[7] The present study represents a further contribution to the chemistry of **1**,[8,9] one of the two major carotenoids in Nature.

Results and discussion

Since allenic carotenoids are stable towards alkali, the alkali sensitivity of **1** is associated with the keto-epoxy moiety. However, it was unknown whether the base reacts initially as a base or as a nucleophile. The following reactions of **1** or protected **1** were studied: (a) with dimethyl sulfide (strong nucleophile/weak base), (b) with NaH (strong base/weak nucleophile) and CH_3I in dry THF for S_N2-type *C*- or *O*-methylation, (c) (as the 3-acetate-5′-TMS ether) with NaH in dry THF followed by (i) CH_3I or (ii) H_2O as an electrophile and (d) with KOH in methanol in different molar ratios, including kinetic studies.

(a) Reaction with dimethyl sulfide. Fucoxanthin provided no new products with dimethyl sulfide as a strong nucleophile/weak base during 23 h.

(b) Reactions initiated by sodium hydride. In order to test the relative acidity of the hydroxy functions and α-hydrogens at C-7 in **1** the available (3*R*,3′*R*)-3,3′-dihydroxy-7,7′,8,8′-tetrahydro-β,β-carotene-8,8′-dione (**3**, Scheme 1) was used as a model. Methylation with NaH/CH_3I in THF provided the 3-methyl ether (**3a**), the 3,3′-dimethyl ether (**3b**) and the 7-methyl-3,3′-dimethyl ether (**3c**) in the ratio 7:40:15, respectively, after 75 h, demonstrating the ease with which methylation of the hydroxy groups takes place, compared with *C*-methylation.

Fucoxanthin under similar conditions provided the methyl ketal 3-methyl ether (**2a**) as an intermediate product and the methyl ketal 3,5′-dimethyl ether (**2b**) as a final product. The products **2a** and **2b** were characterized by VIS and mass spectroscopy. No chromophoric change,

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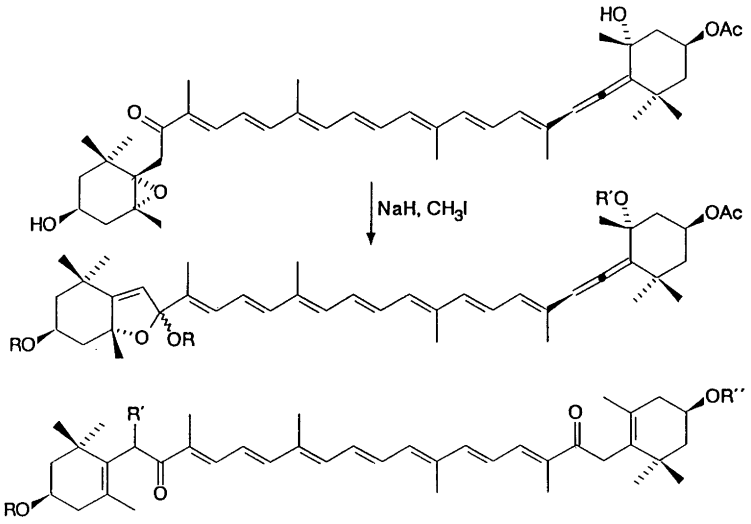


1

2a R = CH_3, R' = H
2b R = R' = CH_3

3 R = R' = R'' = H
3a R = R' = H, R'' = CH_3
3b R = R'' = CH_3, R' = H
3c R = R' = R'' = CH_3

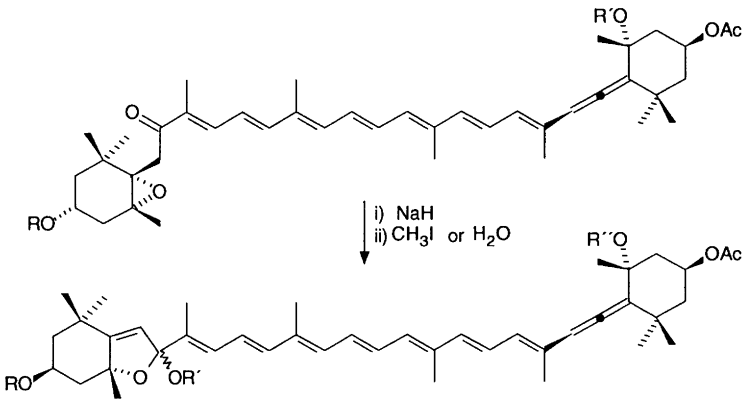
Scheme 1.

as judged by VIS spectroscopy, was observed when **1**, dissolved in dry THF, was treated with NaH, which further indicates that the 3-hydroxy group is more acidic than the C-7 α-hydrogens.

(c) Reactions of protected fucoxanthin initiated by sodium hydride. Fucoxanthin was acetylated and silylated to provide the 3,3'-diacetate-5'-TMS ether (**1b**, Scheme 2). Treatment with NaH in THF followed by CH_3I provided the methyl ketal **2c**, characterized by VIS and mass spectrometry. In an alternative experiment CH_3I was replaced by water as the electrophile, resulting in the rapid formation of the protected hemiketal **2d**, characterized by VIS and mass spectrometry.

(d) Treatment of fucoxanthin with potassium hydroxide in methanol. On a 10 mg scale, fucoxanthin was treated with different concentrations of KOH in methanol, Table 1. Treatment with 0.01 % KOH (molar ratio KOH : **1** = 2.2) or 0.1 % KOH (molar ratio 24) furnished six identified products (**1**, **1c**, **2**, **2e**, **4** and **4a**, Scheme 3). The complex product mixture was obtained under these conditions due to partial hydrolysis of the acetate function. The isofucoxanthin-type products (**4** and **4a**) predominated over the hemiketal products (**2** and **2e**). Similar treatment with 1 % KOH (molar ratio 1750) resulted in complete hydrolysis of the acetate and provided the hemiketal **2e** and isofucoxanthinol (**4a**). With 5 % KOH (molar ratio 2187) only **2c** and **4a** were present after 20 min. Prolonged reaction time gave predominantly **2e**, suggesting that **4a** was an intermediate in the formation of **2e**.

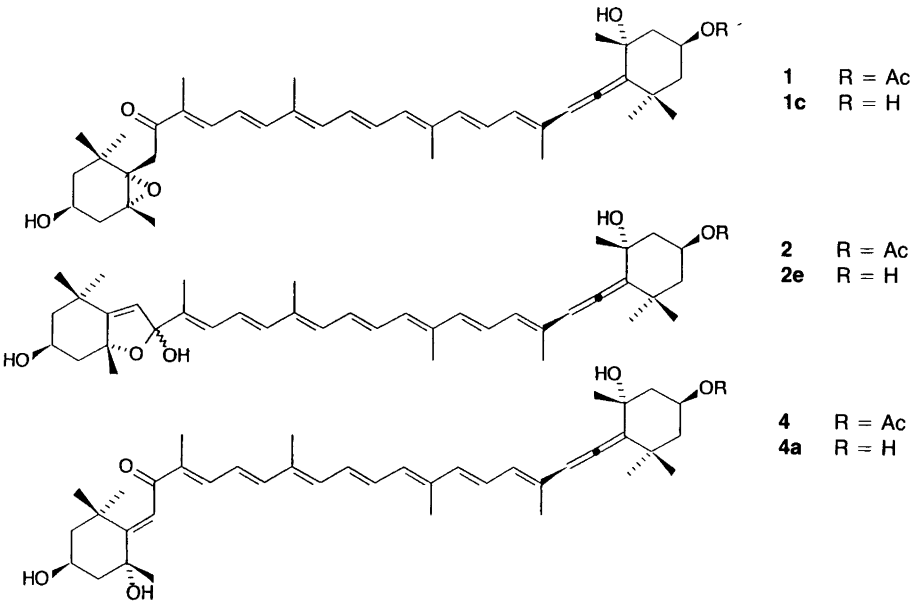
In a kinetic experiment (a) with **1** (5 % KOH, molar ratio 5520), see Fig. 1, the presence of **4a** and **2e** was demonstrated after 1 min. After a recorded interval, pigment loss of 20 % of the total carotenoid remained constant for 20 h. During this period **4a** was transformed into **2e**. The initial pigment loss was ascribed to the preparative TLC procedure. Support for this conclusion was obtained from kinetic experiments (b) and (c) investigating the early phase of this reaction, see the Experimental.



1 R = R' = H
1a R = Ac, R' = H
1b R = Ac, R' = TMS

2c R = Ac, R' = CH_3, R'' = TMS
2d R = Ac, R' = H, R'' = TMS

Scheme 2.



Scheme 3.

The conversion of pure **4a** into **2e** was confirmed in a separate experiment, see Fig. 2. Fucoxanthinol hemiketal was finally shown to be stable towards further treatment with alkali.

The effect of stepwise increases of the molar ratio KOH : **1** in five parallel experiments is shown in Table 2. It was observed that the molar ratio of products **2e** : **4a** increased thirtyfold with increasing KOH : **1** molar ratio. Thus the reaction rate from **1** to the terminal hemiketal **2e** is clearly dependent on the base:substrate ratio, consistent with the results given in Table 1.

The structures of the products resulting from the KOH treatment above were based on the following evidence. Fucoxanthinol was characterized from the R_f-values in three systems and co-chromatography tests with authentic natural **1c**,[10] by VIS, MS and ^{1}H NMR spectroscopy.

Table 1. Results of treatment of fucoxanthin (**1**) with potassium hydroxide in methanol.

Product	Yield after TLC (%)				
	a	*b*	*c*	*d-1*	*d-2*
Fucoxanthin (**1**)	33	20	–	–	–
Fucoxanthinol (**1c**)	8	6	–	–	–
Fucoxanthin hemiketal (**2**)	11	10	–	–	–
Fucoxanthinol hemiketal (**2e**)	3	3	20	18	72
Isofucoxanthin (**4**)	17	4	–	–	–
Isofucoxanthinol (**4a**)	14	7	26	28	1

[a]0.01 % KOH; molar ratio KOH : **1** = 2.2; 40 min. [b]0.1 % KOH; molar ratio KOH : **1** = 24; 35 min. [c]1 % KOH; molar ratio KOH : **1** = 1750; 35 min. [d-1]5 % KOH; molar ratio KOH : **1** = 2187; 20 min. [d-2]5 % KOH; molar ratio KOH : **1** = 2187; 46 h.

The structure of fucoxanthin hemiketal (**2**) followed from VIS absorption data, which were compatible with an aliphatic octaene chromophore, from the mass spectrum which included strong homopyrylium (*m/z* 221) and pyrylium (*m/z* 181) ions, and from IR and ^{1}H NMR data. Fucoxanthinol hemiketal (**2e**) was characterized by means of the same criteria, and also by ^{1}H NMR spectroscopy in two solvents and ^{13}C NMR. The hemiketals **2** and **2e** are extremely sensitive to acid, providing blue oxonium products.[11,12] Chromatographic separation of the C-8 epimeric hemiketals (**2**, **2e**) was not achieved. A ca. 6:4 mixture was evident from the ^{1}H NMR data.

The isofucoxanthins (**4** and **4a**)[8,9] were characterized by means of VIS spectroscopy, IR, MS, CD and from the

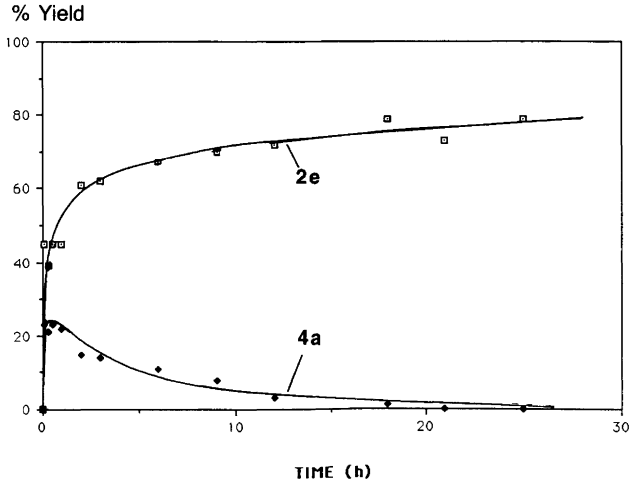


Fig. 1. Reaction of fucoxanthin (**1**) with KOH in methanol. Kinetic experiment (a). Molar ratio KOH : **1** = 5.53×10^3.

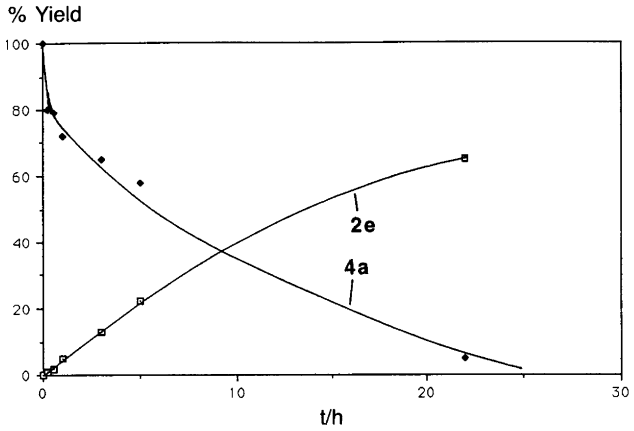


Fig. 2. Reaction of isofucoxanthinol (**4a**) with KOH in methanol. Molar ratio KOH : **4a** = 2.41×10^4.

detailed ^{1}H NMR and ^{13}C NMR data given below. The CD spectra of all-*trans* **4** and **4a** were, as expected, very similar. Different and stronger Cotton effects than for all-*trans* fucoxanthin (**1**)[3,4] may be rationalized in terms of the extended chromophore and of restricted conformational rotation in the isofucoxanthin series, possessing a Δ6 double bond.

The isofucoxanthins have previously been prepared from **1** by treatment with or chromatography on alkaline adsorbents,[8,9] and **4** has been reported to be present in egg yolk.[8] Controlled reaction of **1** with KOH (*c, d*-1, Table 1) is preferred for the preparation of **4a**.

(e) Rationalization of the reactions observed. The reactions treated under (b)–(d) above, as rationalized in Scheme 4, are initiated by an acid/base reaction rather than by a nucleophilic attack. The fucoxanthin end group is known from 2D ROESY ^{1}H NMR studies to have the keto-epoxy *anti* conformation *a*.[13] However, the kinetic experiments required the existence of an equilibrium between rotamers *e* and *b* and/or *a* and *a'*.

Abstraction of an α-proton at C-7 by base (NaH, KOH) provides the enolate ions *b* and *e*, and *b* provides, via the ring-opened alcoholate *c* in protic solvents, the isofucoxanthin end group *d* in a fast, but reversible reaction. A C-6,7 single-bond rotation of *b* to *e*, is followed by ring opening to *f* with the correct configuration for ring closure to *g*, which may be methylated by CH_3I to the methyl ketal *h* or react with protic solvents (CH_3OH, H_2O) to the hemiketal *i*.

The terminal hemiketal/ketal products are the result of the thermodynamically controlled reaction. High KOH : substrate molar ratio, was found to increase the reaction rate to the terminal hemiketal *i*, and use of stronger base (NaH) gave *i* exclusively. The conversion of *c* to *b* or of *e* to *f* is presumed to be the rate-determining step.

Table 2. Treatment of fucoxanthin (**1**) with varying amounts of KOH in methanol for 5 min.

Molar ratio KOH : **1**	Product	Yield (%)	Molar ratio **2** : **4a**
553	**4a**	54	0.1
	2	7	
2763	**4a**	49	0.2
	2	12	
5525	**4a**	27	1.7
	2	45	
11050	**4a**	20	2.4
	2	47	
55253	**4a**	19	2.8
	2	54	

(f) NMR assignments of major products

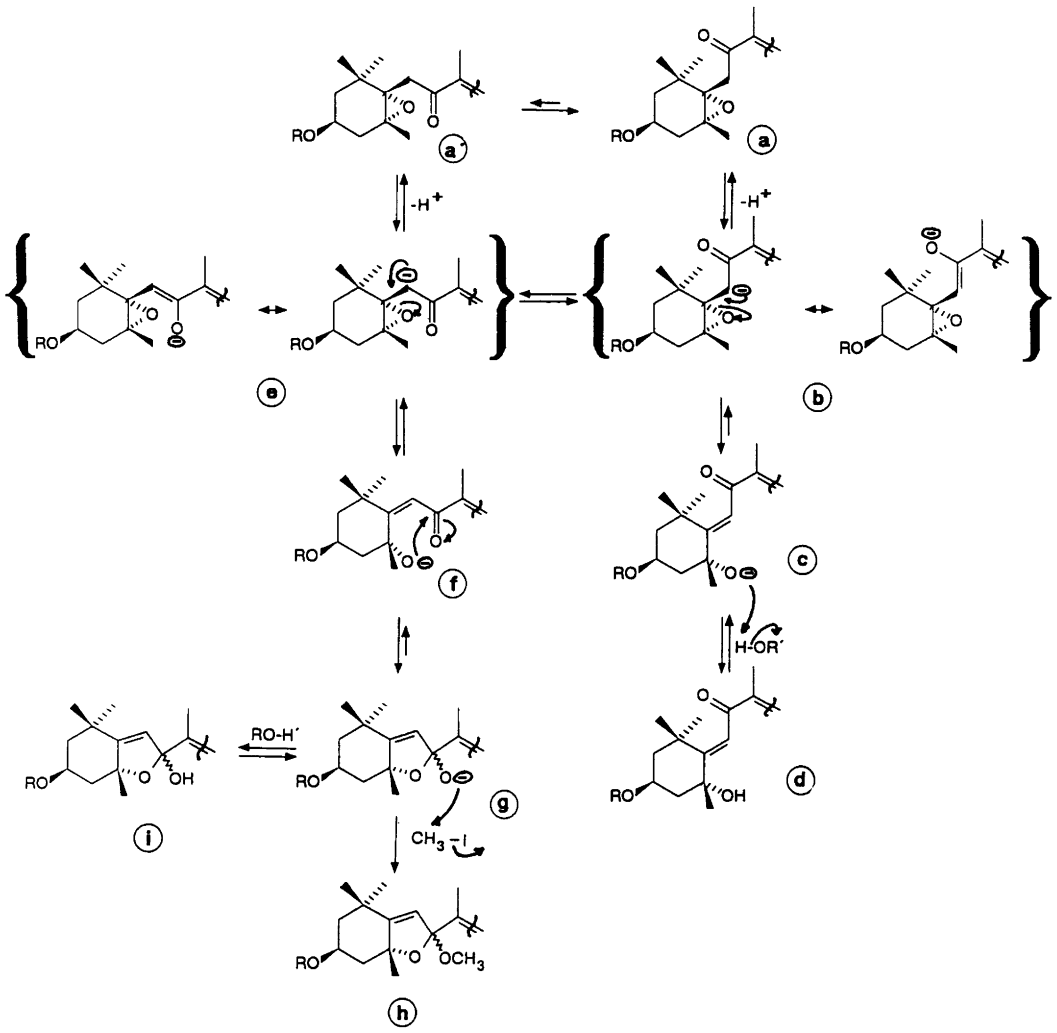
Isofucoxanthinol (**4a**) *and isofucoxanthin* (**4**). Essentially the same approach as in other related cases[13] was applied to the assignment of the ^{1}H and ^{13}C NMR signals of the isofucoxanthins. Assignments are given in Scheme 5.

Fucoxanthin hemiketal (**2**) *and fucoxanthinol hemiketal* (**2e**). The analysis of the ^{1}H and ^{13}C spectra of fucoxanthin hemiketal (**2**) and fucoxanthinol hemiketal (**2e**) was hampered by their instability, and by the presence of stereoisomeric mixtures of *E*/*Z* isomers and a ca. 6:4 mixture of C_8-epimers. 1D ^{1}H and ^{13}C spectra were acquired in two solvents in acid-free $CDCl_3$ and CD_3OD; 2D spectra were also obtained for **2e**.

The signals attributed to the allenic end group for **2** and **2e** were, as expected, virtually identical with those of isofucoxanthin (**4**) and isofucoxanthinol (**4a**), respectively. This unambiguously proved the structure of this part of the molecule.

In the unprimed end of the two hemiketals (**2** and **2e**) a number of tentative assignments are proposed in Scheme 6. Two sets of values refer to the two C-8 epimers. As expected, are some shifts close to those of the carotenoid 5,8-epoxide mutatochrome.[14,15]

(g) Sediment product implications. The isolation of fucoxanthin hemiketal (**2**) from marine sediments has been claimed on the basis of the VIS spectrum and chemical ionization mass spectrum.[16] Fucoxanthin hemiketal (**2**) is a plausible intermediate in the reported degradation of fucoxanthin (**1**) to loliolide in such sediments.[17]



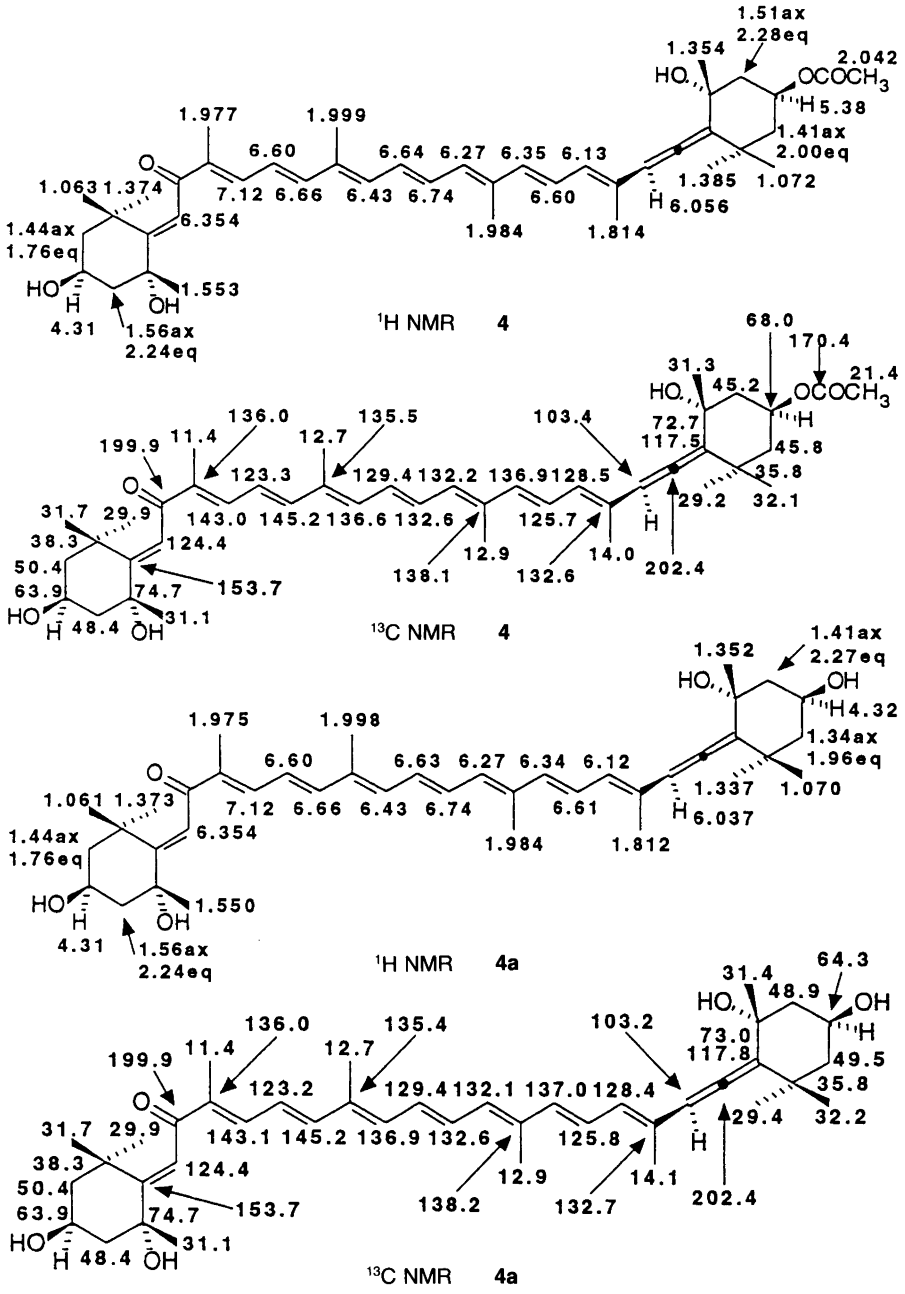
Scheme 4.

Experimental

General methods. General precautions for work with carotenoids were taken. Solvents were of distilled or *pa* quality. Diethyl ether was chromatographed through alumina (neutral) and tetrahydrofuran (THF) was distilled over solid sodium. Sodium hydride was washed with *n*-pentane before use. All reactions were carried out in the dark at room temperature. Reaction mixtures were flushed with N_2-gas. Three different TLC-systems were used. All systems were based on silica gel 60 G (Merck Art. 7731) as the stationary phase. Acetone–*n*-hexane 3:7 (system 1), acetone–*n*-hexane 1:1 (system 2) and ethyl acetate (system 3) were used for development. HPLC was carried out on a Hewlett Packard series 1050 instrument, 5 μ nitrile Techsphere column (25 cm × 4.6 mm), eluent *n*-hexane–isopropyl acetate–acetone–methanol 75.9:17:7:0.1.[4]

VIS spectra were recorded on a Perkin Elmer 552 spectrophotometer; solvents are specified in each case. Spectral fine structure is expressed as % III/II.[18] Mass spectra were recorded on an AEI 902 spectrometer with a direct inlet to the ion source. The ion-source temperature was 210–230 °C. Peak matching of diagnostic ions was performed on the same spectrometer. FT-IR spectra were recorded in KBr disc on a Nicolet 20 SXC FT-IR spectrophotometer and CD spectra on a Jobin Yvon Auto Dicrograph Mark IV in EPA (diethyl ether–isopentane–ethanol 5:5:2) solution at room temperature. ^{1}H NMR, ^{13}C NMR, 2D ^{1}H–^{1}H correlated spectroscopy (COSY), ^{1}H–^{13}C correlated spectroscopy (hetero-COSY), rotating frame Overhauser effect spectroscopy (ROESY) and totally correlated spectroscopy (TOCSY) were carried out on a 400 MHz (100 MHz for ^{13}C) Jeol or Bruker FT instrument and a 500 MHz Bruker FT instrument, generally with $CDCl_3$ as the solvent. The 1D TOCSY, 2D ROESY and ^{1}H-detected ^{1}H,^{13}C-COSY 2D were performed as described elsewhere.[13]

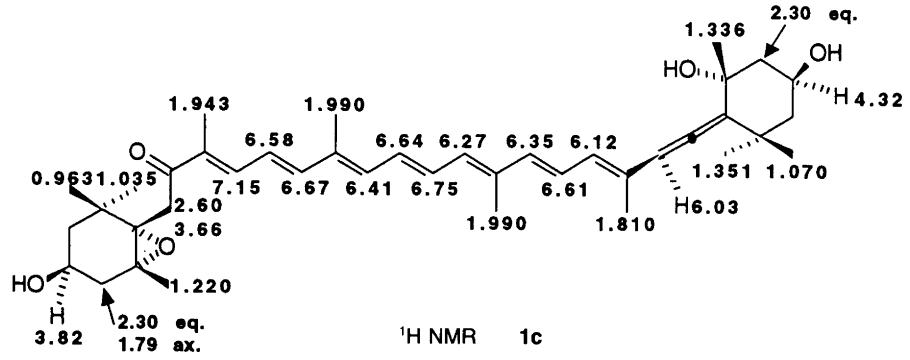
Treatment of fucoxanthin (**1**) *with dimethyl sulfide.* Fucoxanthin (**1**, 1 mg, 1.52×10^{-3} mmol) was dissolved in



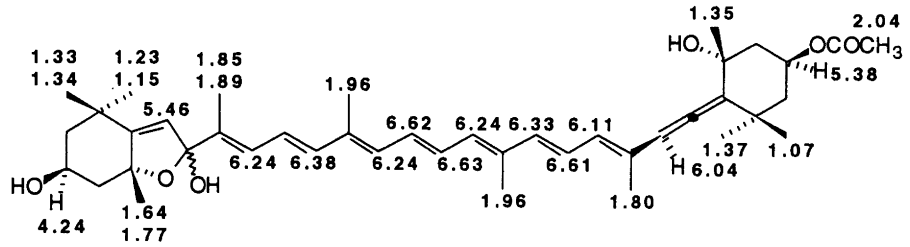
Scheme 5.

CH_2Cl_2 (20 ml) and $(CH_3)_2S$ (7 drops) in CH_2Cl_2 (10 ml) was added. The reaction was monitored by TLC and VIS spectroscopy. No reaction occurred. After 23 h ether and water were added. The ether phase was washed with saturated aq. NaCl and water. Chromatography (TLC system 1) showed a single carotenoid and MS showed only unchanged fucoxanthin (**1**).

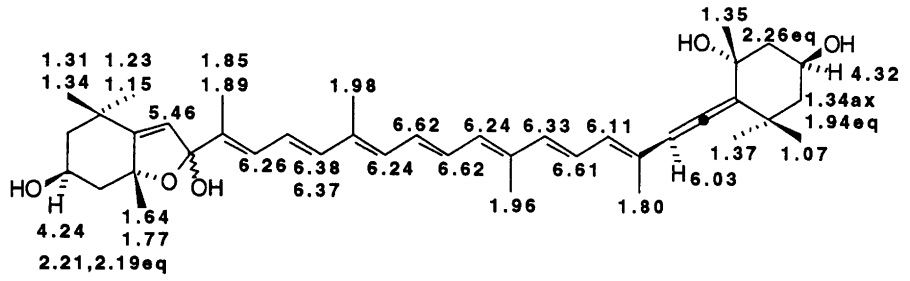
Methylation of (3R,3'R)-3,3'-dihydroxy-7,7',8,8'-tetrahydro-β,β-carotene-8,8'-dione (**3**). (3*R*,3'*R*)-3,3'-Dihydroxy-7,7',8,8'-tetrahydro-β,β-carotene-8,8'-dione [**3**, λ_{max} (acetone) 410, 432.5, 460 nm % III/II = 79; 0.197 mg, 3.3×10^{-4} mmol] was dissolved in dry THF (20 ml) and NaH (0.04 g, 1.67 mmol) was added. The reaction mixture was stirred for 5 min and CH_3I (1.5 ml, 3.42 g, 20 mmol) was added dropwise over 2 min. TLC (system 1) after 18 h showed two methylated products with R_f (system 1) = 0.28 and 0.54, respectively, in addition to unchanged **3**. The reaction mixture was stirred for 75 h, then filtered, and the filtrated was evaporated to dryness on a rotary evaporator (< 30 °C) and dissolved in ether (50 ml). The ether phase was washed with water (3 × 50 ml) and evaporated to dryness and the residue was dissolved in hexane. Three products were isolated after preparative TLC (system 1).



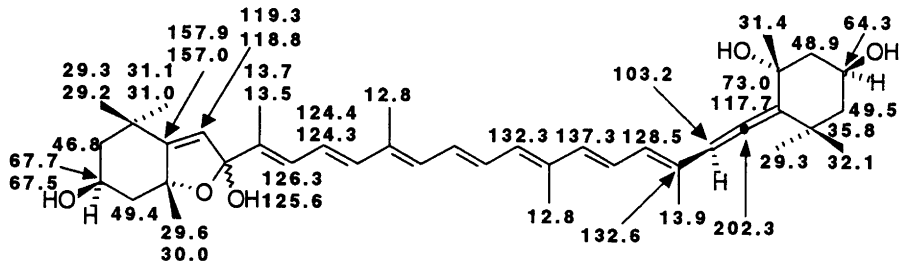
^{1}H NMR **1c**



^{1}H NMR **2**



^{1}H NMR **2e**



^{13}C NMR **2e**

Scheme 6.

3-Hydroxy-3'-methoxy-7,7',8,8'-tetrahydro-β,β-carotene-8,8'-dione (**3a**). Yield 7 μg (3.5 %, $E^{1\%}_{1\,cm}$ = 2500); R_f (system 1) = 0.27; VIS λ_{max} (acetone) 415, 433, 460 nm, % III/II = 50; MS [IP 70 eV; *m/z* (% rel. int.)]: 614 (59, [*M*]), 564 (9, [*M*−18−32]), 313 (45), 265 (54), 239 (74), 211 (63), 183 (59), 177 (100).

3,3'-Dimethoxy-7,7',8,8'-tetrahydro-β,β-carotene-8,8'-dione (**3b**). Yield 40 μg (20 %, $E^{1\%}_{1\,cm}$ = 2500); R_f (system 1) = 0.50; VIS λ_{max} (acetone) 415, 434, 460 nm, % III/II = 45; MS [IP 70 eV; *m/z* (% rel. int.)]: 628 (100, [*M*]), 596 (23, [*M*−32]), 564 (26, [*M*−32−32]), 460 (44), 395 (34).

3,3'-Dimethoxy-7-methyl-7,7',8,8'-tetrahydro-β,β-carotene-8,8'-dione (**3c**). Yield 15 μg (7 %, $E^{1\%}_{1\,cm}$ = 2500); R_f (system 1) = 0.60; VIS λ_{max} (acetone) (412), 435, (457) nm; MS [IP 70 eV; *m/z* (% rel. int.)]: 642 (14, [*M*]), 640 (12, [*M*−2]), 578 (9, [*M*−32−32]), 313 (7), 239 (25), 135 (100).

Treatment of fucoxanthin (**1**) *with NaH in dry THF.* **1** (0.03 mg, 4.6×10^{-5} mmol) was dissolved in dry ether (ca. 10 ml) and NaH (16 mg, 6.7×10^{-4} mol) was added. The reaction was monitored by TLC (system 1) and VIS spectroscopy. TLC showed only one product during 90 min and no chromophoric change was observed.

Methylation of fucoxanthin (**1**). Fucoxanthin (**1**, 1.61 mg, 2.45×10^{-3} mmol) was dissolved in dry THF (50 ml) and NaH (0.08 g, 3.3 mmol) was added. The reaction mixture was stirred for 5 min and CH_3I (2 ml, 4.56 g, 27 mmol) added dropwise over 2 min. The reaction was monitored by TLC (system 1), VIS spectroscopy and MS. TLC after 4 h showed unchanged fucoxanthin (**1**), inseparable from authentic fucoxanthin *ex. Fucus serratus,*[2,3] together with the main product **2a**, which was less polar R_f, (system 1) = 0.30, and traces of an even less polar product **2b**, R_f (system 1) = 0.50. After 148 h the reaction mixture was filtered and left for 24 h at −20 °C. TLC showed one main product **2b**, R_f (system 1) = 0.50 together with unchanged fucoxanthin (**1**) and **2a**.

Fucoxanthin methyl ketal 3-methyl ether (**2a**). Yield not determined (intermediate product only). VIS and mass spectra were recorded of the reaction mixture after 4 h; mainly **2a** (> 80 %). R_f (system 1) = 0.30; VIS λ_{max} (THF) 404, 429, 453, (472) nm; MS [IP 70 eV, *m/z* (% rel. int.)]: 686 (9, [*M*]), 668 (77, [*M*−18]), 654 (28, [*M*−32]), 636 (45, [*M*−18−32]), 626 (17, [*M*−60]), 622 (13, [*M*−32−32]), 608 (10, [*M*−60−18]), 604 (9, [*M*−18−32−32]), 594 (15, [*M*−60−32]), 576 (23, [*M*−60−32−18]), 562 (12, [*M*−60−32−32]), 544 (9, [*M*−60−32−32−18]), 476 (34), 434 (19), 396 (19), 340 (32), 263 (88), 261 (45), 235 (100), 221 (55), 197 (100).

Fucoxanthin methyl ketal 3,5'-dimethyl ether (**2b**). Yield 1.03 mg (60 %, $E^{1\%}_{1\,cm}$ = 2100); R_f (system 1) = 0.47; VIS λ_{max} (THF) 403, 426, 454 nm, % III/II = 78; MS [IP 70 eV; *m/z* (% rel. int.)]: 700 (5, [*M*]), 668 (4, [*M*−32]), 656 (11, [*M*−44]), 640 (7, [*M*−60]), 608 (5, [*M*−60−32]), 576 (8, [*M*−60−32−32]), 544 (6, [*M*−60−32−32−32]), 265 (16), 263 (20), 237 (72, [$C_{14}H_{21}O_3$]), 235 (31, [$C_{15}H_{23}O_2$]), 233 (28, [$C_{16}H_{25}O$]), 221 (28), 205 (58, [$C_{14}H_{21}O$]), 197 (44, [$C_{15}H_{17}$]), 195 (54, [$C_{12}H_{19}O_2$]), 194 (77, [$C_{12}H_{18}O_2$]), 181 (100, [$C_{10}H_{13}O_3$]), 165 (80, [$C_{10}H_{13}O_2$]), 163 (70, [$C_{11}H_{15}O$]).

Fucoxanthin 3-acetate (**1a**). Fucoxanthin (**1**, 2.24 mg, 3.4×10^{-3} mmol) was dissolved in dry pyridine (4 ml) and acetic anhydride (0.4 ml) was added. The reaction was monitored by TLC (system 1). After 24 h ether was added (ca. 50 ml) and then water (ca. 50 ml). The ether phase was washed with saturated aq. NaCl (ca. 50 ml) and water (3 × 50 ml) and evaporated to dryness, and the residue was dissolved in hexane. Fucoxanthin 3-acetate (**1a**) was isolated by TLC (system 1). Yield 1.54 mg, 65 %, $E^{1\%}_{1\,cm}$ = 1660); R_f (system 1) = 0.44; VIS λ_{max} (acetone) (420), 446, 465 nm, % III/II = 3; MS [IP 70 eV; *m/z* (rel. int.)]: 700 (17, [*M*]), 682 (50, [*M*−18]), 664 (19, [*M*−18−18]), 640 (2, [*M*−60]), 622 (31, [*M*−18−60]), 604 (12, [*M*−18−18−60]), 524 (8), 410 (25), 263 (46, [$C_{16}H_{23}O_3$]), 223 (23, [$C_{13}H_{19}O_3$]), 203 (29, [$C_{14}H_{19}O$]), 197 (100).

Fucoxanthin 3-acetate 5'-trimethylsilyl ether (**1b**). Fucoxanthin 3-acetate (**1a**, 1 mg, 1.43×10^{-3} mmol) was dissolved in dry pyridine (4 ml). A mixture of hexamethyldisilane (0.2 ml) and trimethylsilyl chloride (0.1 ml) dissolved in dry pyridine (1 ml) was added dropwise. The reaction was monitored by TLC (system 1). After 24 h the reaction was stopped as described for the acetylation of fucoxanthin above. Yield 0.85 mg (77 %, $E^{1\%}_{1\,cm}$ = 1660. R_f (system 1) = 0.78; VIS λ_{max} (acetone) 445, (464) nm; MS [IP 70 eV; *m/z* (% rel. int.)]: 772 (87, [*M*]), 754 (87, [*M*−18]), 682 (30, [*M*−90]), 664 (40, [*M*−18−90]), 604 (20, [*M*−18−60−90]), 263 (100), 197 (73).

Methylation of fucoxanthin 3-acetate 5'-trimethylsilyl ether (**1c**). **1b** (0.123 mg, 1.6×10^{-4} mmol) was dissolved in dry THF (20 ml) and NaH (0.08 g, 3.33 mmol) was added. The reaction mixture was stirred for 5 min and CH_3I (1 ml, 2.28 g, 13.5 mmol) added dropwise over 1 min. TLC (system 1) after 4 h showed unchanged **1b** (ca. 20 % of total) and a more polar carotenoid, fucoxanthin 3-acetate 5'-trimethylsilyl ether methyl ketal (**2c**), with R_f (system 1) = 0.70. VIS λ_{max} (THF) 403, 426, 454 nm, % III/II = 92; λ_{max} (acetone) 398, 421, 448 nm, % III/II = 90; MS [IP 70 eV; *m/z* (% rel. int.]): 786 (2, [*M*]), 770 (3, [*M*−16]), 754 (15, [*M*−32]), 726 (4, [*M*−60]), 666 (6, [*M*−60−60]), 664 (8, [*M*−32−90]), 576 (5, [*M*−60−60−90]), 263 (29), 234 (85, [$C_{16}H_{26}O$]), 233 (75, [$C_{16}H_{25}O$]), 220 (45), 219 (100, [$C_{15}H_{23}O$]), 205 (84, [$C_{14}H_{21}O$]), 163 (82, [$C_{11}H_{15}O$]).

Fucoxanthin 3-acetate 5'-trimethylsilyl ether hemiketal (**2d**). Fucoxanthin 3-acetate 5'-silyl ether (**1b**, 0.23 mg, 3.0×10^{-4} mmol) was dissolved in dry THF (20 ml) and NaH (0.12 g, 5 mmol) was added. The mixture was stirred for 30 min, filtered and ether (ca. 20 ml) and water (ca. 100 ml) were added. The ether phase was washed with water (3 × 50 ml). No further work-up was necessary. Yield 0.12 mg (61 %, $E^{1\%}_{1\,cm}$ = 1900); VIS λ_{max} (diethyl ether) 396, 420, 447 nm, % III/II = 82, λ_{max} (THF) 402, 425, 453 nm, % III/II = 90; MS [IP 70 eV; *m/z* (% rel. int.)]: 772 (4, [*M*]), 770 (3, [*M*−2]), 756 (4, [*M*−16]), 754 (5, [*M*−18]), 730 (3, [*M*−42]), 712 (2, [*M*−60]), 694 (2, [*M*−18−60]), 664 (3, [*M*−18−90]), 604 (4, [*M*−18−60−90]), 550 (8, [*M*−222]), 265 (23), 266 (23), 205 (100), 197 (42), 165 (47), 163 (52).

Treatment of fucoxanthin (**1**) *with 0.01 % KOH in methanol.* Fucoxanthin (**1**, 9.8 mg, 0.015 mmol) was dis-

solved in 0.01 % KOH in methanol (25 ml), molar ratio KOH : **1** = 2.2. The reaction was monitored by VIS spectroscopy. After 40 min, ether (ca. 50 ml) and water (ca. 100 ml) were added. The ether phase was washed with saturated aq. NaCl solution (ca. 100 ml) and water (3 × 100 ml). Six products were isolated by preparative TLC (system 3), given in order of decreasing R_f-value. Unchanged fucoxanthin (**1**); recovery 3.25 mg (33 %, $E^{1\%}_{1\,cm}$ = 1660), characterized by R_f (system 3), co-chromatography with authentic **1** and VIS spectroscopy. Fucoxanthin hemiketal (**2**); yield 1.00 mg (11 %, $E^{1\%}_{1\,cm}$ = 2000), characterized by R_f (system 3) and VIS spectroscopy. For further characterization see below. Isofucoxanthin (**4**); yield 1.7 mg (17 %, $E^{1\%}_{1\,cm}$ = 1660), characterized by R_f (system 3) and VIS spectroscopy. For full characterization see below. Fucoxanthinol (**1c**); yield 0.71 mg (8 %, $E^{1\%}_{1\,cm}$ = 1660), characterized by R_f (system 3) and VIS spectroscopy. For further characterization see below. Fucoxanthinol hemiketal (**2e**); yield 0.16 mg (3 %, $E^{1\%}_{1\,cm}$ = 2000), characterized by R_f (system 3) and VIS spectroscopy. For further characterization see below. Isofucoxanthinol (**4a**); yield 0.32 mg (3.5 %, $E^{1\%}_{1\,cm}$ = 160), characterized by R_f (system 3) and VIS spectroscopy. For full characterization see below.

Treatment of fucoxanthin (**1**) *with 0.1 % KOH in methanol.* Fucoxanthin (**1**, 9.3 mg, 0.014 mmol) was dissolved in 0.1 % KOH in methanol (25 ml), molar ratio KOH : **1** = 24. The reaction was monitored by VIS spectroscopy and stopped after 35 min with the work-up procedure as described above. Six products were isolated by preparative TLC (system 1), given in order of decreasing R_f-value. Unchanged fucoxanthin (**1**), recovery 1.88 mg (20 %), was characterized by R_f (system 1), co-chromatography with authentic **1**, VIS spectroscopy and MS. Fucoxanthin hemiketal (**2**), yield 0.92 mg (10 %), was characterized by R_f (system 1), VIS spectroscopy and MS. Isofucoxanthin (**4**) and fucoxanthinol (**1c**) were first isolated as a mixture and subsequently separated by TLC (system 2). Isofucoxanthin (**4**), yield 2.08 mg (22 %), was characterized by R_f (systems 1 and 2), VIS, ^{1}H NMR and ^{13}C NMR spectroscopy and MS. Fucoxanthinol (**1c**), yield 0.55 mg (6 %), was characterized by R_f (systems 1–3), co-chromatography tests, VIS, mass and ^{1}H NMR spectroscopy. Fucoxanthinol hemiketal (**2e**) and isofucoxanthinol (**4a**) were first isolated as a mixture and subsequently separated by TLC (system 3). Fucoxanthinol hemiketal (**2e**), yield 0.20 mg (3 %), was characterized by R_f (system 1) and VIS spectroscopy. A mass spectrum was not obtained owing to product instability. Isofucoxanthinol (**4a**), yield 0.64 mg (7 %), was characterized by R_f (systems 1 and 3), VIS, ^{1}H NMR and ^{13}C NMR spectroscopy and MS.

Treatment of fucoxanthin (**1**) *with 1 % KOH in methanol.* Fucoxanthin (**1**, 1 mg, 1.52×10^{-3} mmol) was dissolved in 1 % KOH in methanol (20 ml), molar ratio KOH : **1** = 1750. The reaction was interrupted after 35 min and worked up as described above. Two products were isolated after preparative TLC (system 3). Fucoxanthinol hemiketal (**2e**), yield 0.19 mg (20 %), was characterized by R_f (system 3), VIS spectroscopy and MS. Isofucoxanthinol (**4a**), yield 0.24 mg (26 %), was characterized by R_f (system 3), VIS spectroscopy and MS.

Treatment of fucoxanthin (**1**) *with 5 % KOH in methanol.* Fucoxanthin (**1**, 10 mg, 0.015 mmol) was dissolved in 5 % KOH in methanol (50 ml); molar ratio KOH : **1** = 2187. The reaction was monitored by VIS spectroscopy. An aliquot representing 60 % of the reaction mixture was withdrawn after 20 min and worked up in the usual manner. The reaction (remaining 40 %) was interrupted after 46 h. Two products were isolated from the intermediate sample (i) and only one product from the final reaction mixture (ii). Fucoxanthinol hemiketal (**2a**), yield 1.01 mg (i) (18 %), 3.83 mg (ii) (72 %), was characterized by R_f (system 3), VIS spectroscopy in both acetone and benzene and MS. Isofucoxanthinol (**4a**), yield 1.57 mg (i) (28 %) and trace amounts (ii), was characterized by R_f (system 3), VIS spectroscopy and MS.

Treatment of fucoxanthin (**1**) *with KOH with increasing mole ratio.* Fucoxanthin (**1**, 0.38 mg, 5.8×10^{-7} mmol), in five parallel experiments, was dissolved in different volumes of 5 % KOH in methanol (48, 9.6, 4.8, 2.4 and 0.48 ml) to give a KOH : **1** molar ratio varying from 553 to 55 253. The reactions were interrupted after 5 min, and the products analysed by TLC (system 3) and VIS spectroscopy, see Table 2.

Kinetic experiments with fucoxanthin (**1**). (a) In two parallel experiments fucoxanthin (**1**, 3.17 mg, 4.8×10^{-3} mmol and 4.75 mg, 7.2×10^{-3} mmol) was dissolved in 5 % KOH in methanol (40 ml and 60 ml), to give a molar ratio KOH : **1** of ca. 5520 in each experiment. The reaction was monitored by TLC (system 3) and VIS spectroscopy. Aliquots of 1,2 or 5 ml were withdrawn after different reaction times. Only two products, isofucoxanthinol (**4a**) and fucoxanthinol hemiketal (**2e**), were observed, see Fig. 1.

(b) Fucoxanthin (**1**, 0.62 mg, 9.4×10^{-4} mmol) was reacted with 5 % KOH in methanol (8 ml), molar ratio KOH : **1** = 5644 and aliquots analysed as above after 30 s and 1 min. Result 30 s, 61 % total pigment recovery : unchanged fucoxanthin (**1**, 3 %), fucoxanthinol (**1c**, 2 %), isofucoxanthin (**4**, 9 %), isofucoxanthinol (**4a**, 13 %), fucoxanthin hemiketal (**2**, 12 %) and fucoxanthinol hemiketal (**2e**, 22 %); result 1 min, 61 % total pigment recovery : unchanged **1** (1 %), **4** (6 %), **4a** (17 %), **2** (10 %) and **2e** (25 %).

(c) Fucoxanthin (**1**, 21 μg, 3.2×10^{-2} μmol) was dissolved in 5 % KOH in methanol (3 ml) to give a ratio KOH : **1** 62 500. The reaction was monitored by VIS spectroscopy which revealed the formation of both isofucoxanthinol (**4a**) and fucoxanthinol hemiketal (**2e**) after 5 min. No chromophoric change or drop in absorption intensity was detected during the following 170 h.

Kinetic experiment with isofucoxanthinol (**4a**). Isofucoxanthinol (**4a**, 0.51 mg, 8.3×10^{-4} mmol) was dissolved in 5% KOH in methanol (30 ml) to give a molar ratio KOH : **4a** of 24 100. Aliquots of 5 ml were withdrawn at different times and the reaction was monitored as described for fucoxanthin (**1**) experiment (a) above. Coloured products other than isofucoxanthinol (**4a**) and fucoxanthinol hemiketal (**2**) were not present, see Fig. 2.

Kinetic experiment with fucoxanthinol hemiketal (**2e**). Fucoxanthinol hemiketal (**2e**, 16 μg, 2.6×10^{-2} μmol) was dissolved in 5% KOH in methanol (3 ml) to give a ratio KOH : **2e** of 76 920. VIS spectra were recorded at intervals over a period of 72 h. No chromophoric change was detected.

Fucoxanthin (**1**). R_f = 0.26 (system 1), R_f = 0.32 (system 2) and R_f = 0.77 (system 3); VIS λ_{max} (acetone) (420), 444, 466 nm, % III/II = 5; MS [IP 70 eV; *m/z* (% rel. int.)]: 658 (27, [*M*]), 640 (36, [*M*−18]), 622 (8, [*M*−18−18]), 600 (6, [*M*−58]), 598 (4, [*M*−60]), 580 (8, [*M*−18−60]), 562 (4, [*M*−18−18−60]), 221 (47), 212 (46), 197 (100). For further characterization see Ref. 3.

Fucoxanthinol (**1c**). R_f = 0.08 (system 1), R_f = 0.20 (system 2) and R_f = 0.40 (system 3), inseparable from authentic natural **1c** *ex. Fucus serratus*,[10] VIS λ_{max} (acetone) (420), 445, (465) nm; MS [IP 70 eV; *m/z* (% rel. int.)]: 616 (26, [*M*]), 600 (24, [*M*−16]), 598 (48, [*M*−18]), 582 (16, [*M*−16−18]), 580 (22, [*M*−18−18]), 562 (10, [*M*−18−18−18]), 540 (21, [*M*−76]), 221 (100), 197 (95); IR cm^{-1} 3428s (OH), 3031–2734s (CH), 1929w (allene), 1726w, 1650m (conj. C=O), 1606s, 1530w, 1385s (*gem* Me), 1294w, 1270w, 1149m, 1123m, 1071m, 1052m, 965w (*trans* CH=CH); ^{1}H NMR ($CDCl_3$), see Scheme 6.

Fucoxanthin hemiketal (**2**). R_f = 0.15 (system 1), R_f = 0.28 (system 2) and R_f = 0.77 (system 3). VIS λ_{max} (acetone) 399, 422, 449 nm, % III/II = 70 (benzene) 407, 431, 460, % III/II = 84. MS [IP 70 eV; *m/z* (% rel. int.)]: 658 (4, [*M*]), 656 (4, [*M*−2]), 642 (12, [*M*−16]), 640 (20, [*M*−18]), 624 (8, [*M*−16−18]), 622 (4, [*M*−18−18]), 598 (3, [*M*−60]), 580 (9, [*M*−18−60]), 562 (7, [*M*−18−18−60]), 550 (7), 544 (6, [*M*−16−18−80]), 221 (85), 212 (75), 197 (100), 181 (54); IR cm^{-1} 3430m (OH), 3029–2854s (CH), 1932w (allene), 1725m (acetate), 1584m, 1429m, 1384s (*gem* Me), 1250m (acetate), 1164m, 1121m, 1073m, 964m (*trans* CH=CH); ^{1}H NMR ($CDCl_3$) see tentative assignments, Scheme 6.

Fucoxanthiol hemiketal (**2e**). R_f = 0.05 (system 1), R_f = 0.15 (system 2) and R_f = 0.28 (system 3); VIS λ_{max} (acetone) 399, 421, 448 nm, % III/II = 65. MS [IP 70 eV; *m/z* (% rel. int.)]: 616 (11, [*M*]), 600 (12, [*M*−16]), 598 (27, [*M*−18]), 582 (9, [*M*−16−18]), 580 (21, [*M*−18−18]), 562 (14, [*M*−18−18−18]), 544 (8, [*M*−18−18−18−18]), 221 (91, [$C_{14}H_{21}O_2$]), 197 (100), 181 (66), 165 (91); IR cm^{-1} 3427m (OH), 3029–2732s (CH), 1927w (allene), 1712w, 1654w, 1581w, 1452m, 1367m (*gem* Me), 1282w, 1156m, 1069m, 1042m, 1018m, 964s (*trans* CH=CH); ^{1}H NMR ($CDCl_3$) and ^{13}C NMR, see tentative assignments, Scheme 6, based on COSY and hetero-COSY spectra.

Isofucoxanthin (**4**). R_f = 0.08 (system 1), R_f = 0.23 (system 2) R_f = 0.50 (system 3), t_R = 27 min, flow 1.5 ml min^{-1}; VIS λ_{max} (acetone) (424), 449, (470) nm; MS [IP 70 eV; *m/z* (% rel. int.)]: 658 (23, [*M*]), 642 (17, [*M*−16]), 640 (38, [*M*−18]), 624 (6, [*M*−16−18]), 622 (10, [*M*−18−18]), 616 (7, [*M*−42]), 598 (13, [*M*−60]), 580 (12, [*M*−18−60]), 562 (5, [*M*−18−18−60]), 221 (79), 212 (45), 197 (100), 181 (39), 165 (47); IR cm^{-1} 3422m (OH), 3029–2732s (CH), 1929w (allene), 1723m (acetate), 1631m (conj. C=O), 1603m, 1530w, 1455w, 1383m (*gem* Me), 1270m (acetate), 1219w, 1205w, 1153m, 1123w, 1073w, 1030w, 968w (*trans* CH=CH); HPLC pure all-*trans* CD nm (Δε) 212 (−0.92), 217 (−2.12), 222 (−1.65), 237 (−2.62), 244 (−1.88), 257 (−0.28), 267 (−0.64), 273 (0), 281 (+0.57), 286 (+0.50), 292 (+0.52), 309 (0), 312 (−0.39), 326 (−1.77), 332 (−1.73), 338 (−1.98), 340 (−1.95), 342 (−2.09), 356 (−0.21), 360 (−0.32), 366–374 (0), >374 (+); ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Scheme 5, based on COSY, hetero-COSY, ROESY and TOCSY spectra.

The same approach was used for **4** and **4e** below. Firstly, a few selected 1D TOCSY experiments were performed in order to prove some of the connections between coupled olefinic protons and to locate the position and find the multiplet structure of the methylene protons in both end groups by inverting the signals of H-3 and H-3′ by a means of 180° DANTE pulse. Secondly a 2D ROESY spectrum of both compounds was acquired which gave additional information on the proximity of protons and groups of protons. This helped to confirm the assignments, particularly the stereochemical assignment of methyl groups of the end groups, and provided information on the geometry of the double bonds. Thirdly, the ^{13}C spectra, including DEPT-135, were acquired and assigned with the aid of the ^{1}H-detected ^{1}H,^{13}C-COSY 2D spectra, in each case tuned to one-band $^1J_{CH}$ and long-range $^{2,3}J_{CH}$ couplings. As expected those ^{1}H and ^{13}C data corresponding to identical structural elements were found to be virtually identical.

Isofucoxanthinol (**4e**). R_f = 0.05 (system 1), R_f = 0.10 (system 2), R_f = 0.16 (system 3), t_R = 106 min, flow 1.5 ml min^{-1}; VIS λ_{max} (acetone) (428), 450, (474) nm; MS [IP 70 eV, *m/z* (% rel. int.)]: 616 (8, [*M*]), 614 (2, [*M*−2]), 600 (16, [*M*−16]), 598 (30, [*M*−18]), 596 (8, [*M*−2−18]), 582 (14, [*M*−16−18]), 580 (25, [*M*−18−18]), 578 (10, [*M*−2−18−18]), 564 (6, [*M*−16−18−18]), 562 (10, [*M*−18−18−18]), 560 (3, [*M*−2−18−18−18]), 540 (16, [*M*−76]), 502 (8, [*M*−114]), 221 (100), 197 (51); IR cm^{-1} 3384m (OH), 3029–2733s (CH), 1928w (allene), 1733w, 1634w (conj. C=O), 1601m, 1574w, 1526w, 1461m, 1379m (*gem* Me), 1319w, 1274m, 1217m, 1152m, 1119w, 1070w, 990w, 963w (*trans* CH=CH); HPLC purified all-*trans* CD

nm ($\Delta\varepsilon$) 217 (−1.29), 223 (−0.83), 239 (−1.91), 245 (−0.80), 254 (0), 257 (+0.20), 260 (0), 262 (−0.32), 264 (−0.24), 267 (−0.42), 270 (−0.37), 272 (−0.45), 276 (0), 284 (+0.54), 287 (+0.60), 297 (+0.70), 314 (0), 329 (−1.46), 332 (−1.43), 333 (−1.45), 336 (−1.41), 340 (−1.45), 365 (−0.24), 369 (−0.26), 378 (0), 384 (+0.16), 388 (+0.14), 402 (+1.04), 404 (+1.03), >404 (+); ^{1}H NMR ($CDCl_3$) and ^{13}C NMR, see Scheme 5, based on COSY, hetero-COSY, ROESY and TOCSY spectra.

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