Acta Chemica Scandinavica, 1994: 48: 68–75
Printed in Belgium – all rights reserved

ACTA
CHEMICA SCANDINAVICA
ISSN 0904-213X

Blue Carotenoids. Part 1. Novel Oxonium Ions Derived from Fucoxanthin

Jarle André Haugan and Synnøve Liaaen-Jensen

Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

Haugan, J. A. and Liaaen-Jensen, S., 1994. Blue Carotenoids. Part 1. Novel Oxonium Ions Derived from Fucoxanthin. – Acta Chem. Scand. 48: 68–75.

The reactions of fucoxanthin-derived ketals and hemiketals with dilute acid and of fucoxanthin with weak acid (AcOH) and strong organic (CF_3COOH) or mineral (HCl) acids, have been examined.

The blue products obtained in these reactions were identified as novel carotenoid oxonium ions, characterized by visible absorption spectra and chemical behaviour, including quantitative conversion into cyclic hemiketals of defined structure. The hemiketal products were identified by VIS, IR and mass spectroscopy in comparison with authentic hemiketals or related 5,8-furanoxides. Diagnostic features in the mass spectra of the hemiketals are rationalized.

The reactions observed are rationalized mechanistically. Isofucoxanthins are not intermediates in the reaction of fucoxanthin with acids. The blue oxonium ions were obtained in reasonable yield in competition with electrophilic addition to the polyene chain.

Hitherto blue carotenoids have been classified into two categories (a) neutral carotenoids with a long polyenone chromophore such as violerythrin[1] and (b) carotenoproteins, frequently with astaxanthin as the prosthetic group.[2] A new category (c) carotenoid oxonium ions must now be added.

Strongly coloured oxonium ions are known, such as oxazine dyes[3] and anthocyanidins.[4] Oxonium ions are diagnostically useful fragment ions arising from electron impact of epoxidic carotenoids.[5] However, carotenoid oxonium ions have not previously been isolated, characterized and identified.

The dark blue colour observed by treatment of epoxidic carotenoids with mineral acids or strong organic acids was noted long ago.[6] Moreover, epoxidic and furanoid carotenoids react in the solid state with $HgCl_2$, providing blue-green products, rationalized as electron-delocalized carbocations.[7] Similar blue products obtained from common epoxidic and furanoid carotenoids with $AlCl_3$ were later associated with carotenoid oxonium ion complexes.[8] The structures previously proposed for the blue products were based on VIS spectra and mechanistic considerations only.[7,8]

In this paper studies on the reaction of fucoxanthin (**1**, Scheme 2) and certain derivatives are described, including characterization and identification of new carotenoid oxonium ions and cyclic hemiketals. Preliminary results have been reported.[9]

The present study represents a further contribution to the chemistry of fucoxanthin (**1**),[10–12] one of the two major carotenoids in Nature.

Carotenoids in general are unstable towards strong acid. In this paper it is demonstrated for the first time that reactions with strong organic and conc. mineral acids lead to identifiable coloured carotenoid products.

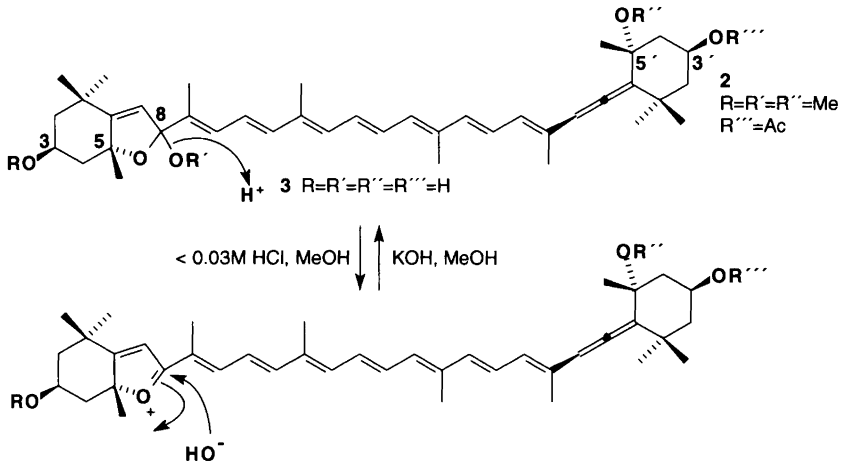
Results and discussion

This study was initiated by the finding that methyl ketals and hemiketals of fucoxanthin (**1**), generated by treatment with base,[12] proved to be extremely acid labile. Blue products were formed upon TLC on silica plates.

The following reactions were studied: (a) treatment of fucoxanthin methyl ketal 3,5′-dimethyl ether (**2**, Scheme 1) and fucoxanthinol hemiketal (**3**, Scheme 1) with weak acid (<0.03 M HCl) and (b) treatment of fucoxanthin (**1**, Scheme 2) with (i) conc. acetic acid, (ii) conc. HCl and (iii) conc. trifluoroacetic acid.

Reactions of fucoxanthin methyl ketal (**2**) *and of fucoxanthinol hemiketal* (**3**) *with dilute mineral acid.* The yellow solution in THF of C-8 epimeric fucoxanthin methyl ketal 3,5′-dimethyl ether (**2**)[12] spontaneously turned blue upon addition of dilute HCl, demonstrated by a bathochromic shift of λ_{max} from 426 nm to 720 nm. The yellow colour was restored upon treatment with KOH. A pigment loss $\leq 3\%$ was calculated from VIS spectra for both processes.

Fucoxanthinol hemiketal (**3**)[12] behaved in the same manner with a λ_{max} shift from 418 nm to 690 nm in methanol, and a pigment recovery of 95% for the forward and backward reaction, see Fig. 1.



Scheme 1.

The reformed yellow product was in this case identical with the initial C-8 epimeric hemiketal **3**, as demonstrated by co-chromatography (TLC), VIS and mass spectra. The reaction sequence is rationalized in Scheme 1, where the blue product is formulated as a strongly electron delocalized oxonium ion. In principle conjugate addition of hydroxide to the blue oxonium ion might also be expected. However, no coloured products with a shorter wavelength chromophore than **3** were observed.

The reversability and high pigment recovery of the colour reaction strongly favours the interpretation involving blue oxonium ions.

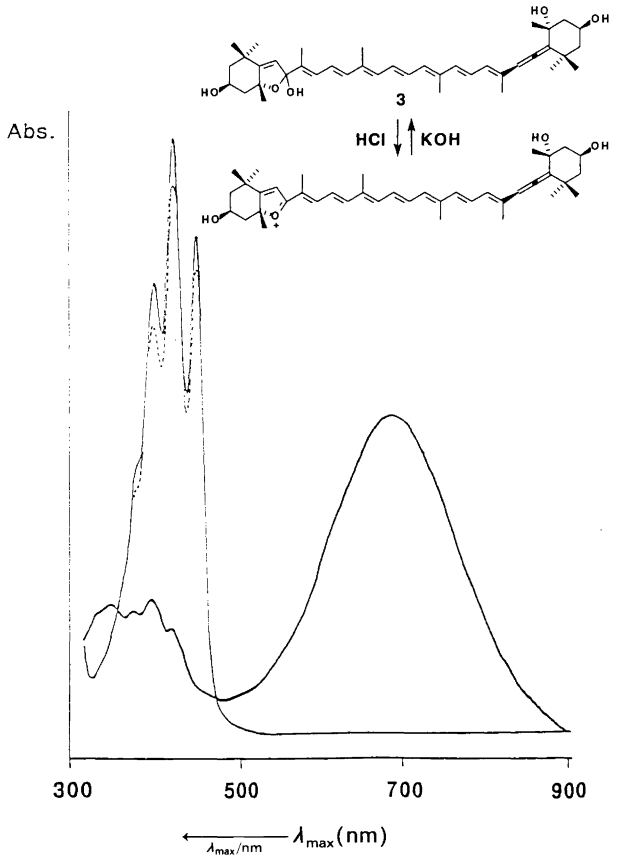


Fig. 1. Treatment of fucoxanthinol hemiketal (**3**, ———) with acid, providing the blue oxonium ion (λ_{max} 690 nm), followed by reversion to fucoxanthinol hemiketal (**3**, – – – –) by treatment with base.

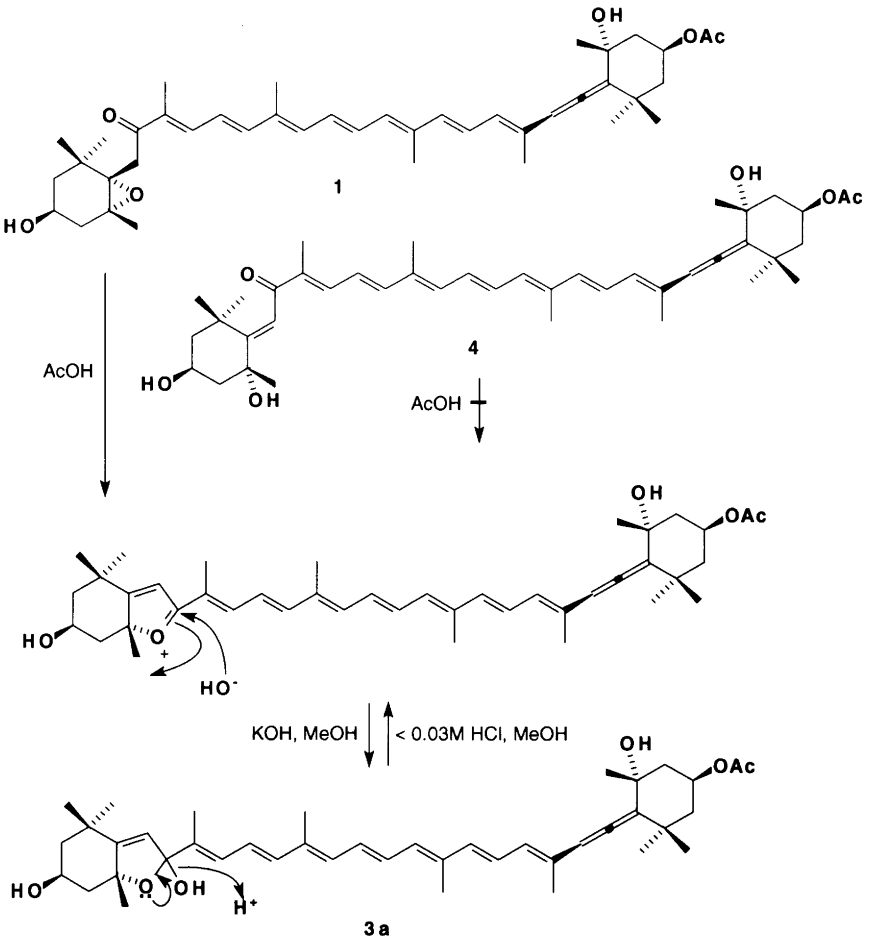
Reactions of fucoxanthin (**1**) *with conc. weak or conc. strong acid.* When fucoxanthin (**1**) was treated with conc. acetic acid the solution slowly turned blue within 90 h with an accompanying bathochromic shift from 449 nm to 720 nm. Upon treatment of this blue solution with H_2O, unchanged **1** (12% yield) and fucoxanthin hemiketal (**3a**, 13%) were isolated and characterized by VIS and mass spectroscopy, see Scheme 2. Three other minor products were not identified.

With regard to the mechanism for the above reaction, isofucoxanthin (**4**)[12] was considered a possible intermediate. However, when isofucoxanthin (**4**, Scheme 2) was treated with conc. acetic acid in a micro-scale reaction, monitored by VIS spectroscopy, no blue products were formed. A plausible reaction mechanism for the conversion of fucoxanthin (**1**) into the blue oxonium ion under acidic conditions is offered in Scheme 3.

When fucoxanthin (**1**) was dissolved in conc. HCl the solution immediately turned from orange to blue. A bathochromic shift of λ_{max} from 444 nm to 686 nm was recorded. After treatment of the blue solution with KOH in methanol until a colour change to yellow was observed, two chlorinated hemiketals were isolated by TLC, see Scheme 4.

3'-Acetoxy-7'-chloro-5,8-epoxy-5,8-dihydro-β,β-carotene-3,8-diol (**5a**, 20% yield) and 7'-chloro-5,8-epoxy-5,8-dihydro-β,β-carotene-3,8,3'-triol (**5b**, 7%) were characterized by VIS, IR and mass spectroscopy as a probable mixture of C-8 epimers. These hemiketals (**5a**, **5b**) provided blue products when treated with dilute acid. It is reasonable to assume that partial hydrolysis of the acetate occurred during the base treatment. Formation of chlorinated products from allenic carotenoids has previously been studied,[13] cf. Scheme 4.

Fucoxanthin also afforded a blue solution when kept in conc. CF_3COOH. A bathochromic shift of λ_{max}



Scheme 2.

from 444 nm to 750 nm was recorded. After treatment of the blue solution with KOH until a colour change to yellow was observed, six coloured products were isolated. Three minor, less polar products were not characterized. One unidentified product had a molecular ion at *m/z* 598, compatible with $C_{40}H_{54}O_4$. A hemiketal function was absent as judged by the absence of formation of blue products upon treatment with dilute acid. The two major coloured products were identified as epimeric 8-hydroxydiadinochrome (**6b**, 5% yield) and its 3′-acetate (**6a**, 0.2%) by VIS and mass spectroscopy, see Scheme 5. Both products were labile towards dilute acid yielding blue products. For characterization of diadinochrome see e.g., Ref. 13.

The conversion of the allenic end group to an acetylenic end group upon treatment with acid has previously been studied,[13] cf. Scheme 5.

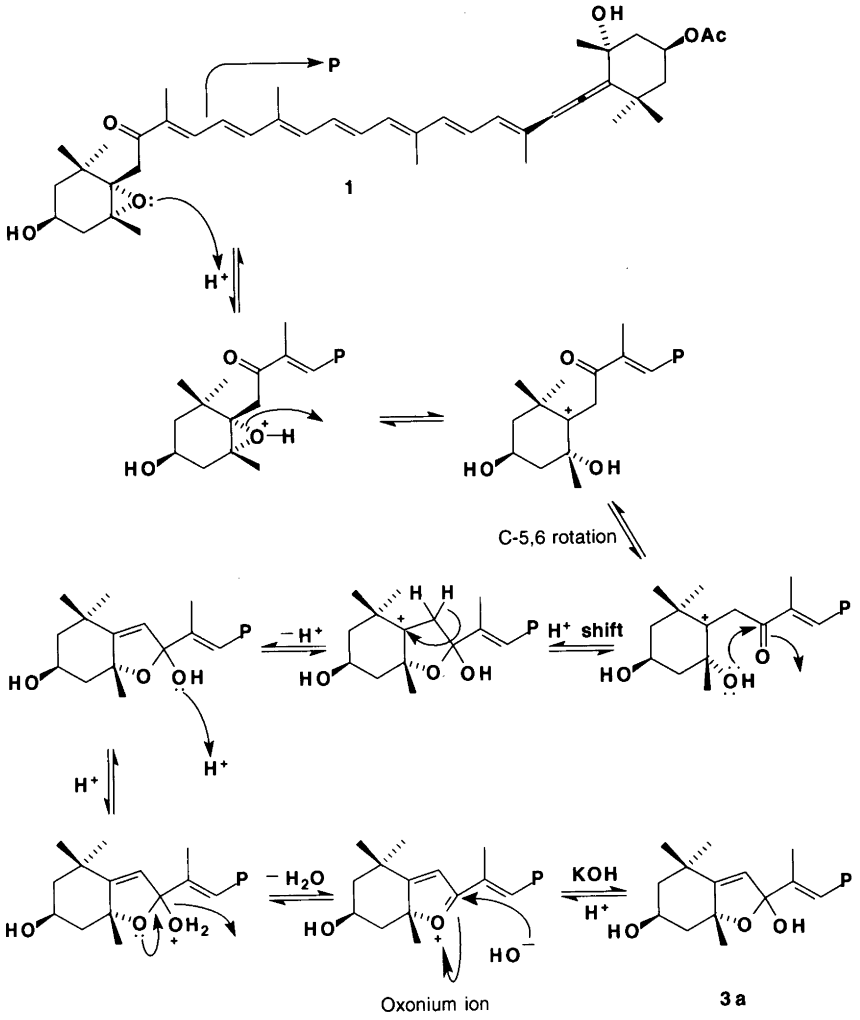
Mass spectroscopy of the hemiketals. Two features in the mass spectra of the hemiketals studied deserve comment. The $M-2$ peak was often stronger than the molecular ion peak, rationalized in Scheme 6. Moreover, the high intensity of the *m/z* 221 and 181 peaks is of diagnostic value. These peaks are ascribed to oxonium ions, see Scheme 6. The suggested mechanism for the formation of the *m/z* 181 ion is supported by a similar fragmentation for isofucoxanthin (**4**).[12]

Further characterization of the blue oxonium ions. ^{1}H NMR spectra of the blue oxonium ions could not be assigned. In comparison with the parent carotenoids several new signals were observed in both the olefinic and methyl regions. Several signals were broad, and part of the olefinic signals was shifted downfield towards 8 ppm. These effects are compatible with a delocalized positive charge in the blue oxonium compounds.

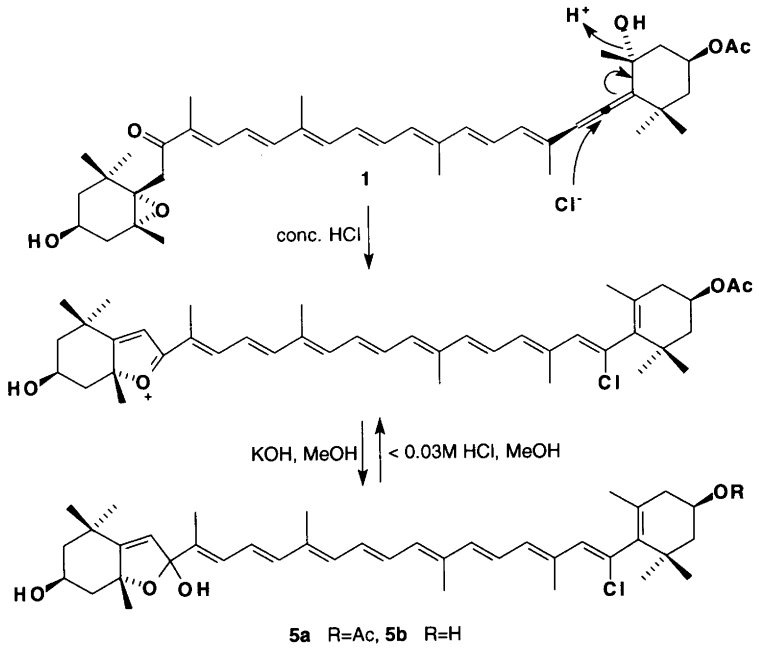
Attempted electrophoresis of fucoxanthin oxonium ions failed. Decomposition occurred within 60 min.

In conclusion, the structural evidence for the novel blue carotenoid oxonium ions rests on VIS absorption spectra and rationalized chemical conversions.

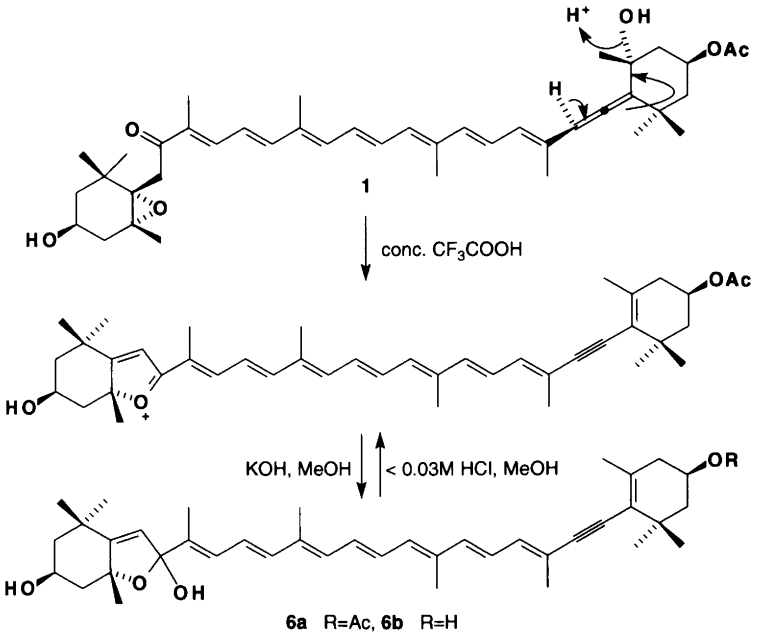
The yields observed in the treatment of fucoxanthin (**1**) with strong organic or mineral acids, judged by the recoveries after the subsequent alkali reactions, were higher than, *a priori*, expected, considering the competing electrophilic addition reactions to the polyene chain resulting in colourless products.



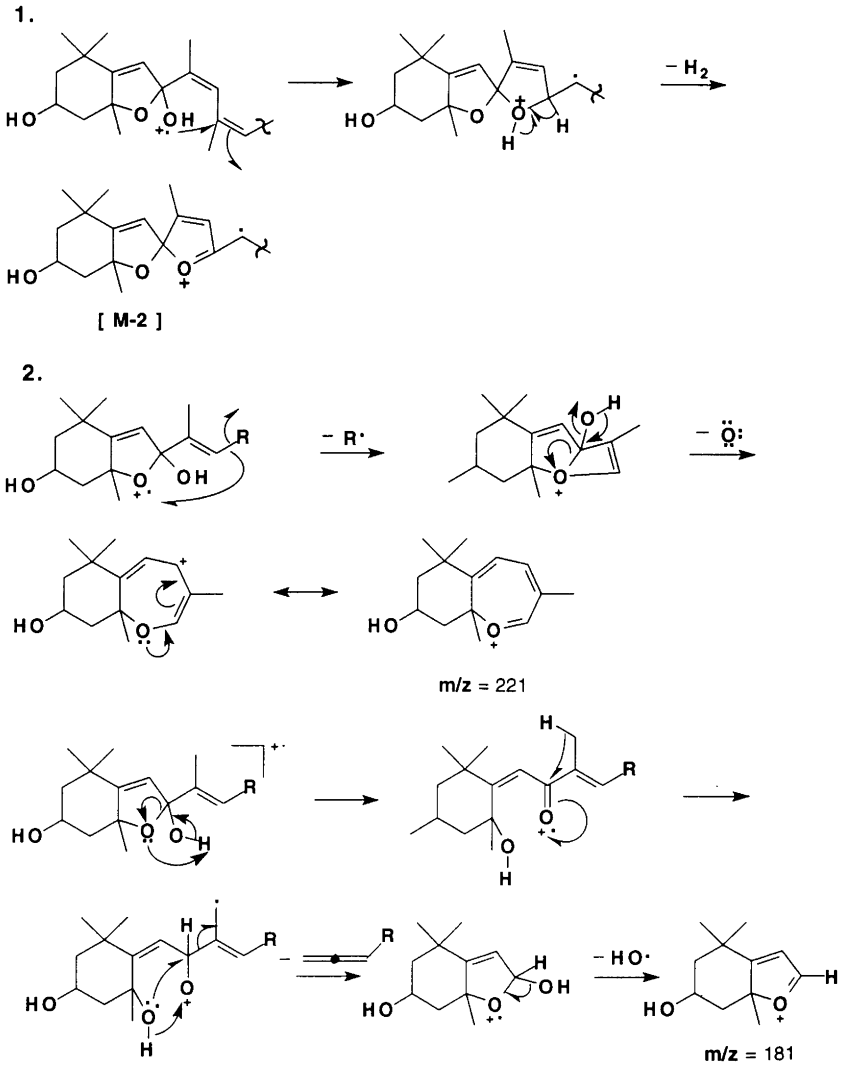
Scheme 3.



Scheme 4.



Scheme 5.



Scheme 6.

Experimental

General methods. General precautions for work with carotenoids were taken. Solvents were of distilled or *p.a.* quality. Diethyl ether was chromatographed through alumina (neutral) and tetrahydrofuran (THF) was distilled over sodium. All reactions were carried out in the dark at room temperature. Reaction mixtures were flushed with N_2 for 20–30 s. Three different TLC systems were used. All systems were based on silica gel 60 G (Merck Art. 7731) as the stationary phase. Acetone–hexane 3:7 (system 1), acetone–hexane 1:1 (system 2) and ethyl acetate (system 3) were used for development.

VIS spectra were recorded on a Perkin Elmer 552 spectrophotometer. Solvents are specified in each case. Spectral fine-structure is expressed as % III/II.[14] Mass spectra were recorded on an AEI 902 instrument with a direct inlet to the ion source at 210–230°C. FT-IR spectra were recorded on a Nicolet 20 SXC spectrophotometer for KBr pellets. ^{1}H NMR spectra were recorded on a 400 MHz Jeol instrument, for solutions in $CDCl_3$.

Acid lability of fucoxanthin methyl ketal 3,5′-dimethyl ether (**2**). To fucoxanthin methyl ketal 3,5′-dimethyl ether (**2**),[12] VIS λ_{max} (THF), 403, 426, 454 nm, % III/II = 78, ca. 15 µg, in dry THF (3 ml) was added HCl (0.3 M solution in MeOH, 1 drop) in a spectrometer cuvette. The solution spontaneously turned from yellow to blue; UV–VIS λ_{max} 720 nm. KOH (10% solution in MeOH, 3 drops) was added and the solution spontaneously turned from blue to yellow; UV–VIS λ_{max} 403, 426, 454 nm, % III/II = 78. The pigment loss in the total sequence was $\leqslant$3% as calculated from the UV–VIS spectra.

Acid lability of fucoxanthinol hemiketal (**3**). Fucoxanthinol hemiketal [**3**,[12] λ_{max} (MeOH) 396, 418, 445 nm, % III/II = 70, 0.41 mg, 6.6×10^{-4} mmol] was dissolved in MeOH (10 ml) and HCl (0.5 M, 2 drops) was added; VIS λ_{max} (HCl–MeOH) 690 nm. After 1 min KOH (5% in MeOH, 1.5 ml) was added. The product was extracted with Et_2O (15 ml), the Et_2O extract was washed with brine (20 ml) and H_2O (2 × 20 ml), and the solvent was evaporated under reduced pressure. Yield 0.39 mg (95%) of **3**; inseparable from authentic **3** on TLC (system 2); VIS λ_{max} (MeOH) 396, 418, 445 nm, % III/II = 70; MS [IP 70 eV; *m/z* (% rel. int.)]: 616 (3, [*M*]), 614 (4, [*M* − 2]), 600 (13, [*M* − 16]), 598 (17, [*M* − 18]), 596 (8, [*M* − 2 − 18]), 582 (12, [*M* − 16 − 18]), 580 (15, [*M* − 18 − 18]), 578 (6, [*M* − 2 − 18 − 18]), 564 (4, [*M* − 16 − 18 − 18]), 562 (5, [*M* − 18 − 18 − 18]), 221 (100), 197 (53), 195 (67), 165 (47).

Treatment of fucoxanthin (**1**) *with conc. CH_3COOH.* Fucoxanthin (**1**, VIS λ_{max} (CH_3COOH) 449 nm, 3.3 mg, 5.02 mmol) was dissolved in conc. CH_3COOH (17 ml). The reaction was monitored by TLC and VIS spectroscopy. After 18 h the reaction mixture was green and after 91 h blue-green, VIS λ_{max} (CH_3COOH) 720 nm. After 91 h H_2O (ca. 100 ml) and Et_2O (ca. 100 ml) were added. The ether phase was washed with H_2O until the hypophase was neutral. The aqueous phase was re-extracted with benzene (ca. 100 ml) and the benzene extract was washed with H_2O until neutral. The organic phases were combined, the solvents evaporated off, and the residue was dissolved in benzene (1 ml) and subjected to preparative TLC (system 1).

Unchanged fucoxanthin (**1**). Yield 0.39 mg (12%, $E^{1\%}_{1\,cm} = 1660$) R_f (system 1) = 0.32, inseparable from authentic **1** (*ex F. serratus*); VIS λ_{max} (acetone) 420, 444, 465 nm; MS [IP 70 eV; *m/z* (% rel. int.)], 658 (42, [*M*]), 642 (16, [*M* − 16]), 640 (47, [*M* − 18]), 624 (14, [*M* − 16 − 18]), 622 (13, [*M* − 18 − 18]), 580 (11, [*M* − 60 − 18]), 562 (9, [*M* − 60 − 18 − 18]), 544 (9, [*M* − 60 − 18 − 18 − 18]), 504 (7), 221 (48), 212 (54), 197 (100).

Fucoxanthin hemiketal (**3a**). Yield 0.43 mg (13%, $E^{1\%}_{1\,cm} = 2400$); R_f (system 1) = 0.20; VIS λ_{max} (acetone) 398, 421, 448 nm, % III/II = 78; MS [IP 70 eV; *m/z* (% rel. int.)], 658 (6, [*M*]), 656 (9, [*M* − 2]), 642 (8, [*M* − 16]), 640 (12, [*M* − 18]), 638 (12, [*M* − 2 − 18]), 624 (8, [*M* − 16 − 18]), 622 (9, [*M* − 18 − 18]), 598 (5, [*M* − 60]), 580 (5, [*M* − 60 − 18]), 562 (7, [*M* − 60 − 18 − 18]), 544 (8, [*M* − 60 − 18 − 18 − 18]), 221 (94), 197 (100), 181 (69). **3a** dissolved in methanol gave a blue solution when treated with dilute HCl.

Three other coloured products were isolated in yields of 6–47 µg. These were unstable and could not be characterized or identified.

Treatment of isofucoxanthin (**4**) *with conc. CH_3COOH.* Isofucoxanthin (**4**,[12] 0.02 mg, 3.0×10^{-5} mmol) was dissolved in conc. CH_3COOH (3 ml) in a spectrometer cuvette. The reaction was monitored by UV–VIS spectroscopy over a period of 75 min. After 5 min the abs. maximum had changed from 450 nm to 462 nm. No further change was observed. No blue product or hemiketal was observed.

Treatment of fucoxanthin (**1**) *with conc. HCl.* Fucoxanthin (**1**, 8.31 mg, 1.26×10^{-2} mmol) was dissolved in conc. HCl (ca. 5 ml) and MeOH (3–4 drops) was added; λ_{max} (HCl–MeOH) 686 nm. After 10 min, KOH in MeOH was added until a colour change from blue to yellow occurred. Water was added and the products extracted with Et_2O. The Et_2O phase was washed with H_2O until neutral. The solvents were evaporated off under reduced pressure and the residue was dissolved in benzene. Two coloured products were isolated by TLC (system 2).

3′-Acetoxy-7′-chloro-5,8-epoxy-5,8-dihydro-β,β-carotene-3,8-diol (**5a**). Yield 1.73 mg (20%, $E^{1\%}_{1\,cm} = 2500$); R_f (system 2) = 0.53; VIS λ_{max} (acetone) 404, 424, 449 nm,

% III/II = 41; MS [IP 70 eV; *m/z* (% rel. int.)]: 678 (2, [*M* + 2]), 676 (5, [*M*]), 674 (7, [*M* − 2]), 660 (13, [*M* − 16], [*M* + 2 − 18]), 658 (8, [*M* − 18]), 656 (5, [*M* − 2 − 18]), 640 (4, [*M* − 36]), 638 (6, [*M* − 2 − 36]), 624 (12, [*M* − 16 − 36]), 622 (13, [*M* − 18 − 36]), 620 (10, [*M* − 2 − 18 − 36]), 594 (17, [*M* − 36 − 18 − 28]), 580 (22, [*M* − 36 − 60]), 564 (15, [*M* − 16 − 36 − 60]), 562 (25, [*M* − 18 − 36 − 60]), 221 (100), 181 (88); IR cm^{-1} 3432 m (OH), 2959–2854 s (CH), 1734 m (acetate), 1460 m, 1386 w (*gem* Me), 1366 m, 1243 m (acetate), 1064 w, 1028 w, 965 s (*trans* CH=CH). **5a** dissolved in methanol yielded a blue solution when treated with dilute HCl.

7'-Chloro-5,8-epoxy-5,8-dihydro-β,β-carotene-3,8,3'-triol (**5b**). Yield 0.56 mg (7%, $E^{1\%}_{1\,cm} = 2500$); R_f (system 2) = 0.44; VIS λ_{max} (acetone) 406, 428, 454 nm, % III/II = 47; MS [IP 70 eV; *m/z* (% rel. int.)]: 636 (2, [*M* + 2]), 634 (5, [*M*]), 632 (6, [*M* − 2]), 620 (7, [*M* + 2 − 16]), 618 (8, [*M* − 16]), 616 (7, [*M* − 18]), 598 (6, [*M* − 36]), 596 (11, [*M* − 2 − 36]), 582 (12, [*M* − 16 − 36]), 580 (18, [*M* − 18 − 36]), 578 (13, [*M* − 2 − 18 − 36]), 564 (7, [*M* − 16 − 18 − 36]), 562 (9, [*M* − 18 − 18 − 36]), 454 (19), 221 (95), 181 (100); IR cm^{-1} 3415 m (OH), 2958–2853 s (CH), 1460 m, 1372 w, (*gem* Me) 1260 w, 1047 m, 1028 m, 965 s (*trans* CH=CH), 810 w (C–Cl), 746 w. **5b** dissolved in methanol provided a blue solution when treated with dilute HCl.

Treatment of fucoxanthin (**1**) *with conc. CF_3COOH.* Fucoxanthin (**1**, 10.51 mg, 1.60×10^{-2} mmol) was dissolved in conc. CF_3COOH (3 ml), VIS λ_{max} (CF_3COOH) 750 nm. After 30 s KOH in MeOH was added until a colour change from blue to yellow occurred. Water was added and the products were extracted with Et_2O. The Et_2O phase was washed with H_2O until neutral. The solvents were evaporated off under reduced pressure and the residue dissolved in benzene. Six coloured products were isolated by TLC (system 2). The three less polar products, all minor, [R_f (system 2) = 0.56–0.71] could not be characterized.

8-Hydroxydiadinochrome-3'-acetate (**6a**). Yield 0.02 mg (0.2%, $E^{1\%}_{1\,cm} = 2500$); R_f (system 2) = 0.50; VIS λ_{max} (acetone) 405, 427, 450 nm, % III/II = 11; MS [IP 70 eV; *m/z* (% rel. int.)]: 640 (3, [*M*]), 622 (5, [*M* − 18]), 580 (15, [*M* − 60]), 564 (12, [*M* − 16 − 60]), 562 (12, [*M* − 18 − 60]), 553 (24), 551 (17), 537 (14), 221 (67), 195 (89), 181 (100). **6a** in methanol provided a blue solution when treated with dilute HCl.

Unknown. Yield 0.36 mg (4%, $E^{1\%}_{1\,cm} = 2500$); R_f (system 2) = 0.46; VIS λ_{max} (acetone) 474 nm; MS [IP 70 eV; *m/z* (% rel. int.)]: 598 (88, [*M*]), 596 (32, [*M* − 2]), 580 (5, [*M* − 18]), 571 (10), 520 (6, [*M* − 78]), 518 (4, [*M* − 80]), 492 (15, [*M* − 106]), 429 (15), 287 (37), 285 (24), 247 (25), 233 (42), 221 (66), 181 (17), 167 (100); IR cm^{-1} 3429 s (OH), 2959–2855 s (CH), 1709 w, 1645 w, 1616 w, 1579 w, 1524 s, 1435 w, 1379 w (*gem* Me), 1215 w, 1158 m, 1062 w, 1029 m, 978 w, 887 w. No blue products were obtained upon treatment with dilute HCl.

8-Hydroxydiadinochrome (**6b**). Yield 0.51 mg (5.3%, $E^{1\%}_{1\,cm} = 2500$); R_f (system 2) = 0.35; VIS λ_{max} (acetone) 405, 426, 451 nm, % III/II = 19; MS [IP 70 eV; *m/z* (% rel. int.)]: 598 (10, [*M*]), 596 (7, [*M* − 2]), 580 (24, [*M* − 18]), 571 (43), 551 (24), 549 (27), 479 (13), 465 (7), 221 (43), 195 (41), 181 (30), 167 (100). **6b** in methanol provided a blue solution when treated with dilute HCl.

^1H NMR of fucoxanthin oxonium ions. Two different procedures were used. (i) Fucoxanthinol hemiketal (**3**) was dissolved in $CDCl_3$ (ca. 1 ml) and conc. CF_3COOD (1 drop) added. A 500 MHz ^1H NMR spectrum was recorded. A number of new broad peaks appeared in both the methyl and olefinic region of the spectrum compared with the spectrum of **3**. Part of the olefinic signals seemed to have been shifted downfield towards 8 ppm. No assignments were possible. (ii) Fucoxanthin (**1**) was dissolved in $CDCl_3$ (ca. 1 ml). Conc. H_2SO_4 was added dropwise to solid NaCl, and the resulting HCl gas was passed through the carotenoid solution until it became dark blue. A 400 MHz ^1H NMR spectrum was recorded. Also in this spectrum, a number of new signals appeared and a downfield shift of some olefinic signals was observed. The two spectra were, however, distinctly different. No assignments were possible. To the NMR sample from procedure (ii) was added 5% KOH in MeOH dropwise until a colour change from blue to yellow was observed. The products were extracted with Et_2O, the Et_2O phase washed with H_2O until neutral pH of the H_2O phase. The solvent was evaporated off under reduced pressure and the residue dissolved in benzene. TLC (system 3) showed three coloured products in a roughly 1:1:1 ratio, all turning blue on the TLC plate when the eluent was allowed to evaporate off, indicating the presence of the 5,8-furano-8-hydroxy moiety in all three products. The three products co-chromatographed with 7'-chloro-5,8-epoxy-5,8-dihydro-β,β-carotene-3,8,3'-triol [**5b**, R_f (system 3) = 0.76], 8-hydroxydiadinochrome [**6b**, R_f (system 3) = 0.58] and fucoxanthinol hemiketal [**3**, R_f (system 3) = 0.30], respectively.

Electrophoresis of fucoxanthin oxonium ions. Four different systems were tested: (i) cellulose acetate with 0.01 M HCl in 0.05 M LiCl, $I = 6$ mA, $U = 180$ V for the first 30 min and $I = 10$ mA, $U = 180$ V for the next 30 min; (ii) Whatman paper No. 1 with 0.01 M HCl in 0.05 M LiCl, $I = 6$ mA, $U = 180$ V for 1 h; (iii) glass-fibre sheets with 0.01 M HCl in 0.05 M LiCl plus 10% isopropyl alcohol, $I = 6$ mA, $U = 80$ V for 1 h; (iv) glass-fibre sheets with 0.02 M HCl, $I = 6$ mA, $U = 60$–70 V for 1 h.

The carotenoid oxonium ions were only partly soluble in the buffer. Addition of isopropyl alcohol to system (iii)

increased the solubility. No migration was observed in any of the systems tested. The blue zone representing the oxonium ion faded and disappeared after 45–60 min.

Acknowledgments. We thank Dr. Helge Kjøsen for discussions on the mass spectroscopic fragmentation of the hemiketals. A research grant from Hoffmann-La Roche, Basel, is gratefully acknowledged.

References

1. Hertzberg, S., Liaaen-Jensen, S., Enzell, C. R. and Francis, G. W. *Acta Chem. Scand. 23* (1969) 3290.
2. Zagalsky, P. *Oceanis 9* (1983) 73.
3. Griffiths, J., Ed., *Developments in the Chemistry and Technology of Organic Dyes*, Critical Reports on Appl. Chem. Vol. 7, Blackwell, Oxford 1984.
4. Harborne, J. B., Mabry, T. J. and Mabry, H. Eds., *The Flavanoids*, Chapman and Hall, London 1975.
5. Baldas, J., Porter, Q. N., Cholnoky, L., Szabolcs, J. and Weedon, B. C. L. *Chem. Commun.* (1966) 852.
6. Karrer, D. and Jucker, E. *Carotinoide*, Birkhäuser, Basel 1948.
7. Yamamoto, H. Y., Chichester, C. O. and Nakayama, T. O. M. *Anal. Chem. 33* (1961) 1792.
8. Monties, B. and Costes, C. *C. R. Acad. Sci. Paris* (1968) 481.
9. Haugan, J. A. and Liaaen-Jensen, S. *Abstr. 9th Int. IUPAC Carotenoid Symp.*, Kyoto 1990, P214.
10. Jensen, A. *Acta Chem. Scand. 20* (1966) 1728.
11. Bonnett, R., Mallams, A. K., Spark, A. A., Tee, J. L., Weedon, B. C. L. and McCormick, A. *J. Chem. Soc. C* (1969) 429.
12. Haugan, J. A., Englert, G. and Liaaen-Jensen, S. *Acta Chem. Scand. 46* (1992) 614.
13. Johansen, J. E. and Liaaen-Jensen, S. *Acta Chem. Scand. 28* (1974) 949.
14. Ke, B., Imsgard, F., Kjøsen, H. and Liaaen-Jensen, S. *Biochim. Biophys. Acta 210* (1970) 139.

Received May 5, 1993.