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Blue Carotenoids. Part 2.* The Chemistry of the Classical Colour Reaction of Common Carotenoid 5,6-Epoxides with Acid

Jarle André Haugan and Synnøve Liaaen-Jensen

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

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The blue colour reaction of common carotenoid 5,6-epoxides has been studied. Examined were the reactions of (i) the monoepoxide neoxanthin with conc. HCl, CF_3COOH or triphenylcarbenium tetrafluoroborate, (ii) the furanoxide diadinochrome with CF_3COOH or triphenylcarbenium tetrafluoroborate and (iii) the diepoxide violaxanthin with conc. HCl or CF_3COOH.

The blue products were identified as carotenoid oxonium ions, providing previously undescribed cyclic carotenoid hemiketals upon treatment with KOH.

The reactions observed have been rationalized mechanistically. Carotenoid furanoxides are intermediates in the reaction of carotenoid 5,6-epoxides to give the blue oxonium ions. The elimination of a hydride ion from C-8 in the furanoxide is the key reaction in the formation of the blue oxonium ions.

The strongly blue colour obtained by treatment of common 5,6-epoxidic carotenoids (end group A, Scheme 1) with acids was observed long ago. Thus the reaction of the diepoxide violaxanthin (**1**) with mineral as well as organic acids was reported in 1931 before its structure was established.[1] Subsequently the blue colour reaction of several epoxidic carotenoids with aq. HCl was observed,[2] including the monoepoxides antheraxanthin (**2**), taraxanthin (= lutein epoxide, **3**)[3] and trollixanthin (= neoxanthin, **4**).[4] Whereas weak acidic conditions promote the conversion of 5,6-epoxides (end group **A**) into 5,8-furanoxides (end group **B**), strong acids are required for the formation of the blue products from the 5,8-furanoxides.[2,5] Lewis acids such as $HgCl_2$, in a solid-state reaction, also proved efficient in experiments with violaxanthin (**1**) and neoxanthin (**4**).[6] Moreover, $AlCl_3$ caused the formation of blue products from epoxidic (**1**) and furanoid (**5**) carotenoids.[7]

Monties and Costes[7] first advanced the hypothesis of blue oxonium ions. Evidence for the existence of blue carotenoid oxonium ions **G** (Scheme 1), obtained from fucoxanthin (**G**, end group **F**) and derivatives thereof, has recently been presented in Part 1.[8]

In this paper further studies on common epoxidic carotenoids (end group **A**) with mineral and organic acids are described, including characterization and identification of novel blue carotenoid oxonium ions and yellow cyclic hemiketals formed from these oxonium ions. Preliminary results have been reported.[9]

Results and discussion

Reactions of neoxanthin (**4**). The following reactions were studied of the allenic, monoepoxide neoxanthin (**4**) (i) with conc. HCl and (ii) of **4** and of its furanoid rearrangement product neochrome (**7**) with conc. CF_3COOH, and (iii) of **4** and **7**-diacetate with triphenylcarbenium tetrafluoroborate (TPCTFB).

(i) When neoxanthin (**4**) in diethyl ether was treated with conc. HCl a bathochromic shift of λ_{max} from 437 nm to 680 nm was recorded. The blue product formed was partly water soluble.

(ii) Neoxanthin (**4**) provided, spontaneously, a blue solution when dissolved in conc. CF_3COOH. After treatment with KOH until a colour change from blue to yellow was observed, two coloured products were isolated. The major product (12% yield) had VIS and mass spectral properties identical with C-8 epimeric 8-hydroxydiadinochromes (**8a**, Scheme 2), previously obtained and characterized by VIS and MS data after similar treatments of fucoxanthin (**6**).[8] The product hemiketal **8a** turned green on the silica TLC plate and blue upon treatment with dilute HCl in methanol. From these findings, the blue product was identified as an acetylenic carotenoid oxonium ion, see Scheme 2.

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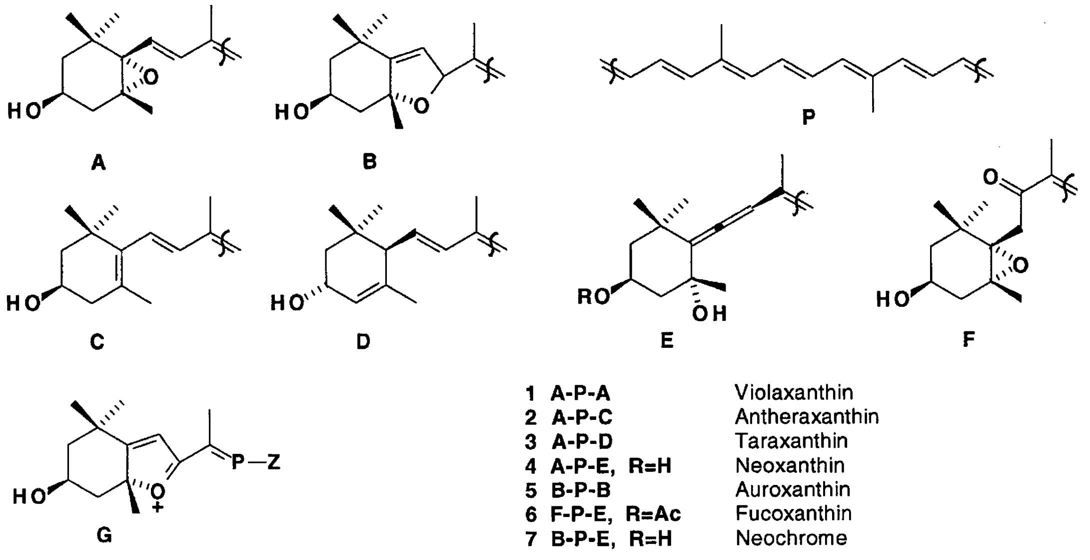
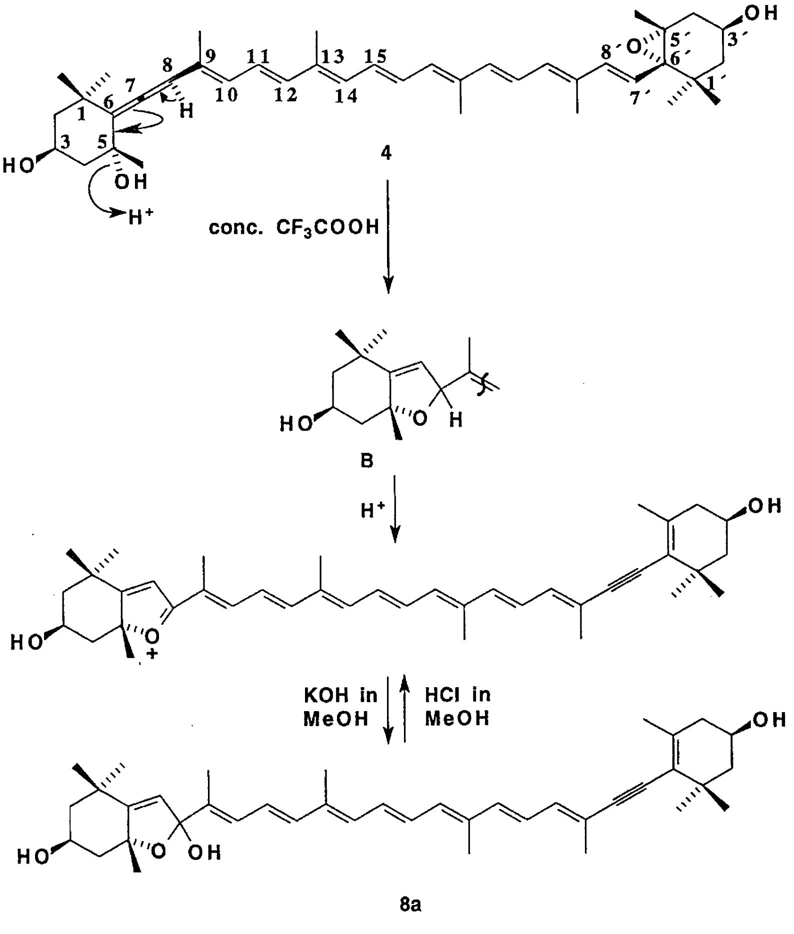


1	**A-P-A**	Violaxanthin
2	**A-P-C**	Antheraxanthin
3	**A-P-D**	Taraxanthin
4	**A-P-E, R=H**	Neoxanthin
5	**B-P-B**	Auroxanthin
6	**F-P-E, R=Ac**	Fucoxanthin
7	**B-P-E, R=H**	Neochrome

Z, cfr. schemes 2, 4-6

Scheme 1.

Three different reaction mechanisms were considered for the formation of blue oxonium ions from common carotenoid 5,6-epoxides. Furanoid intermediates (end group **B**, Scheme 2) were assumed and supported by separate experiments with neochrome (**7**) diacetate, see below: (A) disproportionation with simultaneous oxidation/



Scheme 2.

reduction of two furanoid molecules, (B) oxidation at C-8 by molecular oxygen, generating a good leaving group (^-OH or ^-OOH) and (C) hydride as a rare leaving group. Alternative (A) was ruled out by the absence of formation of coloured products other than the observed hemiketal after alkali treatment of the blue oxonium ion.

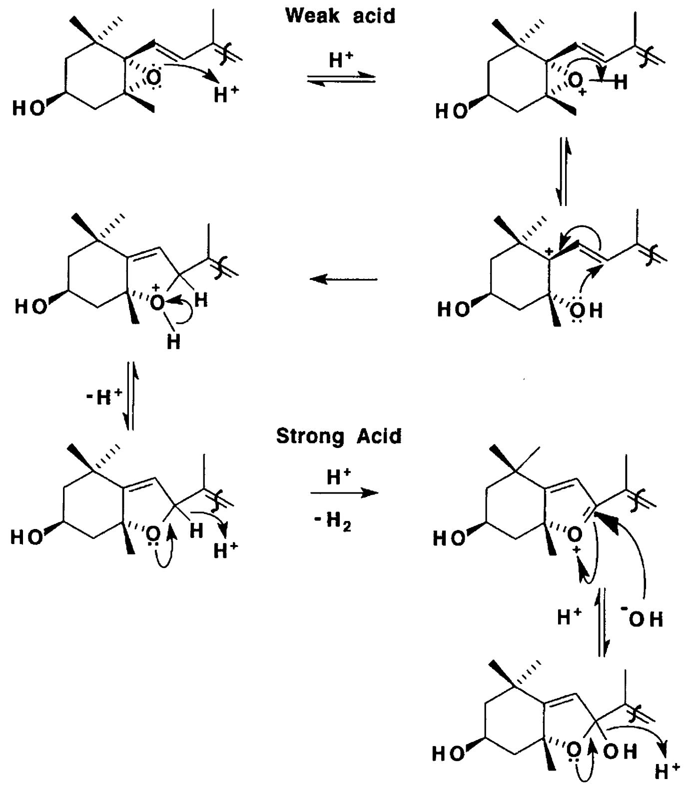
In order to reveal any role played by oxygen in the formation of the oxonium ions two experiments were performed. A sample of neoxanthin (**4**) in CH_2Cl_2 was continuously flushed with N_2 before, during and after the addition of CF_3COOH. The formation of the blue oxonium ions was not prevented. In the second experiment freshly distilled CF_3COOH and a sample of neochrome (**7**) were mixed after repeated removal of oxygen by cooling to − 196 °C, followed by reduction of the pressure to 5.5×10^{-5} atm. A carotenoid:oxygen ratio of 92:1 was calculated and should have resulted in a ca. 1% yield of the blue oxonium ion if oxygen participated in the reaction. However, the yield observed of 8-hydroxydiadinochromes (**8a**) after subsequent alkali treatment was 15%. It was inferred that a reaction mechanism involving hydride ion as leaving group was the most likely alternative, see Scheme 3.

Additional evidence was sought. It is known that triphenylcarbenium tetrafluoroborate (TPCTFB) is a good hydride acceptor.[10] Treatment of neochrome (**7**) diacetate with TPCTFB in dry ether provided the blue oxonium ion, which was converted into the corresponding C-8 epimeric hemiketal by treatment with KOH, see Scheme 4.

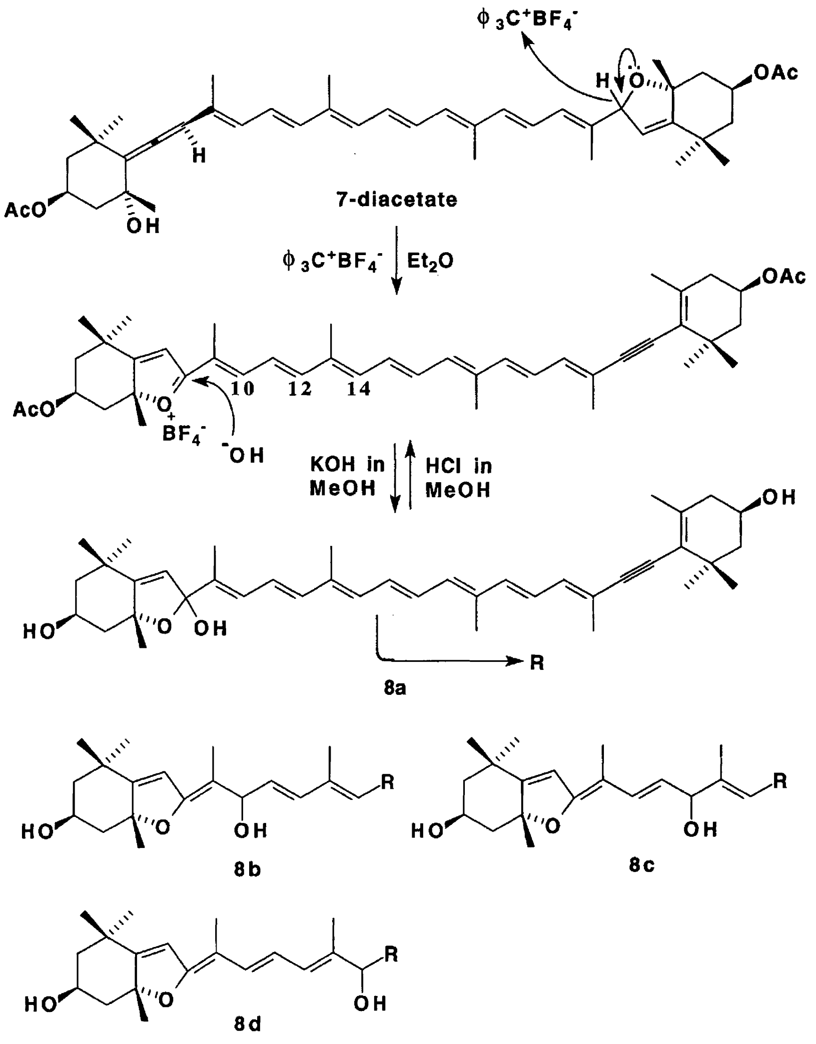
The VIS spectrum of the yellow alkali-treated solution indicated a mixture of products with nonaene-, octaene-, heptaene- and hexaene-like chromophores, yield 8%. The mass spectral fragmentation of the product mixture was compatible with the presence of 8-hydroxydiadinochrome (**8a**). In Scheme 4 the observed product mixture is rationalized in terms of a tight ion pair between the carotenoid oxonium ion and the space-requiring BF_4 anion, partly preventing nucleophilic attack at C-8 due to steric hindrance, and consequently resulting in alternative conjugate addition at C-10, C-12 and C-14, providing **8b,c, d**, respectively. In favour of this interpretation and in contrast with the C-8 hemiketal products, the short chromophore, non-hemiketal products (**8b,c,d**) did not provide blue oxonium ions upon treatment with weak acid.

Treatment of neoxanthin (**4**) with TPCTFB also provided blue oxonium products, converted into hydroxydiadinochromes (**8a,b,c,d**, 5% yield) by alkali treatment. Diadinochrome (**9**, 9%) was also isolated during this reaction sequence, confirming the conversion of allenic to acetylenic carotenoids by TPCTFB.

Reactions of diadinochrome (9). The intermediate nature of diadinochrome (**9**, Scheme 5) was demonstrated by its further conversion by TPCTFB into a blue oxonium



Scheme 3.



Scheme 4.

product. Again alkali treatment of the blue oxonium ion gave a product mixture of hydroxydiadinochromes (**8a–d**, 6%), characterized by VIS and mass spectroscopy, Scheme 5. Coloured products of chromophores shorter than **8a** were ascribed to conjugate addition. The absence of a hemiketal function in these products (**8b,c,d**) was supported by their stability towards dilute acid.

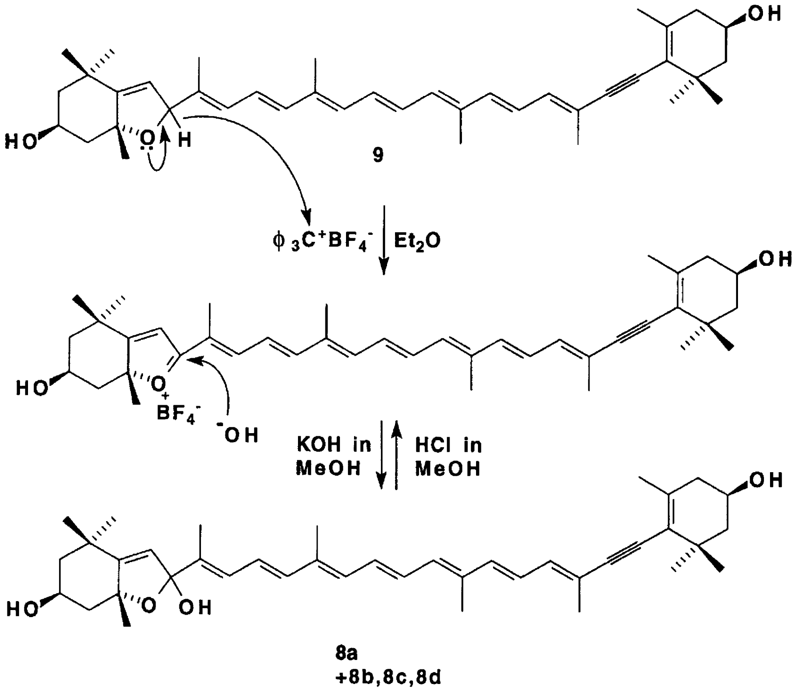
Diadinochrome (**9**) also afforded blue oxonium ions upon treatment with conc. CF_3COOH. After alkali treatment of the blue oxonium ions two coloured products with $\lambda_{max} = 446$ nm were isolated. These products reverted to blue oxonium ions with weak acid, characteristic of hemiketal structures, but could not be further characterized or identified.

*Reactions of violaxanthin (***1***).* The diepoxide violaxanthin (**1**) was kept in conc. HCl for 30 min until a deep blue colour was observed, accompanied by a bathochromic shift of λ_{max} to 644 nm. Subsequent treatment with KOH in MeOH resulted in a colour change from blue to yellow. Two minor products with chromophores longer than violaxanthin (**1**) were not identified. The two major products were identified as epimer mixtures of 8-hydroxyluteoxanthin (**10**, 9% yield) and 8,8′-dihydroxyauroxanthin (**11**, 10%) from VIS and MS data, relative polarity and reaction with dilute mineral acid to blue oxonium ions, see Scheme 6.

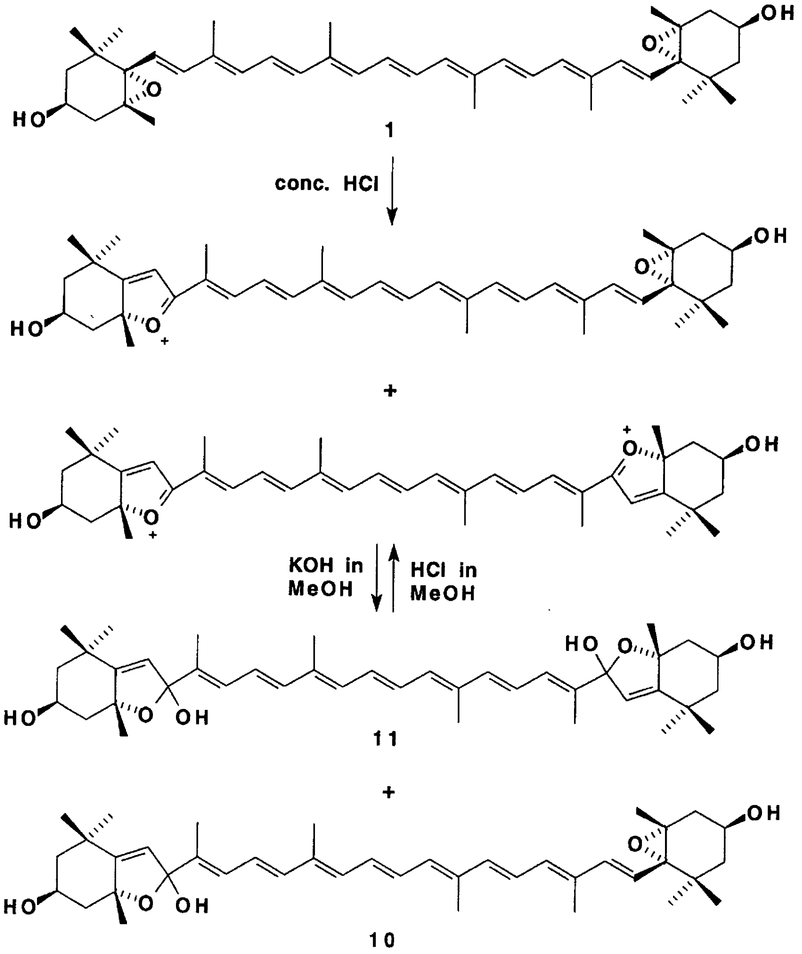
Violaxanthin (**1**) is readily transformed into its difuranoid rearrangement products upon treatment with weak acid.[11] It was therefore unexpected that one unrearranged 5,6-epoxy function was retained in **10**, obviously derived from a mono-oxonium ion. The isolation of the dihemiketal **11** supported the existence of a dioxonium ion as well.

Treatment of violaxanthin (**1**) with conc. CF_3COOH resulted in a faster colour change to blue. However, subsequent alkali treatment provided only one yellow, unidentified product in low yield.

^{1}H NMR spectroscopy of oxonium ions. As previously observed for fucoxanthin (**6**)-derived oxonium ions,[8] the ^{1}H NMR spectra of blue oxonium ions obtained from vio-



Scheme 5.



Scheme 6.

laxanthin (**1**, Scheme 6) and neoxanthin (**4**, Scheme 2), contained several new, broad peaks. Part of the olefinic signals seemed to have shifted downfield relative to the parent carotenoids. No assignments could be made, but the results are compatible with the electron delocalized, ionic structures.

Conclusions. It is concluded that the blue colour observed upon treatment of common 5,6-epoxidic or 5,8-furanoid carotenoids with strong acid is caused by electron-delocalized oxonium ions.

Blue colour reactions with acid have also been reported in the vitamin A series,[12–15] including the well known Carr and Price reaction.[13] No epoxidic functions are present in these substrates, and the products of retinoic acid have been rationalized as carbocations.[15]

Experimental

Materials. Violaxanthin (**1**) and neoxanthin (**4**, mainly as 9′-*Z*, given as all-*E* in Schemes 2 and 4) were isolated from spinach (*Spinacea oleracea*).[16] Diadinoxanthin, isolated from dinoflagellates,[17] were used for the preparation of authentic diadinochrome (**9**) by treatment with weak mineral acid.

Methods. These were as described in Part 1.[8]

*Treatment of neoxanthin (*__4__*) with conc. HCl.* Neoxanthin [**4**, 100 μg, 1.7×10^{-4} mmol VIS λ_{max} (acetone) 414, 438, 466 nm, % III/II = 83; λ_{max} (EtO_2) 414, 437, 465 nm, % III/II = 82] was dissolved in Et_2O. Conc. HCl (ca. 5 ml) was added and a UV–VIS spectrum was recorded of the blue aq. phase, UV–VIS λ_{max} (conc. HCl) 680 nm. The Et_2O phase was light yellow, VIS λ_{max} (Et_2O) 422, 447, 474 nm. The VIS spectrum of the Et_2O phase was broad and indicative of decomposed carotenoid.

*Treatment of neoxanthin (*__4__*) with conc. CF_3COOH.* Neoxanthin (**4**, 0.65 mg, 1.1×10^{-3} mmol) was dissolved in conc. CF_3COOH (3 ml), VIS λ_{max} (CF_3COOH) 745 nm. After 30 s. 10% KOH in MeOH was added until a colour change from blue to yellow was observed. The products were extracted with Et_2O and the Et_2O phase was washed with H_2O until the H_2O phase became neutral. The solvents were evaporated off and the residue was dissolved in benzene. Two coloured products were isolated by preparative TLC (system 1).

Unknown 1. Minor product; R_f (system 1) = 0.43; VIS λ_{max} (acetone) 423, 447, 472 nm. No mass spectrum could be recorded.

*8-Hydroxydiadinochrome (*__8a__*).* Yield 75 μg (12%, $E^{1\%}_{1\,cm}$ = 2500); R_f (system 1) = 0.11; VIS λ_{max} (acetone) 391, 404, 426, 449 nm, % III/II = 10; MS [IP 70 eV; *m/z* (% rel.int.)]: 598 (5, [*M*]), 580 (6, [*M* − 18]), 564 (3, [*M* − 16 − 18]), 562 (3, [*M* − 18 − 18]), 533 (7), 221 (18), 209 (24), 195 (28), 181 (30), 167 (100). IR (KBr) cm^{-1} 3424 m (OH), 2959–2852 s (CH), 2171 w ($C\equiv C$), 1458 w, 1374 w (*gem*, Me), 1261 w, 1055 w, 1026 w, 966 w. **8a** in methanol provided a blue solution when treated with dilute HCl. The C-8 epimeric diadinochromes (**8a**) isolated in the present work were identical (TLC, VIS, MS) with **8a** obtained from fucoxanthins (**6**) in Part 1.[8] The general instability of the hemiketals including **8a** prevented the characterization by ^{1}NMR spectroscopy.

In another experiment neoxanthin (**4**, ca. 100 μg) was dissolved in dry CH_2Cl_2 (ca. 15 ml) and N_2 was passed through the solution. After 40 min CF_3COOH (3–4 ml) was added to the neoxanthin solution, and a yellow to blue colour reaction was observed.

*Treatment of neochrome (*__7__*) with conc. CF_3COOH at 5.5×10^{-5} atm.* All solvents were removed from a sample of neochrome [**7**, 0.58 mg, 9.7×10^{-4} mmol, VIS λ_{max} (acetone) 398, 422, 450 nm, % III/II = 88] on a vacuum line at 5.5×10^{-5} atm. Freshly distilled conc. CF_3COOH was cooled to −196 °C with N_2 (liq.) after which the pressure was reduced to 5.5×10^{-5} atm on the same vacuum line. Then N_2 (gas, 99%) was added until atmospheric pressure was reached. The solid CF_3COOH was allowed to melt, then cooled to −196 °C, and the procedure was repeated two more times. After the fourth evacuation CF_3COOH was allowed to distil and dissolve the carotenoid at −10 to 0 °C. A colour change from yellow to blue was immediately observed and N_2 (gas) was added until atmospheric pressure was reached. To the blue reaction mixture was added 5% KOH in MeOH until a colour change from blue to yellow was observed.

Water was added and the product extracted with Et_2O. The Et_2O phase was washed with water until neutral. Solvents were evaporated off at reduced pressure and the residue was dissolved in benzene. The hemiketal was isolated by TLC (system 1).

8-Hydroxydiadinochrome (8a). Yield 0.087 mg (15%, $E^{1\%}_{1\,cm}$ = 2500). Spectral and chemical properties were as given above.

*Treatment of neochrome (*__7__*) diacetate with CPh_3BF_4.* Neochrome (**7**) diacetate [0.41 mg, 6×10^{-4} mmol, VIS λ_{max} (acetone), 400, 421, 447 nm, % III/II = 70] was dissolved in dry Et_2O (50 ml), and CPh_3BF_4 (0.04 g, 0.12 mmol) was added. The reaction mixture was flushed with N_2 and the reaction was monitored by VIS spectroscopy. After 24 h the reaction mixture was blue, VIS λ_{max} (Et_2O) 675 nm. A solution of 5% KOH in MeOH was added until a colour change from blue to yellow was observed. Water was added and the products were extracted with Et_2O. The Et_2O phase was washed with brine and H_2O until neutral, the solvents were evaporated off at reduced pressure and the residue was dissolved in benzene (1 ml). One coloured product was isolated by TLC (system 1).

Hydroxydiadinochromes (**8a–d**). Yield 29.4 μg (8%, $E^{1\%}_{1\,\mathrm{cm}}$2500); R_f (system 1) = 0.04; VIS λ_{max} (acetone) 340, 362, 384, 405, 425, 448 nm; MS [IP 70 eV; *m/z* (% rel.int.)]: 598 (4, [*M*]), 596 (6, [*M* − 2]), 580 (29, [*M* − 18]), 578 (10, [*M* − 2 − 18]), 562 (7, [*M* − 18 − 18]), 553 (26), 243 (74), 221 (38), 195 (71), 181 (50), 167 (79), 165 (100). An aliquot of hydroxydiadinochromes (**8**) was dissolved in MeOH in a UV cuvette and a VIS spectrum was recorded, VIS λ_{max} (MeOH) 384, 402, 422, 444 nm. 0.3 M HCl (1 drop) was added and a colour change from yellow to blue was observed; VIS λ_{max} (MeOH) 346, 354, 378, 402, 422, 670 nm. 5% KOH in MeOH was added until a colour change from blue to yellow occurred, VIS λ_{max} (MeOH) 340, 362, 384, 402, 444 nm. The pigment recovery was > 95%.

Treatment of neoxanthin (4) with CPh_3BF_4. Neoxanthin (**4**, 0.27 mg, 4.5×10^{-4} mmol) was treated with CPh_3BF_4 (0.04 mg, 0.12 mmol) followed by KOH as described for neochrome **7** diacetate above. Two coloured products were isolated by TLC (system 1).

Diadinochrome (**9**). Yield 23 μg (9%, $E^{1\%}_{1\,\mathrm{cm}} = 2500$); R_f (system 1) = 0.13; inseparable from authentic **9** (*ex Amphindinium carterae*);[17] VIS λ_{max} (acetone) 405, 427, 454 nm, % III/II = 48; MS [IP 70 eV; *m/z* (%rel.int.)] 582 (68, [*M*]), 580 (27, [*M* − 2]), 566 (4, [*M* − 16]), 564 (7, [*M* − 18]), 502 (18, [*M* − 80]), 243 (46), 221 (100), 181 (50), 165 (62).

Hydroxydiadinochromes (**8a–d**). Yield 13 μg (5%, $E^{1\%}_{1\,\mathrm{cm}}$ = 2500). Spectral and chemical properties were as given above.

Treatment of diadinochrome (9) with CPh_3BF_4. Diadinochrome (**9**, 0.38 mg, 6.5×10^{-4} mmol) obtained from diadinoxanthin (0.68 mg, 1.2×10^{-3} mmol) by treatment with 0.03 M HCl in MeOH, was treated with CPh_3BF_4 (0.043 g, 0.13 mmol) as described for neochrome **7** diacetate above. TLC (system 3) indicated the presence four coloured products, three minor yellow and the more polar major green–yellow hydroxydiadinochrome (**8a–d**). The less polar yellow products were isolated together; yield 41 μg ($E^{1\%}_{1\,\mathrm{cm}} = 2500$); R_f 0.54–0.94 (system 3); VIS λ_{max} (acetone) 385, 407, 426 nm. No colour change was observed when 0.3 M HCl (5 drops) was added to a solution of these yellow compounds dissolved in MeOH.

Hydroxydiadinochromes (**8a–d**). Yield 24 μg (6%, $E^{1\%}_{1\,\mathrm{cm}}$ = 2500). Spectral and chemical properties were as given above.

Treatment of diadinochrome (9) with conc. CF_3COOH. Diadinochrome (**9**, 100 μg, 1.7×10^{-4} mmol) was dissolved in conc. CF_3COOH (ca. 2 ml). The mixture immediately turned blue, VIS λ_{max} (CF_3COOH) 730 nm. After 2 min 5% KOH in MeOH was added until a colour reaction from blue to yellow was observed. Water was added, and the products were extracted with Et_2O. The Et_2O phase was washed with H_2O and brine until neutral, the solvents were evaporated off at reduced pressure and the residue dissolved in benzene. Two coloured products were isolated by TLC (system 1).

Unknown 1. Yield 5 μg ($E^{1\%}_{1\,\mathrm{cm}} = 2500$); R_f (system 1) = 0.30 VIS λ_{max} (acetone), 420, 446, 472 nm, % III/II = 3; No MS could be recorded.

Unknown 2. Yield 1 μg ($E^{1\%}_{1\,\mathrm{cm}} = 2500$); R_f (system 1) = 0.24; VIS λ_{max} (acetone) 446, 470 nm, % III/II = 8; No MS could be recorded.

Unknowns 1 and 2 both afforded blue solutions when dissolved in MeOH and treated with dilute HCl.

Treatment of violaxanthin (1) with conc. HCl. Violaxanthin [**1**, 1.9 mg, 3.2×10^{-3} mmol, VIS λ_{max} (acetone) 415, 438, 467 nm, % III/II = 79] was dissolved in conc. HCl (ca. 10 ml). After 30 min, the reaction mixture became deep blue, VIS λ_{max} (conc. HCl) 644 nm. A solution of 5% KOH in MeOH was added until a colour change from blue to yellow was observed, H_2O was added, and the products were extracted with Et_2O. The Et_2O phase was washed with H_2O and brine, solvents were removed at reduced pressure and the residue was dissolved in benzene (ca. 1 ml). Four products were isolated by TLC (system 1). The two less polar, minor products, unknown 3 and 4, were yellow on the TLC sheet, the two major products green.

Unknown 3. Yield 78 μg ($E^{1\%}_{1\,\mathrm{cm}} = 2500$); R_f (system 1) = 0.99; VIS λ_{max} (acetone) 455, 477 nm, % III/II = 17. MS not informative.

Unknown 4. Yield 46 μg ($E^{1\%}_{1\,\mathrm{cm}} = 2500$); R_f (system 1) = 0.72; VIS λ_{max} (acetone) 427, 450, 473 nm; MS [IP 70 eV; *m/z* (% rel.int.)] 548 [*M*?].

8-Hydroxyluteoxanthin (**10**). Yield 166 μg (9%, $E^{1\%}_{1\,\mathrm{cm}}$ = 2500). R_f (system 1) = 0.41; VIS λ_{max} (acetone) 405, 427, 451 nm; MS [IP 70 eV; *m/z* (% rel.int.)] 616 (4, [*M*]), 600 (5, [*M* − 16]), 598 (9, [*M* − 18]), 582 (26, [*M* − 16 − 18]), 580 (17, [*M* − 18 − 18]), 504 (10), 479 (26), 465 (5), 434 (27), 407 (21), 221 (100), 195 (69), 181 (50). **10** in MeOH provided a blue solution when treated with dilute HCl.

8,8′-Dihydroxyauroxanthin (**11**). Yield 205 μg (10% $E^{1\%}_{1\,\mathrm{cm}} = 2500$); R_f (system 1) = 0.26; VIS λ_{max} (acetone) 381, 402, 450 nm, % III/II = 100; MS [IP 70 eV; *m/z* (% rel.int.)] 632 (1, [*M*]), 614 (6, [*M* − 18]), 600 (41, [*M* − 32]), 598 (8, [*M* − 18 − 16]), 584 (16, [*M* − 48]), 568 (25, [*M* − 64]), 520 (10), 504 (10), 221 (100), 181 (42). **11** in MeOH provided a blue solution when treated with dilute HCl.

Treatment of violaxanthin (1) with conc. CF_3COOH. Violaxanthin (**1**, 3 mg, 5×10^{-3} mmol) was dissolved in conc. CF_3COOH (ca. 5 ml). The solution immediately turned blue, and the reaction was monitored by VIS spectroscopy. After 5 min the main absorbance band was at 700 nm, and after 2 h at 687 nm. After 2 h 5% KOH in MeOH was added until a colour change from blue to yellow was observed. Water was added and the products were extracted with Et_2O. The Et_2O phase was washed with H_2O and brine, solvents evaporated and the residue dissolved in benzene. One coloured product was isolated by TLC (system 1).

Unknown 5. Yield 84 μg ($E^{1\%}_{1\,cm} = 2500$); R_f (system 1) = 0.19; VIS λ_{max} (acetone), 407, 428, 452 nm. The product decomposed quickly.

*^1H NMR spectroscopy of oxonium ions obtained from neoxanthin (**4**) and violaxanthin (**1**).* 3 mg samples of neoxanthin (**4**) and of violaxanthin (**1**) were dissolved in CF_3COOD. The solutions spontaneously turned blue. 500 MHz ^1H NMR spectra were recorded. As in the case of fucoxanthin oxonium ions,[8] a number of new broad peaks appeared. Parts of the olefinic signals seemed to have shifted downfield, but no assignments could be made.

*Treatment of carotenoid hemiketals (**8a**, **10**, **11**) with acid followed by base.* All isolated carotenoid hemiketals (**8a**, **10**, **11**) afforded blue oxonium ions when treated with traces of acid. The hemiketals were recovered after treatment of the oxonium ions with base (KOH in MeOH) until a colour change from blue to yellow was observed. The pigments recovery was 95–100%.

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