Animal Carotenoids. 32.* Carotenoids of *Mytilus edulis* (Edible Mussel)

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Nineteen different carotenoids have been isolated from various harvests of *Mytilus edulis* (edible mussels). Besides β,β-carotene (occasional) these were ten acetylenic C_{40}-carotenoids: crocoxanthin-like, anhydro-amarouciaxanthin B, 19′-hexanoyloxyisomytiloxanthin, isomytiloxanthin, alloxanthin, mytiloxanthin, amarouciaxanthin B-like, halocynthiaxanthin, pectenol-like and heteroxanthin; two acetylenic C_{37}-carotenoids: pyrrhoxanthinol and hydrato-pyrrhoxanthinol; four C_{40}-skeletal allenic carotenoids: 19′-hexanoyloxyfucoxanthin, fucoxanthin, 19′-hexanoyloxyfucoxanthinol and fucoxanthinol; two C_{37}-skeletal allenic carotenoids: peridinin and peridininol.

Anhydro-amarouciaxanthin B, 19′-hexanoyloxyisomytiloxanthin (minor occasional) and hydrato-pyrrhoxanthinol constitute new carotenoids.

The characterization comprised TLC and HPLC behaviour, VIS spectrophotometry, ^{1}H NMR (including full assignment of three new carotenoid end groups), CD and mass spectra, as well as chemical derivatizations. Stereochemical considerations are discussed.

Major carotenoids of the edible mussel *Mytilus edulis* are the acetylenic alloxanthin (**6**),[1] mytiloxanthin (**7**)[2,3] and isomytiloxanthin (**5**).[3] The chemical and spectroscopic evidence for these structures has recently been compiled.[4]

The purpose of the present project was (i) to carry out a qualitative and quantitative analysis of the total carotenoid complement of *M. edulis* by modern methods, and (ii) to study the resorption and metabolic transformations of dietary carotenoids in the edible mussel. In this paper we report the physical and chemical studies on which the identification of nineteen different carotenoids from *M. edulis* is based. The quantitative distribution of these carotenoids in *M. edulis* for various harvests, and the resorption and metabolic transformation of dietary carotenoids in the edible mussel are published separately.[5]

Results and discussion

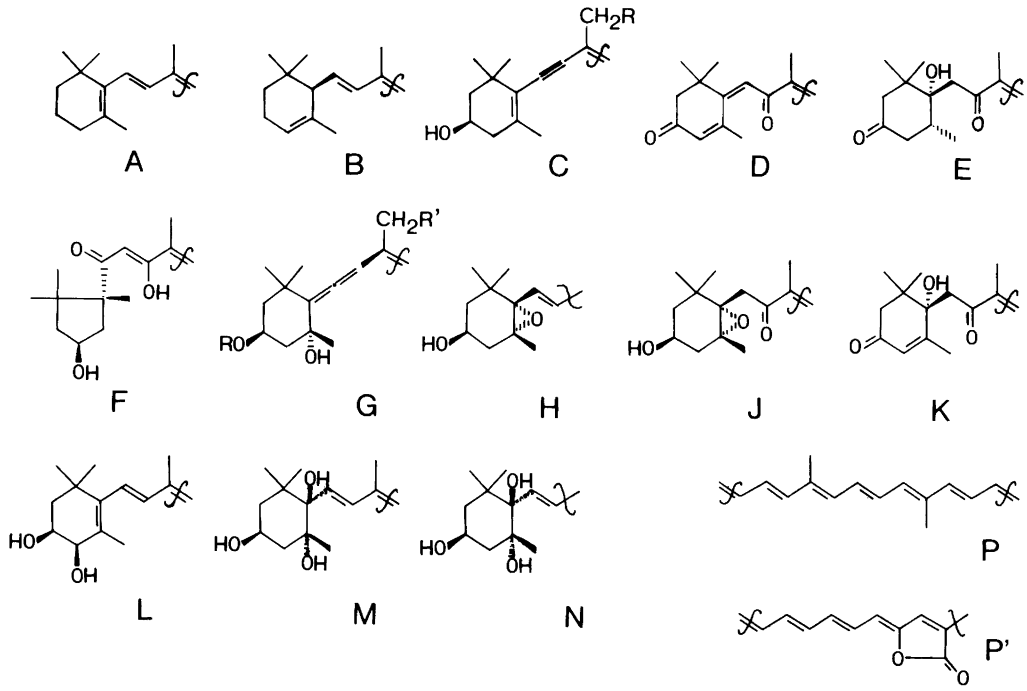
Whereas a grouping according to common structural features is made in conjunction with the food chain studies,[5] the individual carotenoids are treated here in approximate order of increasing polarity, judged by the number and type of polar functional groups and the carbon skeleton (Scheme 1). The order chosen is roughly according to increasing adsorbance in TLC and HPLC, although some interchange in adsorptivity is observed on various adsorbents.

β,β-Carotene (**1**) was occasionally a minor carotenoid, identified by absorption spectra in the visible region (VIS), and mass spectra and chromatography.

Similar criteria were used for the characterization of a minor, crocoxanthin (**2**)-like carotenoid.

Anhydro-amarouciaxanthin B (**3**), isolated from a summer harvest of *M. edulis*, has not previously been characterized. This ketocarote-

*Part 31, see Ref. 35.



1 A–P–A
2 C–P–B, R=H
3 C–P–D, R=H
4 E–P–C, R=$OC(CH_2)_4CH_3$ (=O)
4a E–P–C, R=OH
5 E–P–C, R=H
6 C–P–C, R=H
7 C–P–F, R=H
8 G–P′–H, R=Ac, R′=H
9 J–P–G, R=Ac, R′=$OC(CH_2)_4CH_3$ (=O)
10 J–P–G, R=Ac, R′=H
11 C–P–K, R=H
12 C–P′–H, R=H
13 L–P–C, R=H
14 J–P–C, R=H
15 J–P–G, R=H, R′=$OC(CH_2)_4CH_3$ (=O)
16 M–P–C, R=H
16a **16**–3,3′-diacetate
17 C–P′–N, R=H
17a **17**–3,3′-diacetate
17b **17**–3,3′-diacetate-5-TMS ether
18 G–P′–H, R=R′=H
18a **18**–3,3′-diacetate
19 J–P–G, R=R′=H
CH_2R^1

Scheme 1. Carotenoids from *M. edulis* and some derivatives.

noid is structurally related to amarouciaxanthin B (**11**), isolated from a tunicate.[6] VIS spectra, MS, ^{1}H NMR data and the formation of a monoacetate (**3a**) upon acetylation are consistent with the structure assigned. Relevant ^{1}H NMR models are available.[6–9] The assignment of 3*R*-configuration is based only on analogy with alloxanthin (**6**) and amarouciaxanthin **B**(**11**).[6]

19′-Hexanoyloxyisomytiloxanthin (**4**) was isolated in trace amounts in mixture with isomytiloxanthin (**5**) by TLC and HPLC after feeding the mussels with *Coccolithus huxleyi*, containing 19′-hexanoyloxyfucoxanthin (**9**).[10,11] The presence of **4** was concluded from the MS of (**4** + **5**) and the isolation of an alkaline hydrolysis product with adsorptive, VIS and mass spectral properties compatible with 19′-isomytiloxanthinol (**4a**).

Isomytiloxanthin (**5**) was a general constituent

in most harvests of *M. edulis*. VIS, ^{1}H NMR and mass spectra were consistent with reported data.[3] Except for the central polyene chain, the 400 MHz ^{1}H NMR spectrum may now be fully assigned. Prolonged alkali treatment provided the cross-conjugated anhydro-isomytiloxanthin (**5a**)[3] (Schemes 1 and 2). The product analysis included 400 MHz ^{1}H NMR. No retro aldol cleavage was obtained for isomytiloxanthin (**5**), in contrast to the behaviour of prasinoxanthin[12] and amarouciaxanthins A and B.[6] This may be rationalized by the formation of conjugated C_9-ketones in the three latter cases, whereas the retro aldol product of isomytiloxanthin (**5**) would be a saturated ketone. The ketonic end groups of isomytiloxanthin (**5**), anhydro-isomytiloxanthin (**5a**) and anhydro-amarouciaxanthin B (**3**) have not been fully assigned previously by ^{1}H NMR (see Scheme 2). Anhydro-isomytiloxanthin (**5a**) is structurally a 4,5-dihydro derivative of anhydro-amarouciaxanthin B (**3**), first characterized in the present work.

Alloxanthin (**6**) was also a general constituent.[1] VIS, ^{1}H NMR and mass spectra were as reported,[1] and no separation from an authentic all-*trans* sample was achieved. Present ^{1}H NMR data are consistent with a 9,9′-di-*trans* configuration,[13] and CD data support a 3*R*,3′*R*-configuration, as for alloxanthin from other animal and algal sources.[4]

The enolized β-diketone mytiloxanthin (**7**) with a cyclopentane end group was a major carotenoid,[3] characterized by VIS, ^{1}H NMR, CD and mass spectra. The 3,5-*trans* configuration follows from ^{1}H NMR, and the same chirality of the κ end group as for capsorubin[14] has already been assumed.[3] 9-*cis*-Mytiloxanthin of known absolute configuration, but with no detectable CD, has been prepared by total synthesis.[15] The CD contribution of the alloxanthin end group *C* is small, and the Cotton effect observed here for mytiloxanthin may be ascribed mainly to the contribution from the κ end group *F*.

The C_{37}-skeletal peridinin (**8**), a minor carotenoid component isolated subsequent to feeding of the mussels with *8*-containing dinoflagellates, was characterized by its VIS spectrum, and R_F and R_T values in direct comparison with authentic **8**.

19′-Hexanoyloxyfucoxanthin (**9**) was identified subsequent to feeding with *C. huxleyi*, containing **9**,[10,11] on the basis of R_F and R_T values in direct comparison with authentic **9**, as well as VIS and mass spectra.

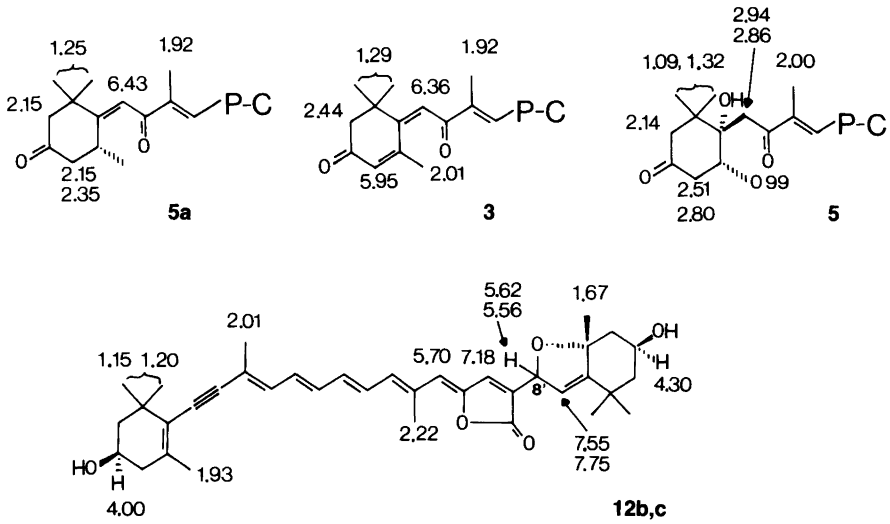
Fucoxanthin (**10**), present subsequent to feeding with **10**-containing diatoms, was identified by the same criteria as for **9**.

Sydnyaxanthin[16] and amarouciaxanthin B,[6] both isolated from tunicates, are presumably identical.[4] Although the former designation has priority, the latter is generally used. An amarouciaxanthin B (**11**)-like carotenoid was a minor metabolite in summer samples. R_F, VIS and MS properties were compatible with those reported for amarouciaxanthin B.

The acetylenic C_{37}-skeletal pyrrhoxanthinol (**12**) is a very minor carotenoid in certain dinoflagellates, accompanied by somewhat larger amounts of pyrrhoxanthin (= pyrrhoxanthinol 3-acetate),[17] and was recently encountered in eggs of a soft coral.[18] Pyrrhoxanthinol (**12**) was isolated in this work from *M. edulis* subsequent to feeding with dinoflagellates, and characterized by VIS, ^{1}H NMR and mass spectra and by the formation of a diacetate (**12a**). Pyrrhoxanthinol (**12**) underwent epoxide-furanoxide rearrangement, giving both C-8′ epimers of the furanoid product (**12b,c**) (Scheme 2), characterized by 400 MHz ^{1}H NMR. The relative configuration of the epoxide end group of **12** from *M. edulis* follows from ^{1}H NMR data in comparison with data for peridinin (**8**)[19] of known absolute configuration. Since no separation was achieved for **12**-diacetate (**12a**) by HPLC from pyrrhoxanthinol diacetate prepared by partial synthesis from peridinin (**8**) acetate of known configuration,[18,20] the (3*R*,3′*S*,5′*R*,6′*R*)-configuration of **12** is deduced.

Pectenol (**13**) has been isolated from the Japanese sea mussel *M. coruscus* and assigned the (3*S*,4*R*,3′*R*)-configuration as a 3,4-*cis* diol by acetonide formation.[21] A minor carotenoid from a summer harvest of *M. edulis* had R_F value, and VIS and mass spectral properties compatible with a dicyclic acetylenic triol. Acetylation provided a triacetate (**13a**), and allylic oxidation with DDQ was positive. From this evidence the present triol was probably identical with pectenol (**13**).

Halocynthiaxanthin (**14**) was first isolated from a sea squirt.[21] The assignment of its relative configuration was based on ^{1}H NMR, and the chirality on biogenetic reasoning.[22] Halocynthiaxanthin (**14**) was isolated from *M. edulis* after feeding with diatoms and was characterized by R_F and R_T



Scheme 2. ^{1}H NMR assignments of some carotenoids.

values, and by VIS and mass spectra. The diacetate (**14a**) was characterized on the basis of the same criteria. The occurrence of a prominent ion in the mass spectrum of **13** at *m/z* 155 may be rationalized by assuming rupture of the C-6,7 bond.

19′-Hexanoyloxyfucoxanthinol (**15**, 3′-desacetyl-19′-hexanoyloxyfucoxanthin), previously characterized as a minor carotenoid from *C. huxleyi,*[10] was isolated from *M. edulis* after feeding with *C. huxleyi.* The present characterization comprised R_F and R_T values, and VIS and mass spectra.

Heteroxanthin (**16**) is an acetylenic carotenoid tetrol with recently revised configuration[23] and is encountered in various microalgae.[24,25] Heteroxanthin (**16**) was occasionally a minor carotenoid in *M. edulis.* The characterization involved R_F and R_T values in comparison with those for authentic **16**, and VIS, ^{1}H NMR and mass spectra. Acetylation provided a diacetate (**16a**) which could not be silylated, in agreement with previous reports,[26] and no epoxide-furanoid rearrangement could be effected. The relative configuration of the triol end group follows from ^{1}H NMR,[23] and the co-chromatography tests and lacking silylation of **16a** are taken as evidence in favour of the same configuration for heteroxanthin from *M. edulis* as for that from algal sources.

Hydrato-pyrrhoxanthinol (**17**) constitutes a C_{37}-skeletal carotenoid, not reported previously, and occurred as a metabolite subsequent to feeding of *M. edulis* with dinoflagellates.[5] This new carotenol was characterized by R_F and R_T values, and by VIS, ^{1}H NMR and mass spectra. Acetylation provided a diacetate (**17a**). 9-*cis* Isomerization occurred readily in solution. As a result of the influence of the butenolide moiety, relevant ^{1}H NMR models for the triol end group are not available. The same absolute configuration as for heteroxanthin (**16**) appears likely, also taking into account the resistance towards silylation of the diacetate **17a**.

Peridininol (**18**) is a minor carotenoid in several dinoflagellates,[16] and was isolated from *M. edulis* after feeding on dinoflagellate diets. The characterization involved R_F and R_T values, and VIS and mass spectra. Acetylation gave a diacetate (**18a**) which provided a mono-trimethylsilyl ether (**18b**). Both derivatives were inseparable from authentic samples.

Fucoxanthinol (**19**) occurs in various algae[24,25] and was occasionally isolated from *M. edulis.* R_F and R_T values in comparison with those for authentic **19**, as well as VIS absorption were employed for the identification.

In conclusion, β,β-carotene, ten acetylenic C_{40}-carotenoids, and two acetylenic C_{37}-carotenoids plus four C_{40}-skeletal allenic and two C_{37}-skeletal allenic carotenoids have been isolated from *M.*

edulis and characterized. The chiralities of these carotenoids appear to be consistent with those for the same or related carotenoids from other animal or algal sources.

Studies demonstrating the metabolic formation of most of these carotenoids in *M. edulis* by structural modification of resorbed, dietary microalgal carotenoids are published elsewhere.[5]

Experimental

Biological material. M. edulis mussels from various harvests were used.[5] The number of mussels from each harvest varied from 60–175.

Isolation of the carotenoids. The methods used were those commonly employed in our laboratory.[27,28] General precautions for work with carotenoids were taken.[29] The mussels were extracted at room temperature with acetone. The combined acetone extract was concentrated, colourless lipids were removed by precipitation from acetone at low temperature and the pigments transferred to ether upon dilution with 5 % aqueous NaCl. The ether extract was concentrated to dryness in the presence of benzene and the residue submitted to chromatography. No saponification step was included.

Chromatography. The following chromatographic systems are referred to: System 1, TLC SiO_2 (trichloroethane:methanol 100:5 if not otherwise stated); System 2, TLC special plates[30] (methanol:ethyl acetate 20:80 if not otherwise stated); HPLC[31] nitrile column (hexane:isopropyl acetate: acetone:methanol 77:17:7:0.5), using a Perkin Elmer Series 2 Liquid Chromatograph equipped with a diode array detector, allowing recording of VIS spectra for each peak during the chromatographic run.

R_F values in System 1 decreased as follows: β,β-carotene (**1**) > crocoxanthin (**2**) > anhydro-amarouciaxanthin (**3**) > isomytiloxanthin (**5**) > alloxanthin (**6**) > mytiloxanthin (**7**) > amarouciaxanthin B (**11**) > pectenol (**13**) > halocynthiaxanthin (**14**) > heteroxanthin (**16**) > peridininol (**18**). R_T-values increased in the following order: β,β-carotene (**1**) < alloxanthin (**6**) < mytiloxanthin (**7**) < 19′-hexanoyloxyisomytiloxanthin (**4**) and isomytiloxanthin (**5**) < 19′-hexanoyloxyfucoxanthin (**8**) < peridinin (**9**) < fucoxanthin (**10**) < pyrrhoxanthinol (**12**) < halocynthiaxanthin (**14**) < 19′-hexanoyloxyfucoxanthinol (**15**) < heteroxanthin (**16**) < hydrato-pyrrhoxanthinol (**17**), and peridininol (**18**) < fucoxanthinol (**19**).

Spectroscopy. The instruments used were as previously stated.[27] Some ^1H NMR spectra were recorded on a Bruker 400 MHz instrument, and CD spectra on a Jobin Yvonne Dicrographe. The spectral fine-structure of VIS spectra are expressed as % III/II.[32] For mass spectra, only diagnostically important or prominent ions are quoted. When lipid contaminants were dominant, no peak intensities are given.

Individual carotenoids

β,β-Carotene (**1**), available amount <0.1 mg; R_F = 0.85 (System 1, hexane), R_T = 1.55, inseparable from authentic **1**; VIS λ_{max} nm (hexane): 446, 472, % III/II = 12; MS *m/z*: 536 (M), 430 (M−106). Possible admixture with β,ε-carotene was not tested.

Crocoxanthin (**2**) *-like.* Available amount <0.1 mg; R_F = 0.75 (System 1, ether); VIS λ_{max} nm (acetone): 446, 422, % III/II = 14; MS *m/z*: 548 (M), 456 (M−92).

Anhydro-amaroucixanthin B (**3**). Available amount 0.3 mg; R_F = 0.50 (System 1), R_F = 0.69 (System 2); VIS λ_{max} nm (hexane): 458, 485, (acetone): 454, (methanol): 466; MS *m/z* (% rel. int.): 578 (100, M), 560 (7, [M−18]), 520 (7, [M−58)], 439 (12), 410 (20), 390 (30), 358 (64); ^1H NMR (100 MHz, $CDCl_3$): δ 1.15s and 1.20s (3H + 3H, Me−16,17), 1.29s (6H, Me−16′,17′), 1.92s (6H, Me−18,19′), 1.97s (3H, Me−19), 2.00s (6H, Me−20,20′), 2.01s (3H, Me−18′), 2.44s (2H, H−2′), 3.99m (1H, H−3), 5.95s (1H, H−4′), 6.36 (1H, H−7′) and 6.27–6.8 m (other conj. olefinic H).

19′-Hexanoyloxyisomytiloxanthin (**4**). Available amount <0.02 mg in mixture with **5**; R_F = 0.68 (System 1, 40 % acetone in hexane), R_T = 5.37; VIS λ_{max} as for **5**; MS in mixture with **5** *m/z* (% rel. int.): 712 (2, M), 694 (2, [M−18]), 281 (100).^1H NMR (400 MHz, $CDCl_3$) in mixture with excess **5** showed a singlet at δ 4.95, attributed to CH_2−19′, besides signals characteristic of **5**.

Hydrolysis with 5% KOH in methanol-ether for 1 h gave isomytiloxanthin-19′-ol (**4a**) as the most polar product, R_F = 0.24 (System 1, 40% acetone in hexane). VIS λ_{max} nm (acetone): 448, (472); MS *m/z* (% rel. int.): 614 (3, M), 155 (48), 149 (100).

Isomytiloxanthin (**5**). Available amount 0.7 mg; R_F = 0.41 (System 1), 0.80 (System 2); R_T = 5.37; VIS λ_{max} nm (acetone): 450, (470); MS *m/z* (% rel. int.): 598 (15, M), 580 (15, [M−18]), 540 (4, [M−58]), 522 (4, [M−72]), 444 (25 [M−154]), 155 (100); ^{1}H NMR (100 MHz, 400 MHz, $CDCl_3$): δ 0.99t (J = 6.8 Hz, 3H, Me−18), 1.09s and 1.32s (3H + 3H, Me−16,17), 1.15s and 1.21s (3H + 3H, Me−16′,17′), 1.48m (1H, H−2′$_{ax}$), 1.85m (1H, H−2′$_{eq}$), 1.93s (3H, Me−18′), 1.94s (3H, Me−19′), 1.99s and 2.01s (6H + 3H, Me−19,20,20′), ca. 2.10m (1H, H−4$_{ax}$), 2.14s (2H, H−2), 2.25m (1H, imp?), ca. 2.51dd and 2.8dd J_1 = 7 Hz, J_2 = 18 Hz, 1H + 1H, H−4$_{ax,eq}$) 2.45dd (1H, H−4′$_{eq}$), 2.86d and 2.94d (J = 14Hz, 1H + 1H, H−7), 3.98 broad s (1H, H−3′), 6.22d (J = 16 Hz, H−10′), 6.27–6.67m (conj. olefinic H) and 6.72d (J = 12 Hz, H−10) (for assignment of the acetylenic end group, see Ref. 8; for previous partial assignment of **5**, see Refs. 15 and 33).

Alkali treatment of **5** with 5% KOH in ether-methanol overnight gave anhydro-isomytiloxanthin (**5a**); R_F = 0.40 (System 1, 30% acetone in hexane); VIS λ_{max} nm (acetone): 465, (490); MS *m/z* (% rel. int.): 580 (7, M), 562 (100 [M−18]), 281 (18, [M^{2+}]), ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.08d (3H, Me−18′), 1.15s and 1.20s (3H + 3H, Me−16,17), 1.25s (6H, Me−16,17), 1.48m (1H, H−2′$_{ax}$), 1.85m (1H, H−2′$_{eq}$), 1.93s (3H, Me−18′), 1.97s (3H, Me−19), 2.00 (3H, Me−19), 2.01s and 2.03s (3H + 3H, Me−20,20′), 2.10m (1H, H−4′$_{ax}$), 2.15s (2H, H−2), 2.15m (1H, H−4), 2.32m (1H, H−4), 2.45dd (1H, H−4′$_{eq}$), 6.43s (1H, H−7), 6.22–6.67m (conj. olefinic).

Alloxanthin (**6**). Available amount 1.5 mg; R_F = 0.38 (System 1), R_T = 4.11, inseparable from an authentic sample of all-*trans* **6**; VIS λ_{max} nm (acetone): (345), (425), 449 and 476, % III/II = 24; MS *m/z*: 564 (100, M), 549 (<1, [M−15]), 458 (<1, [M−106]), 282 (25, M^{2+}); ^{1}H NMR ($CDCl_3$, 100 MHz): δ 1.15s and 1.20s (6H + 6H, Me−16,17,16′,17′), ca. 1.5 (2H, H−2$_{eq}$,2′$_{eq}$), ca. 1.8 (2H, H−2$_{ax}$,2′$_{ax}$), 1.92s (6H, Me−18,18′), 1.96s (6H, Me−20,20′), 2.00s (6H, Me−19,19′), 3.99m (2H, H−3,3′), and 6.1–7.0 (olefinic H), consistent with 9,9′-di-*trans* configuration;[8] CD (EPA) nm (Δε) 210 (−7.0), 280 (−3.0), 325 (−1.8), 492 (0).

Mytiloxanthin (**7**). Available amount 1 mg; R_F = 0.30 (System 1), R_T = 4.81; VIS λ_{max} nm (acetone): 468; MS *m/z*: 598 (75, M), 580 (5, [M−18]), 506 (3, [M−92]), 11 (100), 109 (100); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.85s and 1.18s (3H + 3H, Me−16′,17′), 1.15s and 1.20s (3H + 3H, Me−16,17), 1.34s (3H, Me−18′), 1.47dd (1H, H−2$_{ax}$), 1.55dd (1H, H−4′), 1.72dd (1H, H−2′), 1.92s (<3H, Me−18), 1.99s (9H, Me−20,19′,20′), 2.01s (3H, Me−19), 2.02dd (H−2′, H−4′), 2.44dd (1H, H−4$_{ax}$), 2.88dd (1H, H−4′), 4.00m (1H, H−3), 4.53m (1H, H−3′), 5.85s (1H, H−7′) and 6.4–7.3 (conj. olefinic H). The presence of ca. 70% 9-*trans* and ca. 30% 9-*cis* was evident from the intensity ratios of the Me−18 signal: δ 1.93 for 9-*trans* and δ 1.95s for 9-*cis* (cf. Ref. 13b). CD nm (Δε): 249 (−14), 366 (−13), 440 (−13), 520 (−7.4).

Peridinin (**8**). Available amount <0.05 mg from mussels recently fed dinoflagellates; R_F = 0.37 (System 1, 60% acetone in hexane), R_T = 8.22, inseparable from an authentic sample; VIS λ_{max} nm (acetone): 454.

19′-Hexanoyloxyfucoxanthin (**9**). Available amount <0.05 mg from mussels recently fed *Coccolithus huxleyi*; R_F = 0.30 (System 2, hexane: acetone:*iso*propanol 68.5:30:1.5), R_T = 6.53, inseparable from an authentic sample; VIS λ_{max} nm (acetone): 445, (480); MS *m/z*: 792 (0.2, M), 754 (0.4, [M−18]), 274 (59), 178 (100).

Fucoxanthin (**10**). Available amount <0.05 mg from mussels recently fed diatoms; R_F = 0.70 (System 1, 60% acetone in hexane), R_T = 9.32, inseparable from an authentic sample; VIS λ_{max} nm (acetone): 447, (472).

Amarouciaxanthin B (**11**) *-like*. Available amount <0.1 mg; R_F = 0.14 (System 1); VIS λ_{max} nm (acetone): 450; MS *m/z*; 598 (M), 578 (M−18), 504 (M−92).

Pyrrhoxanthinol (**12**). Available amount 0.8 mg; R_F = 0.48 (System 1, 40% acetone in hexane);

VIS λ_{max} nm (acetone): 454, (472); MS *m/z*: 570 (100, M), 478 (24), 234 (70), 181 (98); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.97s and 1.20s (3H + 6H, Me−16′,17′,18′), 1.14s and 1.19s (3H + 3H, Me−16,17), 1.92s (3H, Me−18), 2.00s (3H, Me−19), 2.22s (3H, Me−20′), 3.9m (2H, H−3,3′), 5.73s (H−12′), 6.2–6.8m (conj. olefinic H), 7.02s (1H, H−10′), 7.15d (1H, H−7′). Other minor signals were indicative of the presence of *cis* isomers.

Standard acetylation provided pyrrhoxanthinol diacetate (**12a**); R_F = 0.71 (System 1, 40 % acetone in hexane); VIS λ_{max} nm (acetone): 453, (475); MS *m/z*: 654 (54, M), 223 (32, furylium), 163 (100). **12a** thus prepared was inseparable by co-chromatography (System 1) from **12a** prepared from peridinin (**8**) acetate by $POCl_3$ treatment.[18]

Furanoid rearrangement of **12** occurred during storage in $CDCl_3$ in the presence of TMS, to **12b,c**, accompanied by 9-*cis* isomerization. ^{1}H NMR ($CDCl_3$, 400 MHz): δ all-*trans* alloxanthin end group: 1.15s and 1.20s (Me−16,17), 1.93s (Me−18) and 2.01s (Me−19); 9′-*cis* alloxanthin end group: 1.19s (Me−16), 1.98s (Me−18), 2.00s (Me−19) with integrals corresponding to 9-*trans*: 9-*cis* ca. 1:4. The two C−8′ furanoid epimers were assigned: Both had δ 7.18s (1H, H−10′), 5.70s (1H, H−12′), 2.22s (3H, Me−20′); furthermore, epimer 1 (8′−*R*): 1.67s (Me−18′), 4.3m (H−3′), 5.62d (H−8′), 7.55 (H−7′), and epimer 2 (8′−*S*): 1.67s (Me−18′), 4.3m (H−3′), 5.54s (H−8′), 7.75s (H−7′). The epimer 1: epimer 2 ratio was ca. 1:1. **12b,c** had R_F = 0.52 (System 1, 40 % acetone in hexane), VIS λ_{max} nm (acetone): 438.

Pectenol (**13**). Available amount 0.1 mg; R_F = 0.14 (System 1), R_F = 0.55 (System 2, compared with diadinoxanthin R_F = 0.64); VIS λ_{max} nm (acetone): (345), (425), 448 and 476, % III/II = 38; MS *m/z*: 582 (42, M), 580 (18, [M−2]), 564 (10, [M−18]), 559 (10), 545 (12), 324 (55), 178 (100).

Acetylation of **13** gave a less polar triacetate (**13a**) with unchanged VIS λ_{max}; MS *m/z*: 708 (50, M), 706 (50, [M−21]), 648 (21, [M−60]), 239 (11), 159 (100). Alkaline hydrolysis of **13a** gave **13**.

DDQ-oxidation of **13**[34] on the μg scale gave a product with a slightly longer chromophore.

Halocynthiaxanthin (**14**). Available amount <0.1 mg; R_F = 0.14 (System 1), 0.24 (System 1, 40 % acetone in hexane), R_T = 10.02; VIS λ_{max} nm (acetone): 448, (473), considered imp.; MS *m/z*: 598 (18, M), 582 (10, [M−16]), 580 (39, [M−18]), 178 (100), 155 (38).

Acetylation gave the diacetate **14a**; R_F = 0.74 (System 1, 40 % acetone in hexane); VIS λ_{max} nm (acetone): 455, (4880); MS *m/z*: 682 (39, M), 664 (4, [M−18]), 622 (27, [M−60]), 576 (5, [M−106]), 178 (100).

The diacetate **14a** gave no TMS ether upon silylation.

19′-Hexanoyloxyfucoxanthinol (**15**). Available amount <0.05 mg from mussels fed on *C. huxleyi*; R_F = 0.20 (System 2, hexane:acetone:isopropanol 68.5:30:1.5), R_T = 10.08; VIS λ_{max} nm (acetone): 444, 468; MS *m/z*: 730 (20, M), 712 (9, [M−18]), 111 (100).

Heteroxanthin (**16**). Available amount 0.1 mg; R_F = 0.50 (System 2, 60 % acetone in hexane), R_T = 25.26; VIS λ_{max} nm (hexane): 338, 432 and 460 (9′-*cis*); (acetone): (420), 443, 472; MS *m/z*: 600 (100, M), 598 (6, [M−2]), 582 (6, [M−18]), 584 (4, [M−2−18]), 564 (1, [M−18−18]), 562 (1, [M−2−18−18]), 508 (2, [M−92]), 291 (6), 221 (12), 181 (10). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.87s and 1.18s (3H + 3H, Me−16,17), 1.15s and 1.20s (3H + 3H, Me−16′,17′), 1.25s (Me−18 and lipid imp.), 1.97s (9H, Me−19,20,20′), 2.00s (3H, Me−19′), 3.95m (1H, H−3′), 4.1m (1H, H−3), 6.1–6.7m (olefinic H).

Treatment with 0.03 M HCl in $CHCl_3$ caused no furanoid rearrangement under conditions where diadinoxanthin was rearranged in a parallel experiment.

Acetylation of **16** gave a less polar diacetate (**16a**) with unchanged VIS λ_{max}; MS *m/z*: 684 (7, M), 624 (1, M−60), 173 (100).

Silylation of **16a** under standard conditions[26] was not effected.

Hydrato-pyrrhoxanthinol (**17**). Available amount 0.22 mg; R_F = 0.22 (System 1, 40 % acetone in hexane), R_T = 26.00; VIS λ_{max} nm (acetone): 452, (472); MS *m/z*: 588 (48, M), 570 (33, M−18), 181 (74), 105 (100); ^{1}H NMR ($CDCl_3$, 100 MHz); δ 0.97s and 1.10s (3H + 3H, Me−16′,17′), 1.14s and 1,20s (3H + >3H (lipid imp.), Me−16,17),

1.42s (3H, Me−18′), 1.93s (3H, Me−18), 2.02s (3H, Me−19), 2.24s (3H, Me−20′), *ca.* 4.1m (H−3, H−3′), 5.74s (1H, H−12′), 6.28d (J = 14 Hz, 1H, H−8′), 6.3−6.9m (conj. olefinic H), 6.81d (J = 14 Hz, 1H, H−7′), 7.10s (1H, H−10′).

Acetylation gave the diacetate **17a** with unchanged VIS λ_{max}; R_F = 0.73 (System 1, 30% acetone in hexane); MS *m/z*: 672 (26, M), 250 (100).

Silylation of **17a** with Sylon BTZ gave the TMS ether **17b** in 5% yield, besides unreacted **17a**. **17b** had unchanged VIS λ_{max}; R_F = 0.83 (System 1; 30% acetone in hexane); MS *m/z*: 744 (26, M), 147 (100).

Peridininol (**18**). Available amount 0.1 mg; R_F = 0.14 (System 1), R_T = 26.71; VIS λ_{max} nm (hexane): 426, 452 and 482; (acetone): 450 (470), 250 (100).

Acetylation provided a diacetate (**18a**) of lower polarity and with unchanged VIS λ_{max}; MS *m/z*: 672 (5, M), 654 (33, [M−18]), 594 (17, [M−18−60]), 197 (100). **18a** was inseparable from peridinin (**8**) monoacetate in System 1 (20% acetone in hexane, R_F = 0.53).

Silylation of **18a** gave a less polar TMS ether (**18b**), inseparable from the TMS ether of peridinin (**8**) monoacetate in the above system.

Fucoxanthinol (**19**). Available amount 0.05 mg; R_F = 0.41 (System 2, 60% acetone in hexane); R_T = 32.09, inseparable from an authentic sample; VIS λ_{max} nm (acetone) 443, (465).

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