Algal Carotenoids. 48.* Structural Assignments of Geometrical Isomers of Fucoxanthin

Jarle André Haugan,[a] Gerhard Englert,[b] Ernst Glinz[b] and Synnøve Liaaen-Jensen[a]

[a] Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway and [b] Pharma Research, New Technologies, F. Hoffmann–La Roche Ltd., CH-4002 Basel, Switzerland

Haugan, J. A., Englert, G., Glinz, E. and Liaaen-Jensen, S., 1992. Algal Carotenoids. 48.* Structural Assignments of Geometrical Isomers of Fucoxanthin. – Acta Chem. Scand. 46: 389–395.

Isolated, crystallized all-*trans* (3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*R*)-fucoxanthin has been submitted to iodine-catalyzed stereomutation. HPLC systems are given for the semipreparative separation of the quasi-equilibrium mixture, which consisted of all-*trans* (42 % of total), 9′-*cis* (20 %), 13′-*cis* (14 %), 13-*cis* (11 %), 9′,13′-*dicis* (6 %), 13,9′-*dicis* (4 %) and, tentatively, of 13,13′-*dicis* (3 %), and 15-*cis* (1 %).

Deduction of the structures of the individual isomers (except 15-*cis*) was based on the assignment of the ^{1}H NMR spectra by 2D ^{1}H–^{1}H correlated spectroscopy (COSY) and by comparison with the known data of the all-*trans* compound. The structure of 9′-*cis* was additionally supported by rotating-frame nuclear Overhauser effect 2D spectroscopy (ROESY).

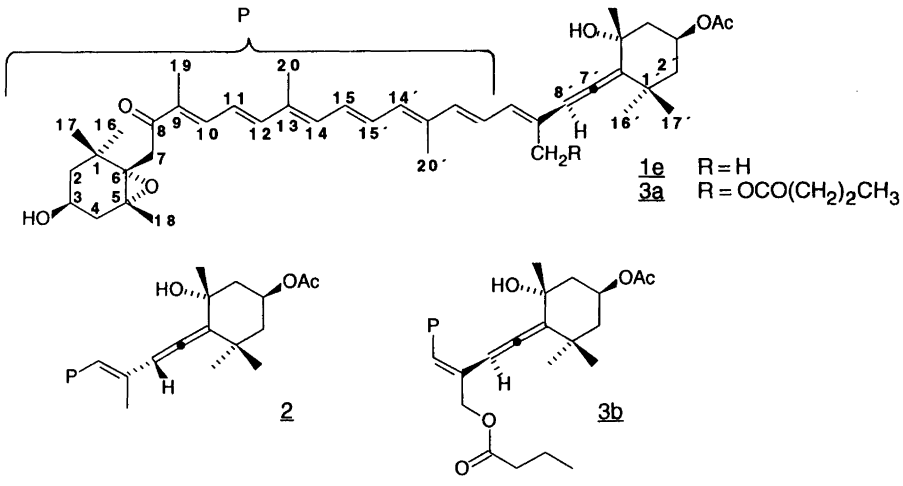
The results are consistent with the visible absorption data (λ_{max} shifts and *cis*-peak intensities) as well as CD data. Mono-*cis* isomers exhibited inverted Cotton effects relative to the all-*trans* isomer, a feature previously associated with conservative CD spectra.

The results confirm our recent finding obtained for related compounds, that the 9′-mono-*cis* isomer had been previously misidentified as the all-*trans* allenic (6′*S*)-isomer.

Fucoxanthin, biosynthesized *de novo* by several algal classes,[1] represents, besides peridinin, one of the most abundant carotenoids in nature.[2] Its constitution was elucidated in the sixties[3–6] and the chirality established by oxidative degradation, chemical and chiroptical correlations as 3*S*, 5*R*, 6*S*, 3′*S*, 5′*R*, 6′*R* (**1e**, Scheme 1).[7]

Subsequently the (i) natural occurrence of and (ii) formation upon iodine-catalyzed stereomutation of the allenic 6′*S* isomer (**2**) were claimed.[8] This identification was later questioned.[9] Detailed ^{1}H NMR data for appropriate models including all-*trans* 19′-butanoyloxyfucoxanthin (**3a**) and its 9′-*cis* isomer (**3b**) strongly suggested that the geometrical 9′-*cis* isomer (**1h**) of fucoxanthin had been misidentified as the optical all-*trans* (6′*S*)-isomer (**2**).[10,11]

In the past, unidentified geometrical isomers of fucoxanthin have been observed, and occasionally referred to as neo A and neo B by a former convention.[12]



Scheme 1.

* Part 47. *Magn. Reson. Chem.* Submitted.

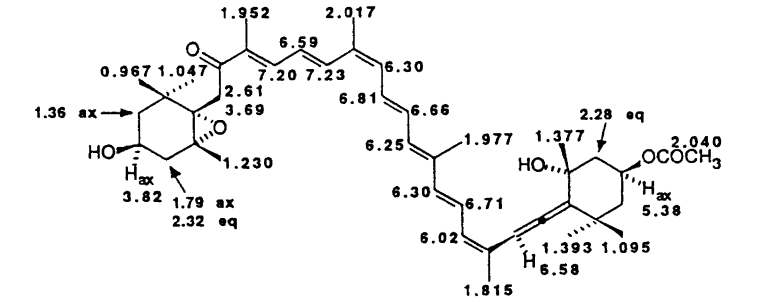
Results and discussion

All-*trans* fucoxanthin (**1e**) was isolated from *Fucus serratus* by an improved procedure[13,14] and fully characterized, including m.p., visible light absorption (VIS), FT-IR, MS, ^{1}H NMR spectroscopy and CD. Detailed ^{1}H and ^{13}C NMR data have recently been reported elsewhere.[11]

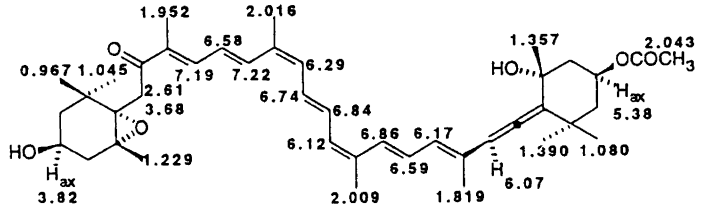
Crystallized **1e** was submitted to iodine-catalyzed stereomutation. Isomerization in benzene or dichloromethane resulted in the same isomeric mixture. The quasi-equilibrium mixture, representing eight isomers (**1a–1h**), was separated by semipreparative HPLC. Individual isomers were characterized by t_R and VIS, CD and ^{1}H NMR (400 MHz) spectra. Separation of isomers **1c** and **1d** was not achieved on a semipreparative or analytical scale, and the ^{1}H NMR spectral analysis was carried out with a 2:1 mixture.

As will be shown below only geometrical isomers of all-*trans* fucoxanthin (**1e**) were detected in the isomerization mixture. With the exception of 15-*cis*, which was tentatively identified by its VIS absorption, the structure of all other geometrical isomers was deduced by ^{1}H NMR spectroscopy. A prerequisite for the configurational assignment of the isomers by ^{1}H NMR was the complete and correct assignment of the signals. Although only small sample quantities of roughly 20 to 100 μg were available, this task could be considerably simplified in all cases by the acquisition of 2D COSY spectra providing the connectivities between coupled protons. This was particularly helpful in the case of the 2:1 mixture of **1d** and **1c**. Here, most of the signals, even of the minor component, could be localized from observed cross-peaks. The chemical shifts of all-*trans* fucoxanthin (**1e**)[10,11] and of six geometrical isomers subjected to ^{1}H NMR analysis are presented in Scheme 2 and 3. In the polyene chain vicinal coupling constants were as expected, around 11 Hz across single bonds and about 15 Hz across double bonds. The AX-type spectra of H-7,7′ indicated a geminal coupling constant of 18.2 ± 0.3 Hz. After completion of the assignments the deduction of the structure of the isomers was based on previous data[15] for the isomerization shifts $\Delta = \delta_{cis} - \delta_{trans}$ (in ppm) of the olefinic protons of more than 40 different C_{40}-carotenoids.[15] This parameter was found to be very indicative of the position of the stereomutated bond.

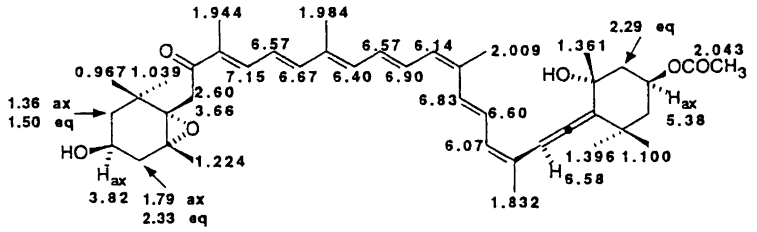
Our results for all-*trans* **1e**, for the mono-*cis* (**1f**, **1g**, **1h**) and di-*cis* (**1b**, **1c**, **1d**) isomers of fucoxanthin are compiled in Table 1.



13,9′-di-*cis* (1b)

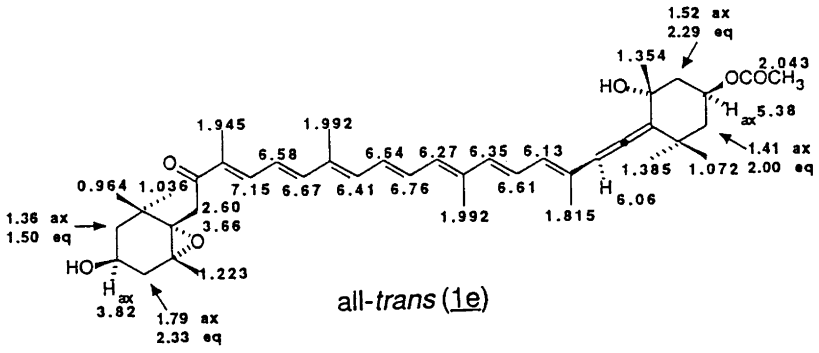


13,13′-di-*cis* (1c)

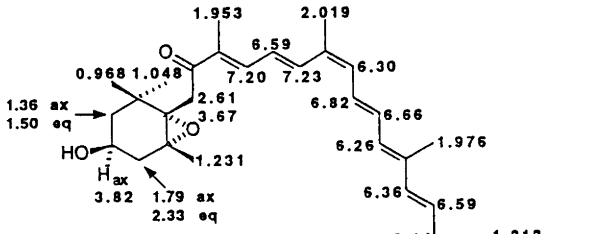


9′,13′-di-*cis* (1d)

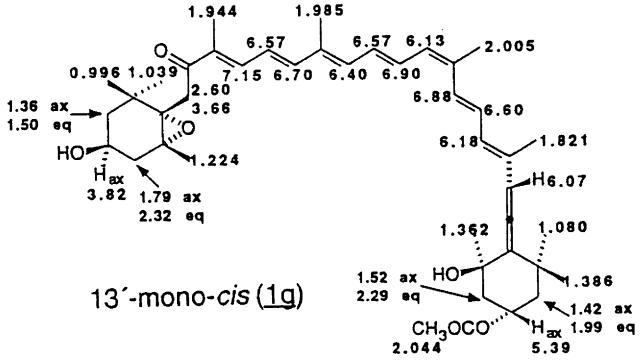
Scheme 2.



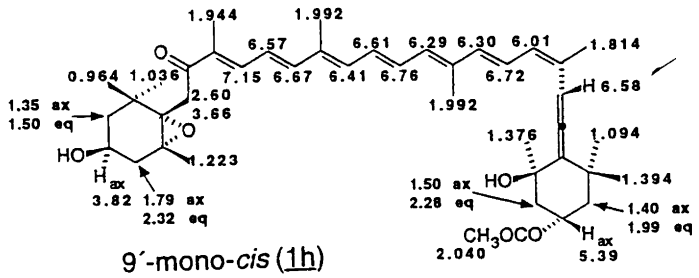
all-*trans* (1e)



13-mono-*cis* (1f)



13′-mono-*cis* (1g)



9′-mono-*cis* (1h)

Scheme 3.

Table 1. Relevant observed isomerization shifts $\Delta = \delta_{cis} - \delta_{trans}$ (in ppm, > 0.03) for the olefinic protons in the ^{1}H NMR spectra of mono- and di-*cis*-isomers of fucoxanthin relative to the all-*trans*-isomer.

Olefinic protons	13-mono-*cis* (**1f**)	13′-mono-*cis* (**1g**)	9′-mono-*cis* (**1h**)	13,9′-di-*cis* (**1b**)	13′,13′-di-*cis* (**1c**)	9′,13′-di-*cis* (**1d**)
H-10	0.05			0.05	0.04	
H-11						
H-12	0.56	0.03		0.56	0.55	
H-14	−0.11			−0.11	−0.12	
H-15	0.18	−0.07		0.17	0.10	−0.07
H-15′	−0.10	0.14		−0.10	0.08	0.14
H-14′		−0.14			−0.15	−0.13
H-12′		0.53	−0.05	−0.05	0.51	0.48
H-11′			0.11	0.10		
H-10′		0.05	−0.12	−0.11	0.04	−0.06
H-8′			0.52	0.52		0.52

It is seen that for steroisomers with Δ 13 or Δ 13′ *cis* bonds a downfield shift of ca. 0.5 pp, is observed for H-12 or H-12′, respectively. Isomers with a Δ 9′ *cis* bond exhibit a downfield shift of ca. 0.5 ppm for H-8′. The Δ-values of the three di-*cis* isomers are fairly additive from the corresponding mono-*cis* values, since the stereomutated bonds are sufficiently separated from each other.[15]

The stereochemical assignments of the methyl protons of the two end groups were previously deduced from 2D ROESY experiments with all-*trans* **1e** and related compounds[11] and confirmed here by a corresponding experiment with 9′-*cis* **1h**. A qualitative picture of the measured through-space connectivities for this isomer is shown in Fig. 1. Each arrow represents the two cross-peaks found in the 2D spectrum, indicating that the two protons or groups of protons are spatially close to each other. Thus, strong ROE cross-peaks between H-8′ and H-11′ and between H-19′ and H-10′ clearly proved the Δ 9′-*cis* geometry. Medium-to-strong cross-peaks between protons on each side of the conjugated olefinic chain helped unambiguously to prove the *trans* geometry of the remaining double bonds. The geminal methyl protons of C-1 (as well as C-1′) could be distinguished from each other, since only the axial methyl protons showed a strong through-space contact to the axial H-3 (and H-3′). Since both the high- and low-field protons H-7$_l$ and H-7$_h$ at 3.66 and 2.60 ppm gave strong through-space contacts to H-10 it is concluded that the carbonyl at C-8 must be s-*trans* as depicted in Schemes 2 and 3 and Fig. 1. Protons H-18 gave a strong through-space interaction with H-7$_h$, whereas H-16 and H-17 had cross-peaks of medium intensity with H-7$_l$. These results point to a preferred angular space for rotation around the C-6/C-7 single bond.

In order not to overload the picture several other observed medium-to-strong through-space contacts are omitted, namely between the geminal protons at C-2, C-2′, C-4, C-4′, as well as between methyl protons of the end groups with neighbouring protons on the same side of the ring. All these results were completely analogous to those previously obtained for related compounds.[11]

Interestingly, no isomers with the 9-*cis* configuration next to the keto group were encountered. Obviously 9-*cis* represents a thermodynamically disfavoured configuration. ^{1}H NMR spectroscopy, also supported by VIS and CD data discussed below, demonstrated that the 9′-*cis* isomer (**1h**) with 0.5 ppm downfield shift for the allenic proton has been misidentified[8] as the all-*trans* (6′*S*)-isomer (**2**). VIS-absorp-

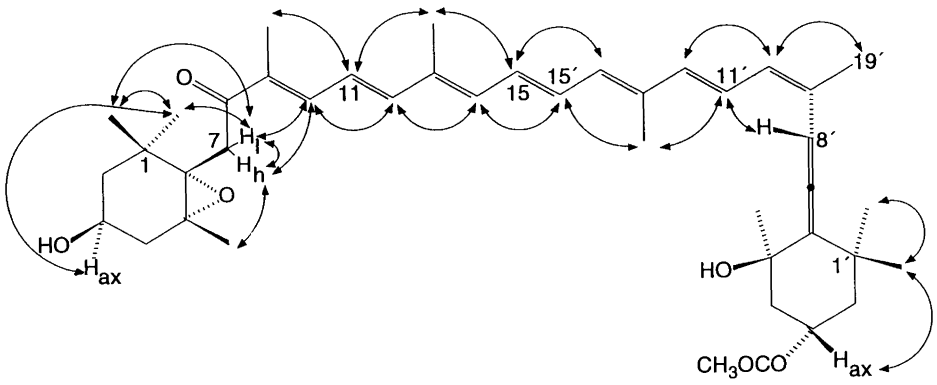


Fig. 1. Rotating-frame nuclear Overhauser effect (ROE) 2D spectroscopy of 9′-*cis*-fucoxanthin (**1h**). Each arrow connecting protons or groups of protons corresponds to a cross-peak in the 2D spectrum and hence indicates close spatial proximity.

Table 2. Composition of the iodine-catalyzed stereomutation mixture of (3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*R*)-fucoxanthin and absorption characteristics in visible light of the individual geometrical isomers.

Geometrical isomer	In HPLC eluent 3[a]			
	% of total	VIS λ_{max}/nm	% III/II	% D_B/D_{II}
15-*cis* (**1a**)	1	325, (420), 439, (458)		55
13,9′-di-*cis* (**1b**)	4	329, (415), 435, (459)		40
13,13′-di-*cis* (**1c**)	3	329, (418), 435, (457)		26
9′,13′-di-*cis* (**1d**)	6	329, (420), 437, (461)		34
all-*trans* (**1e**)	42	330, (427), 445, 471	6	7
13-*cis* (**1f**)	11	329, (420), 437, (463)		45
13′-*cis* (**1g**)	14	329, (422), 441, (466)		52
9′-*cis* (**1h**)	20	327, (424), 443, 469	2	12

[a]Hexane–isopropyl acetate–1-propanol–*N*-ethyldiisopropylamine 83.9 : 14 : 2 : 0.1.

tion properties for a compound previously identified as 13-*cis* (**1f**)[8] suggest steric impurity, whereas previously reported VIS properties for 13′-*cis* (**1g**)[8] are compatible with the present findings.

The relative abundance of each geometrical isomer in the iodine-catalyzed stereomutation mixture is given in Table 2. The sequence all-*trans* (**1e**) > 9′-*cis* (**1h**) > 13′-*cis* (**1g**) > 13-*cis* (**1h**) > 9′,13′-di-*cis* (**1d**) > 13,9′-di-*cis* (**1b**) > 13,13′-di-*cis* (**1c**) > 15-*cis* (**1a**) reflects the relative configurational stability of the mono-*cis* and di-*cis* isomers.

VIS absorption data are presented in Table 2. Mono-*cis* isomers (**1a**, **f**, **g**, **h**) exhibited hypsochromic shifts of 2 nm (**1h**, 9′-*cis*), 4 nm (**1g**, 13′-*cis*), 6 nm (**1a**, 15-*cis*) and 8 nm (**1f**, 13-*cis*) for the main λ_{max} relative to the all-*trans* isomer (**1e**) recorded in the HPLC eluent (mainly hexane). Di-*cis* isomers (**1b**, **c**, **d**) showed generally larger, hypsochromic shifts of 8 nm (**1d**) and 10 nm (**1b** and **1c**), consistent with previous generalizations.[12] The term % III/II[16] describes spectral fine-structure and the term % D_B/D_{II} [16] the relative intensity of the so-called *cis*-peak.[12] Conjugated keto carotenoids have in general reduced spectral fine-structure.[12,17] In hexane all-*trans*-fucoxanthin (**1e**) had a % III/II value = 40. For the other geometrical isomers values from 0–35 % were recorded.

The intensity of the *cis*-peak has generally been assumed to depend on the angular shape of the polyene chain and to be higher for carotenoids with a near-to-central *cis*-bond.[12] The observed *cis*-peak intensities 15-*cis* (**1a**) > 13′-*cis* (**1g**) > 13-*cis* (**1f**) > 13,9′-di-*cis* (**1b**) > 9′,13′-di- (**1d**) > 13,13′-di-*cis* (**1c**) > 9′-*cis* (**1h**) > all-*trans* (**1e**) follow roughly the predicted order, cf. Table 2 and Schemes 2 and 3.

CD spectra were obtained for the all-*trans* (**1e**), the mono-*cis* isomers 9′-*cis* (**1h**), 13-*cis* (**1f**), 13′-*cis* (**1g**) and the 2:1 mixture of the di-*cis* isomers 9′,13′-di-*cis* (**1d**) + 13,13′-di-*cis* (**1c**). Conservative carotenoid CD spectra are characterized by a sequence of 5–6 relatively sharp Cotton effects of alternating sign, the rotatory strength of which roughly add up to zero. In general typical conservative spectra invert upon all-*trans* to mono-*cis* isomerization.[18] Previously the CD spectrum of fucoxanthin (**1e**) has been classified as non-conservative.[18] However, as seen from Fig. 2 the CD spectra, albeit with low $\Delta\varepsilon$ values, have conservative features in the sense that inversion of the Cotton effects roughly results from all-*trans* to mono-*cis* isomerization.

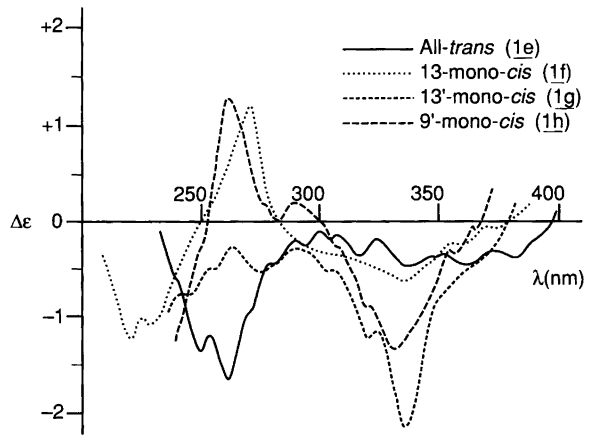


Fig. 2. CD spectra in EPA at room temperature of all-*trans*- (**1e**), 13-*cis*- (**1f**), 13′-*cis*- (**1g**) and 9′-*cis*- (**1h**) fucoxanthin.

Experimental

General methods. General precautions for work with carotenoids were taken. Solvents were of p.a. quality. Five different systems were used for analytical and semipreparative HPLC separations. System 1 was based on an Ultrasphere 5 CN column, system 2 and 5 on a Brownlee Labs. Si Speri-5 Column, system 3 (semipreparative) on a semipreparative Ultrasphere TH cyano column and system 4 on a Spherisorb S 5-W column. Eluents used were, for HPLC systems 1–3, hexane–isopropyl acetate–acetone–methanol 75.9 : 17 : 7 : 9 : 0.1 (eluent 1) and for systems 4 and 5 hexane–isopropyl acetate–isopropyl alcohol–*N*-ethyldi-isopropylamine 83.9 : 14 : 2 : 0.1 (eluent 2). Two instruments were used : a Perkin Elmer Series 2 LC equipped with a PYE 402 detector and a Hitachi 2000 D Chromato-

integrator (systems 1–3) and a Hewlett Packard HPLC system with diode array detector and on-line recording of VIS spectra (systems 2, 4 and 5). The detection wavelength for semipreparative HPLC (system 3) was 490 nm, and the detailed procedure is described elsewhere.[14]

VIS spectra were recorded on a Perkin Elmer 552 spectrophotometer with acetone or hexane as solvents. CD spectra were recorded on a Jobin Yvon Auto Dicrograph Mark IV or Mark II. EPA (diethyl ether–isopentane–ethanol 5 : 5 : 2) was used as the solvent. 1D ^{1}H NMR and 2D ^{1}H–^{1}H correlated (COSY) spectra were recorded on a 400 MHz AM-400 Bruker FT-instrument with $CDCl_3$ as the solvent.

The 2D COSY spectra (absolute value) were measured using standard Bruker software with spectral widths in F_1 and F_2 of only ca. 600 Hz covering in F_2 solely the olefinic range between approximately 5.9 and 7.4 ppm. Parameters: acquisition of typically 1K data points and 250 experiments, zero-filling to 1K in F_1 and sine-bell filtering in both directions prior to Fourier transformation. Total measuring time between 12 and 16 h depending on the sample quantity available, which was roughly estimated to be between 20 and 100 μg. The small spectral width in both directions ensures that a very good digital resolution of ca. 0.6 Hz/pt, and hence an excellent resolution in the crowded olefininc range is available. As has previously been shown[19] the connectivity between in-chain methyl groups and coupled olefinic protons is deducible from cross-peaks folded along F_1 into the acquired region.

*Isolation and characterization of all-*trans *fucoxanthin* (**1e**). Fucoxanthin was isolated from *Fucus serratus* as described elsewhere.[13,14] Crystallized **1e** had m.p. 164 °C (evacuated tube); R_f (TLC, silica plates, 0.5 mm, Kieselgel 60 Merck, hexane–acetone 7 : 3) = 0.26; t_R (system 4, flow 1.5 ml min^{-1}) = 19.0 min, t_R (system 3, flow 4.0 ml min^{-1}) = 15.2 min; VIS λ_{max} (hexane): (428), 446, 475 nm, % III/II = 40; (acetone): (420), 444 ($E^{1\%}_{1CM}$ = 1660), 467, % III/II = 5; FT-IR (KBr, ν_{max} cm^{-1}): 3483 (OH), 3030–2856 (CH), 1930 (allene), 1732 (C=O, acetate), 1654 (conjugated C=O), 1607, 1576, 1530, 1471, 1456 (CH_2), 1385 and 1367 (*gem.* Me), 1335, 1261, 1245 (C–O, acetate), 1201, 1175–1157, 1071, 1053, 1032, 958 (*trans* disubstituted C=C), 917; MS (210 °C, 70 eV) *m/z* (ion, intensity relative to base peak in %): 658 (*M*, 23), 640 (*M*−18, 37), 622 (*M*−18−18, 26), 580 (*M*−18−60, 21), 578 (*M*−80, 7), 562 (*M*−18−18−60, 13), 560 (*M*−18−80, 6), 488 (9), 484 (7), 482 (8), 237 (27), 221 (43), 212 (51), 197 (100); ^{1}H NMR ($CDCl_3$) see Scheme 2; CD (EPA = diethyl ether – isopentane – ethanol 5 : 5 : 2) nm (Δε): 214 (−3.12), 224 (−0.78), 233 (−0.75), 251 (−1.68), 261 (−1.76), 272 (−1.00), 301 (−0.34), 308 (−0.40), 317 (−0.32), 325 (−0.43), 333 (−0.22), 354 (−0.48), 367 (−0.53), 378 (−0.35), 383 (−0.40), 395 (0), > 395 (+).

Small-scale stereomutation of **1e**. Crystallized **1e** (0.4 mg) was isomerized in two parallel experiments, dissolved in CH_2Cl_2 or benzene (50 ml). Iodine (1–2 % of the carotenoid) was added and the solutions exposed to dim sunlight for 3 h. The isomerizations were monitored by VIS spectroscopy and HPLC. Both solvents resulted in the same isomeric composition. In benzene solution VIS λ_{max} 0 h: (440), 460, (483) nm, % D_B/D_{II} = 8; 3 h: (435), 457, (480), nm, % D_B/D_{II} = 14 and in CH_2Cl_2 solution VIS λ_{max} 0 h: 452, (470) nm, % D_B/D_{II} = 14; 3 h: 452 (470) nm, % D_B/D_{II} = 15.

Large-scale stereomutation of **1e**. Crystallized **1e** (20.7 mg) was dissolved in benzene (500 ml) and iodine (0.3 mg) was added. The solution was exposed to sunlight. The isomerization was monitored by HPLC. After 2 h the same photostationary equilibrium mixture as in the small-scale experiments was reached. The solution was concentrated to 1 ml and separated into six peaks, collected as six fractions by semipreparative HPLC – system 3, cf. details given elsewhere.[14] t_R values (system 3, flow 4.0 ml min^{-1}): fraction 1 (**1a**) 12.5 min, fraction 2 (**1b**) 13.1 min, fraction 3 (**1c** + **1d**) 13.8 min, fraction 4 (**1e**) 15.2 min, fraction 5 (**1f**) 15.8 min, fraction 6 (**1g** + **1h**) 16.6 min. VIS, ^{1}H NMR and CD spectra were recorded for fractions 2–6; for fraction 1 the VIS spectrum only was recorded. According to ^{1}H NMR data fractions 3 and 6 each contained a mixture of two stereoisomers. The stereoisomers in fraction 6 were subsequently separated by HPLC – system 4. Attempts to separate the two stereoisomers in fraction 3 were unsuccessful.

*15-*cis*-Fucoxanthin* (**1a**), tentative assignment fraction 1, available 8 μg (1 % of total); t_R (system 4, flow 1.5 ml min^{-1}) = 11.2 min, (system 3, flow 4.0 ml min^{-1}) = 12.5 min; VIS λ_{max} (acetone) 324, 436, (458) nm, % D_B/D_{II} = 33; (hexane) 327, (418), 442, (463) nm, % D_B/D_{II} = 45; (HPLC eluent 2) 325, (420), 439 (458) nm, % D_B/D_{II} = 55.

*13,9'-Di-*cis*-fucoxanthin* (**1b**), fraction 2, available 0.2 mg (4 % of total); t_R (system 4, flow 1.5 ml min^{-1}) = 15.3 min, (system 3, flow 4.0 ml min^{-1}) = 13.1 min; VIS λ_{max} (acetone) 325, (410), 433 (452) nm, % D_B/D_{II} = 41; (hexane) 328, (420), 438, 463 nm, % III/II = 2, % D_B/D_{II} = 36; (HPLC eluent 2) 329, (415), 435, (459) nm, % D_B/D_{II} = 40; ^{1}H NMR assignments Scheme 2.

*13,13'-Di-*cis*-fucoxanthin* (**1c**), fraction 3, available ca. 0.1 mg (3 % of total) in a ca. 1:2 mixture with **1d**, inseparable by HPLC systems 1–5; t_R (system 4, flow 1.5 ml min^{-1}) = 16.4 min (cf. 16.8 min for **1d**), (system 3, flow 4.0 ml min^{-1}) = 13.8 min; VIS λ_{max} (eluent 2) 329, (418), 435, (457) nm, % D_B/D_{II} = 26; 1H NMR assignments Scheme 2; CD in mixture with **1d**, see below.

*9',13'-Di-*cis*-fucoxanthin* (**1d**), fraction 3, available ca. 0.2 mg (6 % of total) in ca. 2:1 mixture with **1c**; t_R (system 4, flow 1.5 ml min^{-1}) = 16.8 min, (system 3, flow 4.0 ml min^{-1}) = 13.8 min; VIS λ_{max} (eluent 2) 329, (420), 437,

(461) nm, % D_B/D_{II} = 34; ^{1}H NMR assignments Scheme 2.

A ca. 2:1 mixture of **1d** + **1c** had VIS λ_{max} (acetone) 328, 434, (456), % D_B/D_{II} = 28; (hexane) 327, (420), 438, 465 nm, % III/II = 14, % D_B/D_{II} = 26; CD (EPA) nm Δε: 241 (−0.26), 251 (−0.50), 262 (−0.28), 277 (−0.59), 293 (0), 331 (+0.94), 349 (0), 353 (−0.12), 367 (−0.03), 324 (−0.04), 378 (0), 396 (+0.19), 411 (+0.03), > 411 (+).

All-trans-*fucoanthin* (**1e**), fraction 4, available 1.95 mg (42 % of total); t_R (system 4, flow 1.5 ml min^{-1}) = 19.0 min, (system 3, flow 4.0 ml min^{-1}) = 15.2 min; VIS λ_{max} (acetone) (420), 444, 467 nm % III/II = 6; (hexane) 329, 423, 448, 476 nm, % III/II = 40, % D_B/D_{II} = 8; (eluent 2) 330, (427), 445, 471 % III/II = 6, % D_B/D_{II} = 7; ^{1}H NMR assignments Scheme 3; CD (EPA) nm (Δε) 233 (−0.11), 250 (−1.36), 254 (−1.21), 261 (−1.65), 280 (−0.46), 281 (−0.47), 290 (−0.22), 293 (−0.24), 300 (−0.10), 305 (−0.20), 307 (−0.17), 316 (−0.28), 323 (−0.23), 337 (−0.48), 352 (−0.35), 362 (−0.49), 375 (−0.33), 378 (−0.34), 382 (−0.39), 396 (0), > 396 (+), see Fig. 2.

13-cis-*Fucoxanthin* (**1f**), fraction 5, available 0.18 mg (11 % of total); t_R (system 4, flow 1.5 ml min^{-1}) = 25.2 min, (system 3, flow 4.0 ml min^{-1}) = 15.8 min; VIS λ_{max} (acetone) 331, (414), 438, (458) nm, % D_B/D_{II} = 41; (hexane) 329, (420), 442, 467, % III/II = 8, % D_B/D_{II} = 38; (HPLC eluent 2) 329, (420), 437, (463) nm, % D_B/D_{II} = 45; ^{1}H NMR assignments Scheme 3; CD (EPA) nm (Δε) 221 (−1.22), 225 (−1.02), 229 (−1.08), 250 (0), 271 (+1.19), 283 (0), 335 (−0.61), 354 (−0.22), 357 (−0.24), 369 (−0.06), 372 (−0.07), 379 (0), > 379 (+), see Fig. 2.

13'-cis-*Fucoxanthin* (**1g**), fraction 6, available 0.4 mg (14 % of total) in a ca. 2:3 mixture with **1h**. Isomers **1g** and **1h** were inseparable in HPLC-systems 1–3, but separated in systems 4 and 5. Isomer **1g** had t_R (system 4, flow 1.5 ml min^{-1}) = 28.4 min, (system 3, flow 4.0 ml min^{-1}) = 16.6 min; VIS λ_{max} (eluent 2) 329, (422), 441, (466) nm, % D_B/D_{II} = 52; ^{1}H NMR assignments Scheme 3; (EPA) nm (Δε): 224 (−0.74), 245 (−0.74), 255 (−0.50), 258 (−0.29), 275 (−0.53), 289 (−0.30), 293 (−0.31), 304 (−0.52), 306 (−0.51), 320 (−1.23), 325 (−1.17), 336 (−2.15), 378 (0), > 378 (+), see Fig. 2.

9'-cis-*Fucoxanthin* (**1h**), fraction 6, available 0.6 mg (20 %) of total in a ca. 3:2 mixture with **1g**, cf. subsequent separation from **1g** above. Isomer **1h** had t_R (system 4, flow 1.5 ml min^{-1}) 32.0 min, (system 3, flow 4.0 ml min^{-1}) = 16.2; VIS λ_{max} (eluent 2) 327, (424), 443, 469 nm, % III/II = 2, % D_B/D_{II} = 12; ^{1}H NMR assignments, see Scheme 3; CD (EPA) nm (Δε) 261 (+1.39), 276 (+0.21), 282 (+0.03), 289 (+0.20), 301 (0), 304 (−0.15), 321 (−0.89), 331 (−1.35), 354 (−0.30), 364 (−0.04), 367 (0), > 367 (+), see Fig. 2.

A 3:2 mixture of **1h** and **1g** had VIS λ_{max} (acetone) 330 (418), 441, 460 nm, % D_B/D_{II} = ,27; (*n*-hexane) 329, (422), 444, 472 nm, % III/II = 35, % D_B/D_{II} = 17; CD (EPA) nm (Δε) 238 (−1.27), 263 (0), 277 (+0.67), 302 (+0.04), 310 (+0.09), 315 (0), 330 (−0.33), 341 (−0.45), 377 (0), > 377 (+).

References

1. Bjørnland, T. and Liaaen-Jensen, S. In: Green, J., Leadbeater, B. S. C. and Diver, W. L., Eds., *Chromophyte Algae: Problems and Perspectives,* Clarendon, Oxford 1989, p. 37.
2. Strain, H. H., Svec, W. A., Wegfahrt, P., Rapoport, H., Haxo, F. T., Norgård, S., Kjøsen, H. and Liaaen-Jensen, S. *Acta Chem. Scand., Ser. B 30* (1976) 109.
3. Bonnett, R., Spark, A. A., Tee, J. L. and Weedon, B. C. L. *Proc. Chem. Soc.* (1964) 419.
4. Jensen, A. *Acta Chem. Scand. 20* (1966) 1728.
5. Jensen, A. *Carotenoids in Norwegian Brown Seaweeds and Seaweed Meals.* Report No. 31. Norw. Institute of Seaweed Research, Tapir 1966.
6. Bonnett, R., Mallams, A. K., Spark, A. A., Tee, J. L., Weedon, B. C. L. and McCormick, A. *J. Chem. Soc. C* (1969) 469.
7. DeVille, T. E., Hursthouse, M. B., Russell, S. W. and Weedon, B. C. L. *Chem. Commun.* (1969) 1311.
8. Bernhard, K., Moss, G. P., Tóth, G. and Weedon, B. C. L. *Tetrahedron Lett.* (1974) 3899.
9. Bjørnland, T., Skjetne, T. and Liaaen-Jensen, S. *Abstr. 8th Int. Carot. Symp.,* Boston 1987, P3.
10. Bjørnland, T., Englert, G., Bernhard, K. and Liaaen-Jensen, S. *Tetrahedron Lett. 30* (1989) 2577.
11. Englert, G., Bjørnland T. and Liaaen-Jensen, S. *Magn. Reson. Chem. 28* (1990) 519.
12. Zechmeister, L. cis–trans *Isomeric Carotenoids, Vitamins A and Arylpolyenes,* Springer, Wien 1962.
13. Haugan, J. A. and Liaaen-Jensen, S. *Phytochemistry 28* (1989) 2797.
14. Haugan, J. A., Aakermann, T. and Liaaen-Jensen, S. *Adv. Enzymol. In press.*
15. Englert, G. In: Britton, G. and Goodwin, T. W., Eds. *Carotenoid Chemistry and Biochemistry,* Pergamon, Oxford 1982, p. 107.
16. Ke, B., Imsgard, F., Kjøsen, H. and Liaaen-Jensen, S. *Biochim. Biophys. Acta 210* (1970) 139.
17. Vetter, W., Englert, G., Rigassi, N. and Schwieter, U. In: Isler, O. Ed., *Carotenoids,* Birkhäuser, Basel 1971, Chap. 4.
18. Sturzenegger, V., Buchecker, R. and Wagniére, G. *Helv. Chim. Acta 63* (1980) 1074.
19. Englert, G., Glinz, E. and Liaaen-Jensen, S. *Magn. Reson. Chem. 26* (1988) 55.

Received August 2, 1991.