Pergamon

Tetrahedron 56 (2000) 9047–9056

TETRAHEDRON

Gyroxanthin—the First Allenic Acetylenic Carotenoid☆

Terje Bjørnland,† Anne Fiksdahl, Tore Skjetne, Jostein Krane and Synnøve Liaaen-Jensen*

Organic Chemistry Laboratories, Department of Chemistry and Biology, Norwegian University of Science and Technology, N-7491, Gløshaugen, Trondheim, Norway

Received 12 May 2000; accepted 14 July 2000

Abstract—Selected recent studies on marine allenic and acetylenic carotenoids are focused on. The structure elucidation of gyroxanthin, (3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*R*)-5,6-epoxy-6,7,7′,8′-tetradehydro-5,6,5′,6′-tetrahydro-β,β-carotene-3,19,3′,5′-tetrol, is reported in detail and in historical perspective. Gyroxanthin occurs as a diester (19-dodecanoate 3′-acetate) in the dinoflagellate *Gymnodinium galatheanum*.

Introduction

The field of carotenoids, including isolation, structure elucidation, the application of modern spectroscopic methods, total synthesis, biosynthesis and metabolism, has been reviewed in a current book series.[1–4] Carotenoids contribute significantly to the bright colours of the marine environment, particularly in tropical waters, and serve different functions. Various aspects of marine carotenoids were surveyed in 1978 in one of Scheuer's monographs.[5]

Acetylenic and allenic carotenoids are common in many marine organisms. Acetylenic carotenoids are synthesized de novo only in microalgae. The marine, allenic carotenoid peridinin (**1**, Fig. 1) from phytoplankton and fucoxanthin (**2**) from macroalgae and phytoplankton are the carotenoids produced in largest quantity in Nature.

Allenic and acetylenic carotenoids have served as useful chemosystematic markers in algae[4–7] and as food chain indicators.[4] Certain filter-feeding marine animals possess the ability to convert allenic carotenoids metabolically to acetylenic analogues.[8] Chemical and stereochemical aspects of allenic and acetylenic carotenoids were reviewed in 1990.[9]

A more recent example of a complex allenic carotenoid is (3*S*,5*R*,3′*S*,5′*R*,6′*S*)-13′-*cis*-7′,8′-dihydroneoxanthin-20′-al 3′-β-lactoside (**3**, Fig. 1), which is a cross-conjugated carotenal.[10] The total synthesis of optically active peridinin (**1**) and fucoxanthin (**2**) have been successfully accomplished in recent years.[11,12] Studies on the chemistry of fucoxanthin (**2**) have been extended. Treatment with bases provides yellow hemiketals[13] and acids lead to the formation of blue oxonium ions.[14] In this context a recently isolated 4-keto-19′-hexanoyloxyderivative of fucoxanthin (**2**) with a strongly oxygenated end group, showed unexpected chemistry including facile cleavage to a C_{31}-skeleton apocarotenone.[15] Allenic carotenoids possess an *R*-configured chiral axis. Attention has been focused on the possible natural occurrence of *S*-allenic carotenoids.[16,17] Studies on allenic *R*/*S* isomerization have been conducted[18–20] and diphenyl diselenide has been shown to be an excellent promotor for the photochemical stereoisomerization of allenic carotenoids,[21] also of acid-labile allenic epoxide-containing carotenoids such as neoxanthin (**4**, Fig. 1).[22] Allenic isomerization is always accompanied by more facile geometrical isomerization.[19–22]

Marine acetylenic carotenoids include structurally interesting representatives such as the C_{37} butenolide pyrrhoxanthin (**5**),[23] bastaxanthin (**6**), a carotenoid sulphate from sponges,[24] and oxabicycloheptane-derivatives such as eutreptiellanone (**7**),[25] Fig. 2. Recently the total syntheses of optically inactive pyrrhoxanthin (+/−**5**)[11] and of all-*E* (3*R*,3′*R*)-diatoxanthin (**8**) were achieved,[26] as well as of the thermodynamically more stable 9*Z* isomer of **8**.[27]

In this paper is presented the structure elucidation of gyroxanthin, including experimental details, the first example of an allenic, acetylenic carotenoid. The work is treated in a historical perspective, demonstrating the development in the carotenoid field. Gyroxanthin diester was first isolated in the late seventies as the minor 'Carotenoid 2' from monoalgally cultured material of the dinoflagellate *Gyrodinium* sp.-A.[28] Chromatographic, VIS

☆ Algal Carotenoids 65, no. 64 Ref. 15.

Keywords: gyroxanthin; carotenoid; allenic; acetylenic; *Gyrodinium*.
* Corresponding author. Tel.: +47-73-594099; fax: +47-73-594256; e-mail: slje@chembio.ntnu.no
† Present address: Department of Chemistry and Biotechnology, P.O. Box 5040, Agricultural University of Norway, N-1432 Ås, Norway.

0040–4020/00/$ - see front matter
PII: S0040-4020(00)00757-2

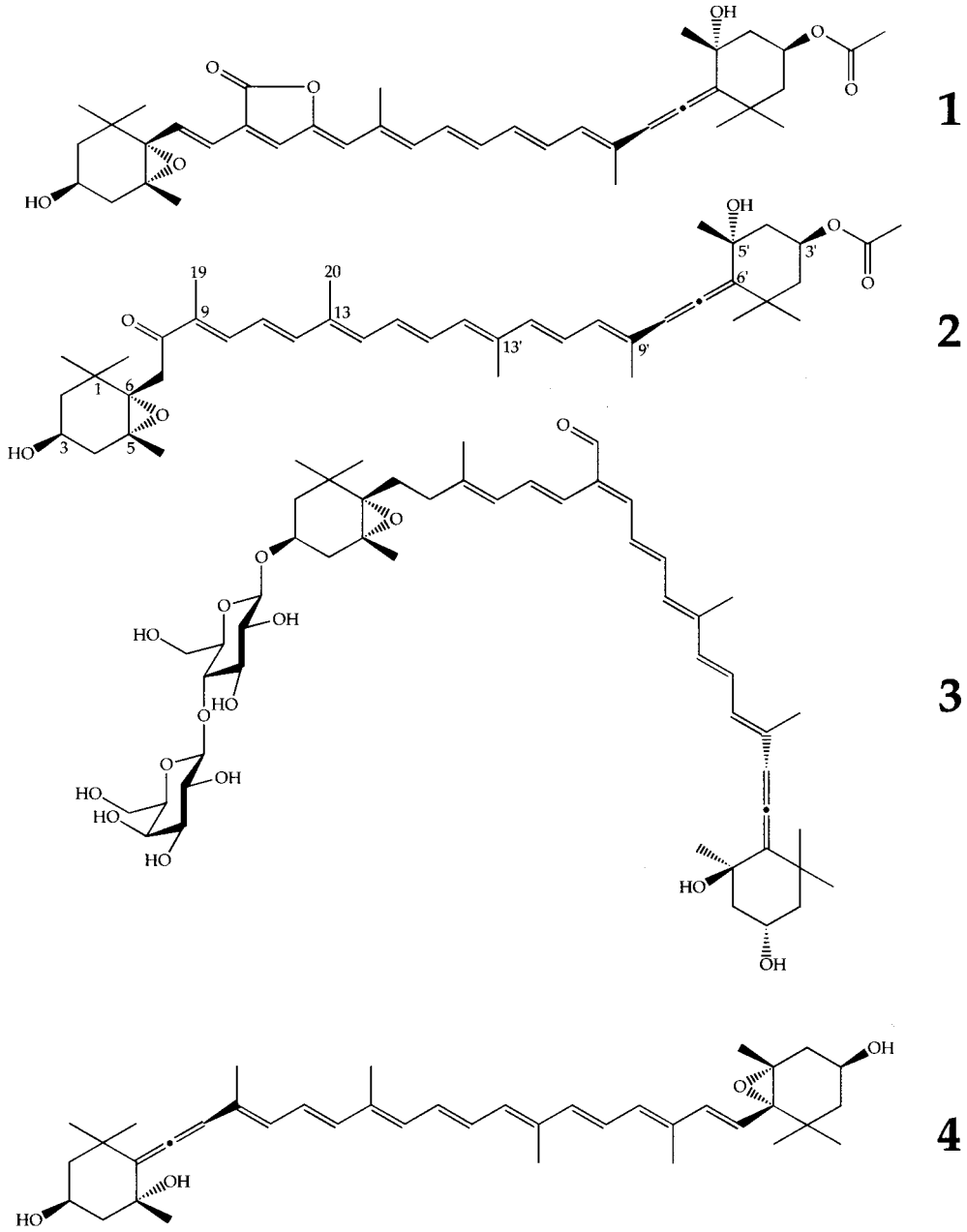

Figure 1. Structures of selected marine, allenic carotenoids.

and mass spectral properties of the native ester and its alkaline hydolysis product were not compatible with the data for previously known algal carotenoids, and the name gyroxanthin was suggested for the free xanthopyll. Later it was recognized that the algal isolate was identical with *Gymnodinium galatheanum* Braarud (Tangen, K. Personal Communication), described from material collected during the Galathea expedition.[29]

Results and Discussion

Gyroxanthin diester, isolated separately from two different isolates, was a minor carotenoid of *G. galatheanum*, accounting for 11% (isolate 76-D) of the total carotenoids.[28] The xanthophylls accompanying gyroxanthin diester were identified by methods including VIS, MS and ^{1}H NMR spectroscopy as the acetylenic diatoxanthin (**8**), diadinoxanthin (**9**), halocynthiaxanthin 3′-acetate (**10**) and 19′-acyloxyhalocynthiaxanthin 3′-acetate (**10a**) and the allenic fucoxanthin (**2**), fucoxanthin 3-acetate (**2a**), 19′-hexanoyloxyfucoxanthin (**11**) and 19′-butanoyloxyfucoxanthin (**11a**),[28,30,31] Fig. 3.

Micro scale chemical derivatization in combination with VIS, MS and NMR data in conjunction with biosynthetic analogies (Fig. 3), lead to plausible structures of gyroxanthin diester (**12**) and free gyroxanthin (**15**), Fig. 4.

Natural gyroxanthin diester (**12**) provided one acetate (**13**) upon standard acetylation and a di(trimethylsilyl) ether (**14**) upon silylation, and therefore possessed one *prim*/*sec* and

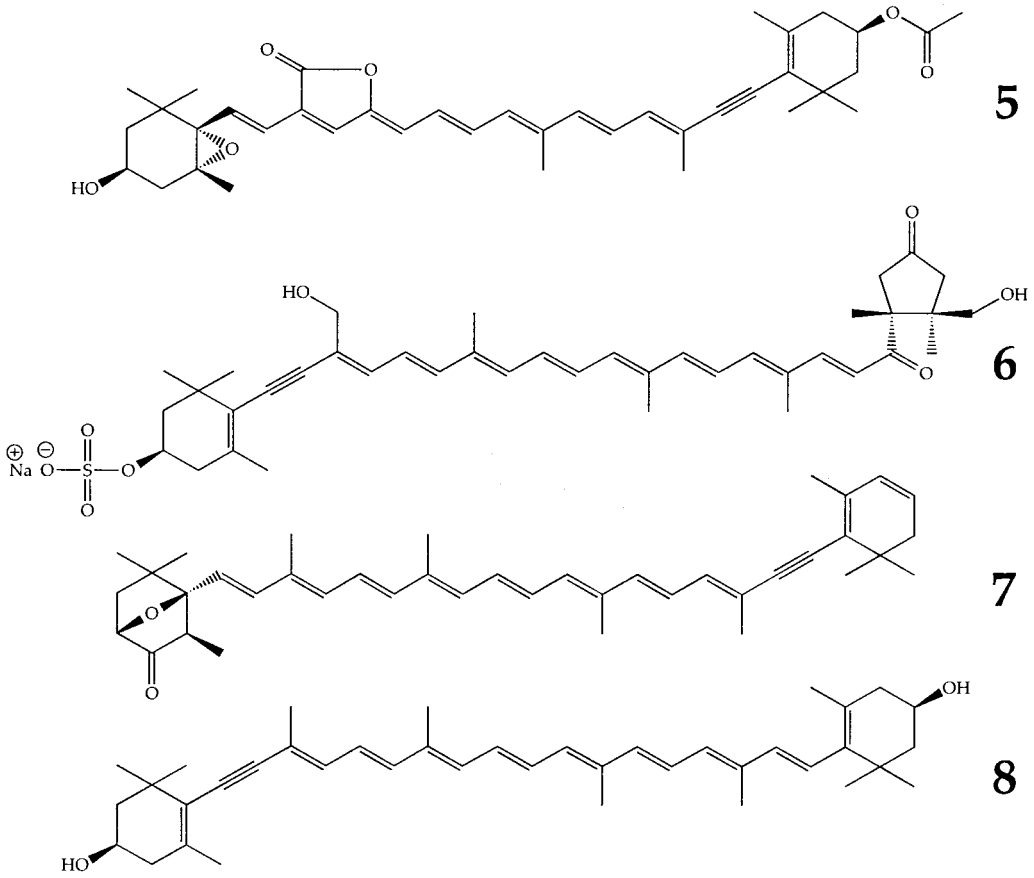

Figure 2. Structures of selected, marine acetylenic carotenoids.

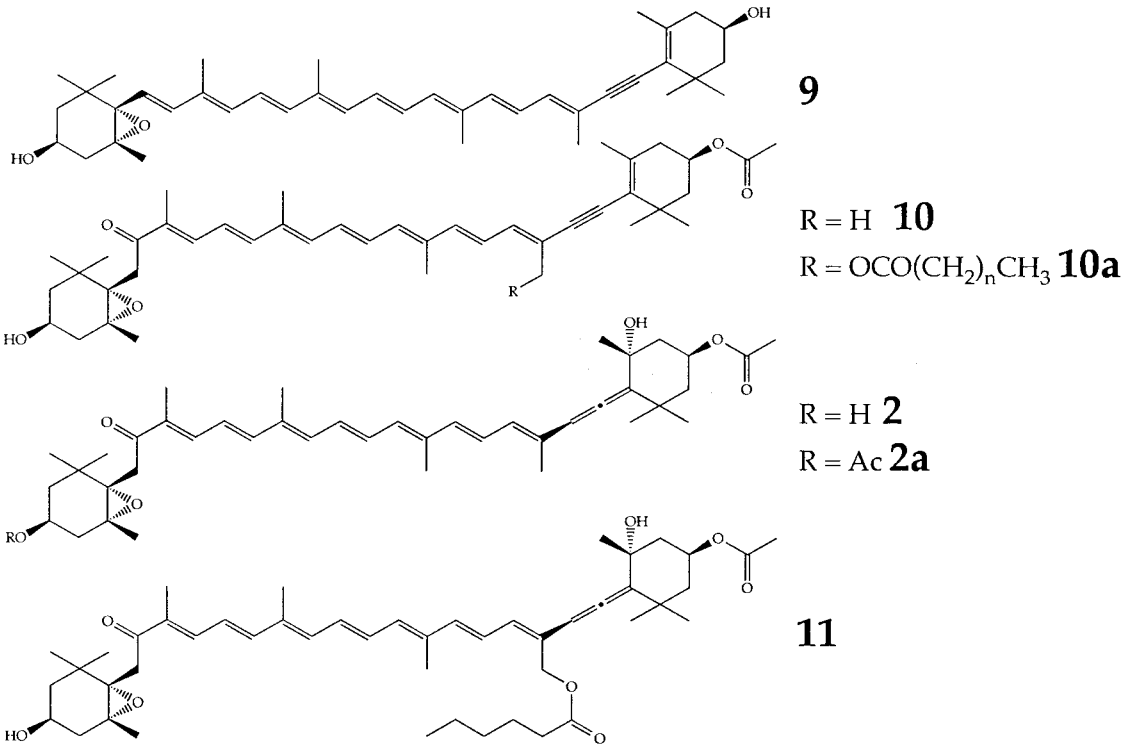

Figure 3. Allenic and acetylenic xanthophylls accompanying gyroxanthin diester (**12**) in *G. galatheanum*, also including **8** (Fig. 2).

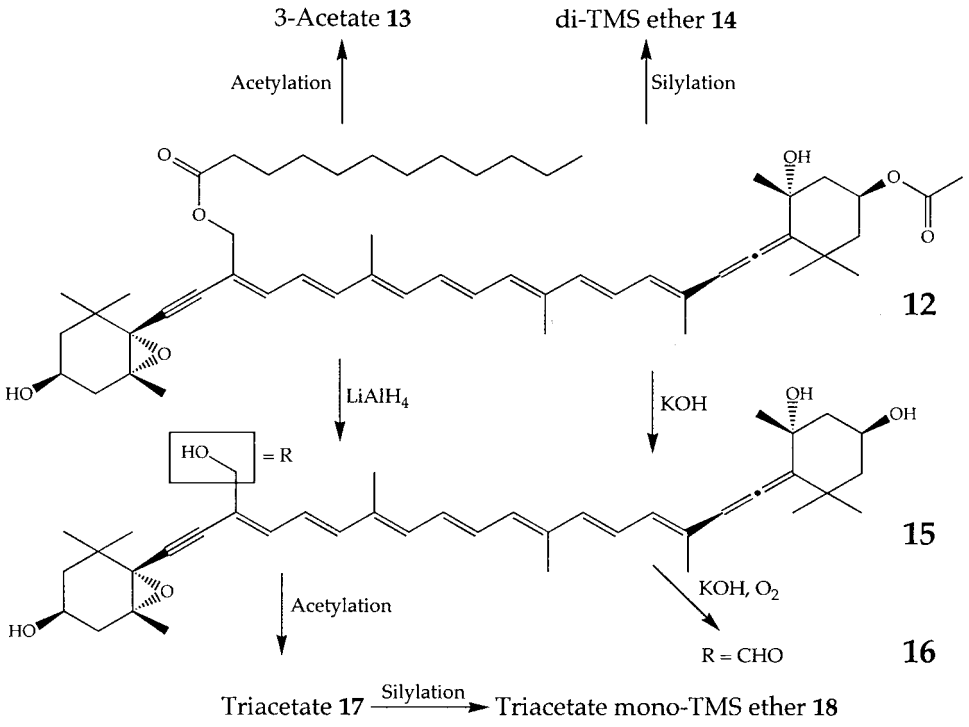

Figure 4. Chemical derivatizations of natural gyroxanthin diester (**12**).

one *tert* hydroxy group. In a kinetic silylation experiment with 19′-hexanoyloxyfucoxanthin (**11**) 3-acetate as parallel it was shown that one hydroxy group in gyroxanthin diester was silylated fast (*prim*/*sec*), whereas the second one was silylated as slowly as the *tert* C-5′ hydroxy group of **11** 3-acetate. The presence of one *prim*/*sec* and one *tert* hydroxy group in gyroxanthin diester was compatible with its chromatographic adsorption between fucoxanthin acetate (**2a**) and diatoxanthin (**8**).

Alkaline hydrolysis of gyroxanthin diester (**12**) gave the more polar derivative gyroxanthin (**15**), $C_{40}H_{54}O_5$, which provided a triacetate **17** upon standard acetylation. The latter (**17**) further gave a mono(trimethylsilyl) ether **18** upon silylation. This accounted for four of the five oxygen functions of gyroxanthin (**15**) as three *prim*/*sec* and one *tert* hydroxy group. It could be inferred that natural gyroxanthin diester (**12**) has two *prim*/*sec* hydroxy groups esterified.

Support for the natural diester **12** being a monoacetate came from its mass spectrum with characteristic losses of 60 mass units and a ^{1}H NMR 3 proton singlet at δ 2.04 which disappeared upon saponification to gyroxanthin (**15**). The second ester function was, judged by the mass spectrum of the natural gyroxanthin diester, mainly an ester with a saturated $C_{12:0}$ fatty acid (**12**), partly also with a $C_{14:0}$ fatty acid (**12a**). That one esterified hydroxy group was primary, followed from the characteristic shift of a 2 proton singlet from δ 4.78 in the ^{1}H NMR spectrum of the natural diester (**12**) to δ 4.23 for gyroxanthin (**15**). Further support was obtained from a pink autoxidation product gyroxanthal (**16**), obtained as a by-product upon alkali treatment of the diester **12**. Relative polarity by TLC and the round-shaped ca. 25 nm bathochromically shifted VIS spectrum of **16** were compatible with the formation of a cross-conjugated in-chain aldehyde function. Similar facile formation of cross-conjugated carotenals has been observed for *prim* in-chain carotenols.[32,33]

Mild $LiAlH_4$-reduction of gyroxanthin diester (**12**) gave a single product identical with gyroxanthin (**15**) obtained from the alkaline hydrolysis. Consequently gyroxanthin diester (**12**) contained no carbonyl functions apart from the two ester groups, and the fifth oxygen attached to its carbon skeleton was inert. However, no common epoxide–furanoxide rearrangement[1] was obtained upon treatment of **12** with weak acid.

The IR spectrum of gyroxanthin diester (**12**) showed the presence of an allene bond (1930 cm^{-1}) and the ^{1}H NMR spectra of **12** and **15** were compatible with the presence of an allenic fucoxanthin (**2**) type end-group. Two typical in-chain methyl singlets were observed (δ 1.94 and δ 1.96). The *prim* esterified hydroxy group of **12** exhibited the same chemical shift as in 19′-hexanoyloxyfucoxanthin (**11**). A 3 proton singlet at δ 1.80 was attributed to the third in-chain methyl group of a common dicyclic carotenoid. The special chemical shift was tentatively ascribed to a neighbouring allene bond.

A 7 nm hypsochromic shift without loss of spectral fine-structure was observed going from gyroxanthin diester (**12**) to gyroxanthin (**15**). In-chain substituted carotenoids are known to give stable *cis*-isomers[32] and the spectral changes observed were compatible with 9-*trans* to 9-*cis* isomerization. Thus the ^{1}H NMR spectrum of gyroxanthin diester (**12**) exhibited two $-CH_2O-$ acyl singlets at δ 4.78 and 4.64 in ratio 4:1, ascribed to 9-*trans* and 9-*cis* configuration, respectively.[34] Saponification caused a single

signal for CH_2OH at δ 4.23. Considering established changes in chemical shift (0.44 ppm) from esterified to free *prim* carotenol in related systems[34] the observed value for **15** was consistent with complete isomerization to 9-*cis* during the saponification procedure.

The observed tendency to obtain the preferred 9-*cis* configuration was further supported by the established preference of 9-*cis* configuration in acetylenic carotenoids.[35,36] The acetylenic bond was also consistent with the mass spectral fragmentation pattern: Observed losses of 92 mass units (toluene) and lacking losses of 106 mass units (xylene) upon electron impact of gyroxanthin and all its derivatives may be accounted for by a triple bond in 7,8-position, cfr. the established sites for toluene and xylene expulsion from carotenoids on electron impact.[37] The presence of the acetylenic bond also rationalized the negative epoxide test. However, the expected, frequently weak absorption for the acetylenic bond in the IR spectrum[35] around 2170 cm^{-1} could not be detected. Support for the acetylenic bond was obtained from the partly assigned ^{13}C NMR spectrum of a partly 9-*cis* isomerized diester **12**; signals at δ 86.1 and 88.1 for C-7 and C-8, cf.[38]

There were still uncertainties related to the structure of gyroxanthin diester (**12**). These were mainly related to the end group with the new and unique combination of a 5,6-epoxidic and a 7,8-acetylenic structural element next to a substituted in-chain hydroxy function. Moreover, the stereochemistry of gyroxanthin (**15**) and its derivatives was not firmly established. Isolation of the pure all-*trans* **12** and 9-*cis* **12** geometrical isomers and the application of 1 and 2D ^1H NMR techniques, FT-IR and CD spectroscopy served to substantiate the previous assignments.

Gyroxanthin diester from isolate 76-D and 76-E showed the same mass spectral properties. This included diagnostic peaks resulting from elimination of neutral fragments with 18, 60, 92 (but not 106), 200 and 228 m.u. and combinations of these. Of special importance was the *m/z* 212 ion. In a detailed study of the electron impact fragmentation of peridinin (**1**)[39] this ion was shown to originate from the allenic part of the molecule. This result was later extended to include a series of structurally related allenic carotenoids with and without a chemically derived in-chain methyl group adjacent to the allene bond.[40] The *m/z* 212 ion was therefore a clear indication that the derivatized in-chain methyl group in gyroxanthin diester was not next to the allene bond but in position 9, 13 or 13′. Based on the relative intensities of the molecular ions the relative proportion of laurate and myristate esters was similar in the two isolates. The identity of the acid moieties as long-chain *n*-fatty acids was deduced from the MS and the ^1H NMR data.

1D and 2D ^1H NMR of all-*trans* **12** allowed assignment of chemical shifts for all protons in the molecule, Fig. 5. All-*trans*-gyroxanthin diester (**12**) had the full complement of proton signals previously reported for the allenic end-group of fucoxanthin (**2**).[41,42] This included the Me-singlet for the acetate group (δ 2.06), the broad multiplet for H-3′$_{ax}$ (δ 5.38) and the singlet for the allenic proton H-8′ (δ 6.06). This consistency implies the same relative configuration for the three stereogenic carbons and the allenic axis in this end-group of **12** and fucoxanthin (**2**). For generic reasons the enantiomeric end-group with 6′*R*-chirality is preferred. No 6*S*-allenic carotenoids have so far been detected in nature.[17]

From the ^1H-^1H 2D spectra of **12** it was possible to firmly locate the triple bond, now disclosed by a weak FT-IR-absorption at 2209 cm^{-1}, to the 7,8-position: in the central chain segment C-9′ to C-13′ there was a long-range cross-peak between Me-19′ and H-10′, which allowed assignment

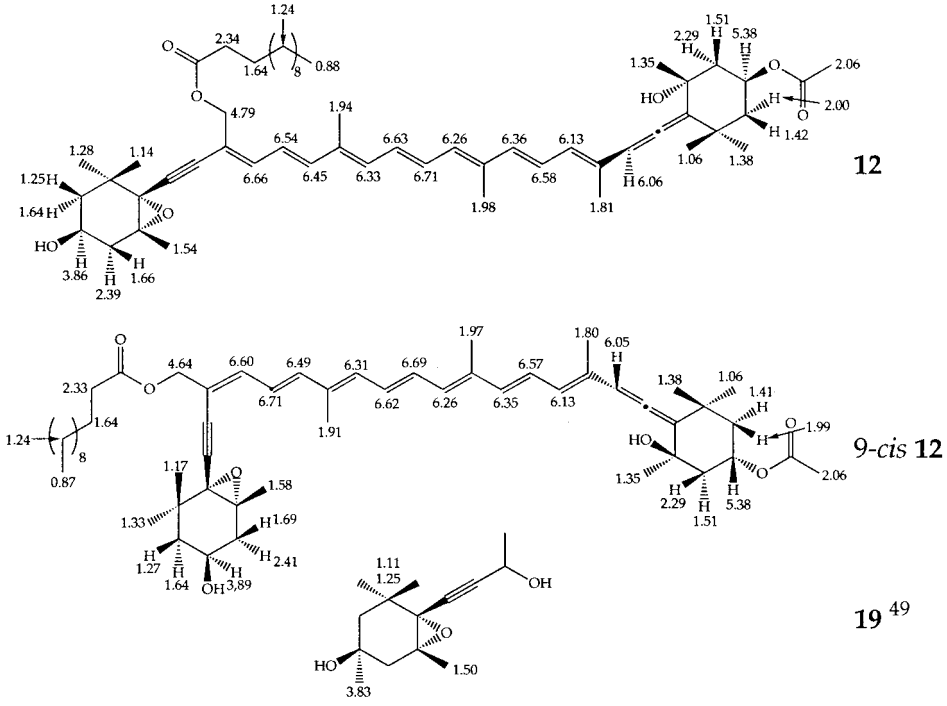

Figure 5. ^1H NMR assignments of all-*trans* gyroxanthin diester (**12**), 9-*cis* **12** and the synthetic model **19**.[49]

of the δ 6.13 doublet to H-10′. Chemical shifts for H-11′ and H-12′ were then given by the connectivities of the 2D spectrum. In the next segment (C-13′ to C-13) both Me-groups (Me-20′ and Me-20) had long-range cross-peaks with one proton each within the segment. Together with the connectivities for the olefinic region this allowed chemical shift assignments for all protons in this segment. By comparison with the ^{1}H NMR data for fucoxanthin[41,42] the Me-singlet at δ 1.98 is assigned to Me-20′. In the last segment (C-9 to C-13) there was a weak long-range cross-peak between CH_2-19 and H-10, which together with the connectivities within this segment accounted for the rest of the olefinic proton signals. C-7 and C-8 therefore had to be connected by a triple bond.

The ^{1}H–^{1}H 2D NMR spectra also gave support for an epoxide function in the 5,6-position. Both rings carried one proton which gave rise to a broad multiplet, typical for methine protons of a secondary alcohol or ester function. In the allenic part of the molecule this proton showed the same coupling pattern as in fucoxanthin (**2**).[41,42] In the acetylenic end this proton showed a strong coupling with one and a weak coupling with two vicinal protons. Each of these vicinal protons gave a strong geminal coupling to one proton of their own. Accordingly, both C-2 and C-4 carried two protons each, and this located the hydroxy group at C-3 and the epoxide function in the 5,6-position. The coupling pattern of the ring protons was further supported by simple decoupling experiments at δ 3.86 (H-3$_{ax}$) and δ 2.39 (H-4$_{eq}$). The left end-group in gyroxanthin diester, now established with a 5,6-epoxide and a 7,8-acetylenic function, has previously not been reported for naturally occurring carotenoids.

Gyroxanthin diester was slowly transformed to a yellow slightly more polar (by TLC) carotenoid during chromatographic manipulations and storage. Based on ^{1}H NMR spectrometric evidence from partly transformed native gyroxanthin diester a 9-*cis* structure for this product was assumed. Firm evidence for this structure based on spectrometric studies of a TLC-pure sample could be obtained.

After extraction and purification by repeated TLC the all-*trans* **12** and the 9-*cis* isomer were obtained in yields of 0.87 mg (33%) and 1.80 mg (67%), respectively. The mass spectrum of 9-*cis* **12** revealed the same molecular weight as for native gyroxanthin diester (**12**). The similarity in fragmentation pattern, including relative intensities of the diagnostic peaks, confirmed the same structure formula. Stereochemical differences, not revealed by MS, were evident from the VIS, CD and ^{1}H NMR: The VIS properties of **12** and 9-*cis* **12** were comparable regarding spectral fine structure. For 9-*cis* **12**, however, λ_{max} showed a hypsochromic displacement of 4 nm, and the *cis*-peak, $D_B/D_{II}(\%)$[43]=10, was small. The small hypsochromic displacement, the similarity in spectral fine structure and the low intensity of the *cis*-peak confirmed the assignment of a geometrical isomer with a single *cis*-bond near the end of the chromophore.[44] The position of the *cis*-bond was established by ^{1}H NMR: complete chemical shift assignments based on 1D and 2D ^{1}H NMR were made along the same lines as already described for the all-*trans* isomer **12**, Fig. 5. The spectral data for the all-*trans* and 9-*cis* isomers were similar, including an identical set of signals for an allenic fucoxanthin-type end-group.[38] There were, however, increasing small shift differences for the olefinic protons towards the left end of the polyene chain, which were also apparent for the ring methyl groups (Me-16,17,18) and CH_2-19 and Me-20 as well. These differences were as would be expected for a 9-*cis* geometrical isomeric relationship. The isomerization shifts $\Delta=\delta_{cis}-\delta_{trans}$ in ppm located the *cis*-bond to the 9-position, see Englert.[38]

We are now turning to the chiroptical properties of all-*trans* gyroxanthin diester (**12**) and 9-*cis* **12**. Certain carotenoids exhibit socalled conservative CD spectra with several maxima and minima where the Cotton effect is integrating to around zero.[45] Unexpectedly, conservative CD spectra, albeit of low intensity, were observed. Moreover, opposite Cotton effects for the all-*trans* and 9-*cis* isomer were evident, Fig. 6. When attempting to rationalize the Cotton effects of the gyroxanthin diesters the following data should be kept in mind. The bisallenic carotenoid mimulaxanthin exhibits a weak, non-conservative CD.[46] Judged by the

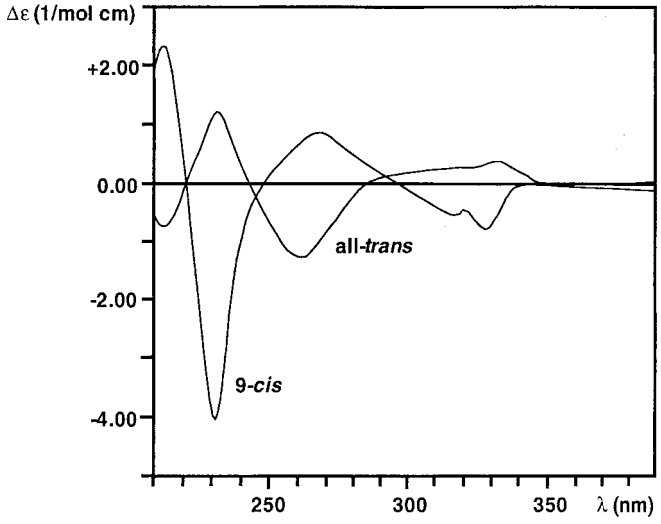

Figure 6. CD spectra of all-*trans* gyroxanthin diester (**12**) and of 9-*cis* **12** in EPA solution at room temperature.

modification of the strong conservative CD in the series zeaxanthin-diatoxanthin (**8**)-alloxanthin the introduction of 7,8 (7′,8′) triple bond(s) causes a gradual reduction of the intensity of the Cotton effect. A similar effect is seen for astaxanthin-7,8-didehydroastaxanthin.[47] The 3-hydroxy-5,6-epoxy end group is known to cause a strong conservative CD, governed by the epoxy function.[47] In-chain substituted carotenoids may cause modifications of the Cotton effect.[47] Gyroxanthin diester (**12**) is a 7,8-didehydro-derivative of neoxanthin (**4**) with an in-chain substituent. Qualitatively there is a similarity in the CD spectra of the gyroxanthin diesters (**12**, 9-*cis* **12**) and the neoxanthins (**4**, 9-*cis* **4**)[48] which may be taken to support the same absolute configuration of the epoxy group in **4** and **12**. Since the 3-hydroxy group does not contribute noticably to the Cotton effect of carotenoid 5,6-epoxides, the configuration at C-3 is not settled by CD. However the chemical shifts in the ^{1}H NMR spectra of the methyl groups and the C-3 methine proton in 15 versus the synthetic model **19**,[49] Fig. 5, lend support to the same relative configuration for the epoxidic end group.

The above conclusion was substantiated by NOE experiments with all-*trans* **12**, supporting the relative configuration and a preferred twisted chair form of the epoxidic end group, see Experimental.

Conclusion

The complete structure of gyroxanthin diester (3*S*,5*R*, 6*S*,3′*S*,5′*R*,6′*R*)-7,8-didehydro-19-hydroxy-neoxanthin 3′-acetate 19-laurate, the first allenic acetylenic carotenoid containing a novel epoxidic acetylenic in-chain substituted end group, is considered proved by chemical and spectroscopic evidence (VIS, FT-IR, MS, ^{1}H NMR, ^{13}C NMR and CD).

Experimental

Biological material

Culture conditions, harvesting and extraction of *Gymnodinium galatheanum* Braarud were as previously described for the synonymous *Gyrodinium* sp.-A.[28] Isolate 76-D provided 12 mg **12** after chromatography. Isolate 76-E provided pure **12** and 9-*cis* **12**.

General methods

General precautions for work with carotenoids were taken.[1] Methods were as generally employed in the Trondheim laborarory.[1,2,50] Saponification, acetylation, silylation, $LiAlH_4$-reduction and epoxide tests were carried out by standard procedures.[33] ^{1}H NMR spectra were recorded in $CDCl_3$ on Bruker 400 and 500 MHz instruments. For the mass spectra only important diagnostic peaks above *m/z* 197 are cited. CD spectra were measured on a Jobin Yvon Auto Dicrograph Mark IV in EPA (Et_2O–*i*-C_5H_{12}–C_2H_5OH 5:5:2; v/v) solution. If not otherwise specified TLC was carried out on Si gel (0.5 mm) with developing solvents stated in each case.

Isolation of the carotenoids

The carotenoids were separated by TLC-1 on Si gel–$CaCO_3$ 1:1; (w/w) as adsorbent[51] and developed with light petrol–Me_2CO–C_6H_6–*i*-PrOH 75:20:4:1 (v/v). Rechromatography was performed by TLC-2 with Si gel–$Ca(OH)_2$–MgO–$CaSO_4$ 10:4:3:1 (w/w) as adsorbent.[51] The characterization and quantification of the xanthophylls **2**, **2a**, **8**, **9**, **10**, **10a**, **11** and **11a**[28,30,31] are not included here. From isolate 76-E the separation of **12** and 9-*cis* **12** was achieved on the further refined systems[52] TLC-3 (Si gel–$CaCO_3$ 4:1; w/w, developed with *n*-C_6H_{14}–Me_2CO–*i*-PrOH 68.5:30:1.5; v/v) followed by TLC-4 (Si gel–kieselguhr–$Ca(OH)_2$–MgO 14:16:9:9; w/w, using the same mobile phase as for TLC-3).

All-*trans* gyroxanthin diester (12). Available amount of chromatographically pure diester (TLP-3 and TLP-4) from isolate 76-E was 0.87 mg (0.203 mg/g lipid-extracted dry-wt). VIS. λ_{max} (nm in *n*-C_6H_{14}): 418, 442 and 470, III/II(%)[43]=76; λ_{max} (nm in Me_2CO): (423), 444 and 472, III/II(%)=55. For quantitative calculations the average extinction coefficient $E_{1\ cm}$ (1%)=2500 was anticipated for the free xanthophyll, corresponding to $E_{1\ cm}$ (1%)=1830 for the diester. MS. *m/z* (rel. int. in %): 866 $[M_1]^+$(1), 848 $[M_1-18]^+$(3), 838 $[M_2]^+$(16), 820 $[M_2-18]^+$(30), 804 (1), 802 (1), 788 $[M_1-18-60]^+$(0.6), 778 $[M_2-60]^+$(0.5), 760 $[M_2-18-60]^+$(5), 746 $[M_2-92]^+$(1), 744 (1), 728 $[M_2-18-92]^+$(0.7), 640 (6), 638 $[M_1-228,\ M_2-200]^+$ (6), 620 $[M_1-18-228,\ M_2-18-200]^+$(6), 582 (5), 264 (12), 236 (19), 212 (allenic end-group) (36), 200 (36) and 197 (allenic end-group) (100). ^{1}H NMR. δ-values (500 MHz, $CDCl_3$, D-locked): 0.88t (*J*=6.9 Hz, 3H, Me of laurate and myristate), 1.06s (3H, Me-17′), 1.14s (3H, Me-17), 1.24s (ca. 18H, CH_2 of laurate and myristate), 1.25 (H-2_{ax}), 1.28s (ca. 3H, Me-16), 1.35s (3H, Me-18′), 1.38s (3H, Me-16′), 1.42 (H-$2'_{ax}$), 1.51 (H-$4'_{ax}$), 1.54s (3H, Me-18), 1.64m (H-2_{eq}, β-CH_2 of laurate and myristate), 1.66 (H-4_{ax}), 1.81s (3H, Me-19′), 1.94s (3H, Me-20), 1.98s (3H, Me-20′), 2.00 (H-$2'_{eq}$), 2.06s (3H, acetate–Me at C-3′), 2.29d (broad) (J_{gem}=13 Hz, 1H, H-$4'_{eq}$), 2.34t (*J*=7.7 Hz, 2H, α-CH_2 of laurate and myristate), 2.39dd (J_{gem}=14 Hz, $J_{4eq,3ax}$=5 Hz, 1H, H-4_{eq}), 3.86m (1H, H-3_{ax}), 4.79s (2H, CH_2-19), 5.38m (1H, H-$3'_{ax}$), 6.06s (1H, H-8′), 6.13d ($J_{10',11'}$=11.1 Hz, 1H, H-10′), 6.26d ($J_{14',15'}$=11.5 Hz, 1H, H-14′), 6.33d ($J_{14,15}$=11.3 Hz, 1H, H-14), 6.36d ($J_{12',11'}$=14.8 Hz, 1H, H-12′), 6.45d ($J_{12,11}$=14.7 Hz, 1H, H-12), 6.54dd ($J_{11,10}$=11.4 Hz, $J_{11,12}$=14.6 Hz, 1H, H-11), 6.58dd ($J_{11',10'}$=11.3 Hz, $J_{11',12'}$=14.3 Hz, 1H, H-11′), 6.63dd ($J_{15,14}$=ca. 11.3 Hz, $J_{15,15'}$=ca. 14.3 Hz, 1H, H-15), 6.66d ($J_{10,11}$=11.3 Hz, 1H, H-10) and 6.71dd ($J_{15',15}$=14.5 Hz, $J_{15',14'}$=11.1 Hz, 1H, H-15′); chemical shifts and connectivities were confirmed by decoupling experiments of H-4_{eq}, H-3_{ax} and H-12. Difference NOE experiments[53] were conducted. The resonances at 1.25 and 1.66 ppm were positively effected by the methyl group at 1.54 ppm (cf. Fig. 5). Irradiation at 1.14 ppm caused NOE to 1.64 ppm, but no effect to 1.25 ppm, compatible with a twisted chair form where the protons at 1.64 ppm come closer to the irradiated position than those at 1.25 ppm. CD. $\Delta\epsilon$ (EPA) (nm): 213 (−0.8), 220 (0), 232 (+1.2), 243 (0), 260 (−1.3), 285 (0), 334 (+0.4) and ca. 354 (0), Fig. 6.

9-*cis* Gyroxanthin diester (9-*cis* 12). Available amount of chromatographically pure diester (TLP-3 and TLP-4) from isolate 76-E was 1.80 mg (0.422 mg/g lipid-extracted dry-wt). VIS. λ_{max} (nm in *n*-C_6H_{14}): 325, 414, 438 and 465, III/II(%)=75, D_B/D_{II}(%)[43]=10; λ_{max} (nm in Me_2CO): (420), 440 and 467, III/II(%)=60. The same extinction coefficient, $E_{1\ cm}$ (1%)=1830, as for the all-*trans* isomer was used for calculation of wt amount. FT-IR. ν_{max} (cm^{-1} in KBr) (128 scans): 3428vs (broad) (OH); 2958s, 2924s and 2853s (CH); 2209vw (acetylene) cf.[54]; 1930m (allene); 1737vs (ester carbonyl); 1670w, 1607w (conj. C=C); 1578w; 1534m; 1456s (CH_2); 1378s and 1365s (*gem*. CH_3); 1247s (C–O of ester); 1162s (tert. OH); 1113w and 1030m (C–O–); 966s (*trans* disubst. C=C); 894m and 857m (trisubst. C=C); 721m. MS. *m/z* (rel. int. in %): 866 $[M_1]^+$(0.3), 848 $[M_1-18]^+$(0.9), 838 $[M_2]^+$(9), 820 $[M_2-18]^+$(30), 804 (0.8), 802 (0.6), 778 $[M_2-60]^+$(0.3), 760 $[M_2-18-60]^+$(2), 746 $[M_2-92]^+$(2), 728 $[M_2-18-92]^+$(0.3), 638 [M_1-228, $M_2-200]^+$(5), 620 [$M_1-18-228$, $M_2-18-200]^+$(5), 212 (allenic end-group) (30) and 197 (allenic end-group) (100). ^1H NMR. δ-values (500 MHz, $CDCl_3$, D-locked): 0.87t (J=6.7 Hz, 3H, Me of laurate and myristate), 1.06s (3H, Me-17′), 1.17s (3H, Me-17), 1.24s (>20H, CH_2 of laurate, myristate and contaminants), 1.27 (H-2_{ax}), 1.33s (3H, Me-16), 1.35s (3H, Me-18′), 1.38s (3H, Me-16′), 1.41 (H-$2'_{ax}$), 1.51 (H-$4'_{ax}$), 1.58s (3H, Me-18), 1.64m (H-2_{eq}, β-CH_2 of laurate and myristate), 1.68 (H-4_{ax}), 1.80s (3H, Me-19′), 1.91s (3H, Me-20), 1.97s (3H, Me-20′), 1.99 (H-$2'_{eq}$), 2.06s (3H, acetate–Me at C-3′), 2.29d (broad) (J_{gem}=13 Hz, 1H, H-$4'_{eq}$), 2.33t (J=7.6 Hz, 2H, α-CH_2 of laurate and myristate), 2.41ddd (J_{gem}=14 Hz, $J_{4eq,3ax}$=5 Hz, $J_{4eq,2ax}$=ca. 1.5 Hz, 1H, H-4_{eq}), 3.89m (1H, H-3_{ax}), 4.64s (2H, CH_2-19), 5.38m (1H, H-$3'_{ax}$), 6.05 (1H, H-8′), 6.13d ($J_{10',11}$=11.3 Hz, 1H, H-10′), 6.26d ($J_{14',15'}$=11.2 Hz, 1H, H-14′), 6.31d ($J_{14,15}$=11.1 Hz, 1H, H-14), 6.35d ($J_{12',11'}$=15.0 Hz, 1H, H-12′), 6.49d ($J_{12,11}$=15.1 Hz, 1H, H-12), 6.57dd ($J_{11',10'}$=11.3 Hz, $J_{11',12'}$=14.7 Hz, 1H, H-11′), 6.60d ($J_{10,11}$=11.2 Hz, 1H, H-10), 6.62dd ($J_{15,14}$=10.9 Hz, $J_{15,15}$=14.0 Hz, 1H, H-15), 6.69dd ($J_{15',14'}$=ca. 11 Hz, $J_{15',15}$=ca.14 Hz, 1H, H-15′) and 6.71dd ($J_{11,10}$=11.1 Hz, $J_{11,12}$=14.8 Hz, 1H, H-11). CD. $\Delta\epsilon$ (EPA) (nm): 213 (+2.3), 221 (0), 232 (−4.0), 249 (0), 270 (+0.9), 298 (0), 330 (−0.8) and ca. 343 (0), Fig. 6.

Gyroxanthin diester 3-acetate (13). Standard acetylation of **12** gave **13**; R_f 0.55 (40% Me_2CO–*n*-C_6H_{14}; v/v), less polar than **12**. VIS. λ_{max} nm (Me_2CO) 412, 435 and 462, III/II(%)=45. MS. *m/z* (rel. int. in %) 908 $[M_1]^+$(0.8), 880 $[M_2]^+$(0.9), 848 $[M_1-60]^+$(3), 213 (100), 197 (89).

Gyroxanthin diester di(trimethylsilyl)ether (14). Standard silylation of **12** gave **14**; R_f=0.80 (40% Me_2CO–*n*-C_6H_{14}; v/v). MS. *m/z* (rel. int. in %) 1010 $[M_1]^+$(0.3), 982 $[M_2]^+$(0.5), 892 $[M_2-90]^+$(1), 197 (31), 181 (100). In a kinetic silylation experiment with **12** and **11** 3-acetate, monitored by TLC, the following percentage composition of total carotenoid was estimated: 5 min: 100% **12** mono-TMS ether, 100% unreacted **11** 3-acetate; 10 min: 90% **12** mono-TMS ether and 10% **14**, 70% unreacted **11** 3-acetate and 30% **11** 3-acetate mono-TMS ether; 30 min: 40 % **12** mono-TMS ether and 60% **14**, 100% **11** 3-acetate mono-TMS ether; 60 min: 10% **12** mono-TMS ether and 90% **14**.

Gyroxanthin (15). Compound **15** was obtained by standard saponification (4 h) with KOH or by $LiAlH_4$ treatment of **12**; R_f=0.07 (40% Me_2CO–*n*-C_6H_{14}; v/v). VIS. λ_{max} (nm in Me_2CO) 408, 434 and 461, III/II(%)=69. MS. *m/z* (rel int. in %) 614 $[M]^+$(25), 596 $[M-18]^+$(27), 578 $[M-18-18]^+$(18), 562 $[M-16-18-18]^+$(9), 546 $[M-16-16-18-18]^+$(4), 522 $[M-92]^+$(3), 197 (100). *IR*. ν_{max} (cm^{-1} in KBr): 3400 (OH), 2980, 2850 (CH), 1930 (allene), 1455 (CH_2), 1380 (*gem*. metyl), 1100, 1045 (*tert*OH), 970 (*trans* disubst. C=C), 955, 850 (trisubst. C=C), 725. ^1H NMR. (400 MHz, $CDCl_3$, δ-values): 1.06s/1.34s (3+3H, Me-16′/17′), 1.14s/1.17s and 1.17s/1.35s (6H, Me-16/17 in all-*trans* and 9-*cis*), 1.35s (3H, Me-18′), 1.50s/1.56s (3H, Me-18 in all-*trans* and 9-*cis*), 1.67m (H-2,2′), 1.81s (3H, Me-19′), 1.86s/1.92s (3H, Me-20 in all-*trans*/9-*cis*), 1.97s (3H, Me-20′), 2.29d/2.46d (2H, O*H* at 3 and 5′), 2.43dd (1H, J_{gem}=10 Hz, J_{vic}=4 Hz, H-4), 3.50s/3.78m (2H, O*H* in 19 and 3′), 3.89m (H-3), 4.22s/4.325s (2H, CH_2-19 in 9-*cis*/all-*trans*), 4.33m (H-3′), 6.05s (1H, H-8′), 6.09d (J=11.4 Hz, 1H, H-10′), 6.22d (J=11.0 Hz, 1H, H-10), 6.26d (J=11.3 Hz, H-14′), 6.31d (J=15.0 Hz, 1H, H-12′), 6.24d (J=15.2 Hz, 1H, H-14), ca. 6.43 (H-12), 6.55dd (J=11.4 Hz, J=15.0 Hz, 1H, H-11′), 6.55–6.65m (2H, H-11,15′), 6.77dd (J=15.2 Hz, J=11.0 Hz, 1H, H-15). Chemical shifts and connectivities were confirmed by decoupling experiments of H-2, H-4, H-3, H-10′, H-12′, H-11 and H-14, H-15, H-14′ and H-10. Signals for O*H*-3,5′,19′ and 3′ at δ 2.29, 2.46, 3.50 and 3.78 disappeared upon shaking with D_2O. ^{13}C NMR. (100 MHz, $CDCl_3$, δ-values) tentative assignments: 12.6/12.8 (Me 20,20′), 13.9 (Me-19′), 24.6 (Me-18), 29.6 (Me-18′), 31.2/32.1 (Me-16′/17′), 31.4/31.6 (Me-16,17), 36.0 (C-1,1′), 45.4 (C-2), 45.8 (C-4′), 46.6 (C-2), 49.3 (C-4), 53.7 (C-19), 64.1 (C-3,3′), 65.7 (C-5′), 72.9 (C-5), 82,4 (C-6), 86.1(C-7), 88.1 (C-8), 103.1 (C-8′), 117.5 (C-6′), 122.0 (C-9), 125.1 (C-11), 125.7 (C-11′), 128.3 (C-10′), 132.2 (C-9′), 137.1 (C-12′), 140.2 (C-10), 202.2 (C-7′), 129.7, 131.0, 132,2, 134.4, 135.7, 137.1 (C-12,13,14,15,13′,14′ and 15′); unassigned signals: 48.9, 68.4, 76.7. Standard epoxide test in Et_2O/HCl caused no change in the VIS spectrum.

Gyroxanthal (16). Compound **16** (8% of recovered carotenoid) was obtained as a by-product upon alkaline hydrolysis of **12** to **15** (92%); R_f=0.12 (40% Me_2CO–*n*-C_6H_{14}; v/v). VIS. λ_{max} (nm in Me_2CO) 460.

Gyroxanthal (16) 3-acetate. Obtained as a minor compound upon TLC of 12; R_f=0.20 (40% Me_2CO–*n*-C_6H_{14}; v/v). VIS. λ_{max} (nm in Me_2CO) 461. MS. *m/z* (rel. int. in %) 636 $[M-18]^+$(9), 618 $[M-18-18]^+$(13), 576 $[M-18-60]^+$(16), 197 (100). Alkaline hydrolysis provided **16**.

Gyroxanthin triacetate (17). Compound **17** was obtained by standard acetylation of **15**; R_f=0.50 (40% Me_2CO–*n*-C_6H_{14}; v/v). VIS. λ_{max} (nm in Me_2CO) (414), 437 and 464, III/II(%)=43. MS. *m/z* (rel. int. in %) 740 $[M]^+$(6), 722 $[M-18]^+$(9), 706 $[M-16-18]^+$(3), 698 $[M-42]^+$(4), 680 $[M-60]^+$(5), 662 $[M-18-60]^+$(5), 648 $[M-92]^+$(7), 620 $[M-60-60]^+$(8), 604 $[M-16-60-60]^+$(7), 239 (100), 211 (66), 197 (72).

Gyroxanthin triacetate mono(trimethylsilyl)ether (18). Compound **18** was obtained upon silylation of 17;

R_f=0.75 (40% Me_2CO–*n*-C_6H_{14}; v/v). VIS. λ_{max} (nm in Me_2CO) (412), 435 and 463, III/II(%)=40; MS. *m/z* (rel. int. in %) 812 $[M]^+$(2), 745 $[M-67]^+$(1), 722 $[M-90]^+$(1), 604 (12), 239 (100), 211(75).

Acknowledgements

We thank Dr G. Englert, formerly Hoffmann-La Roche, Basel, for the ^1H NMR spectrum of **15**. This work was in part supported by a grant from Hoffmann-La Roche, Basel, to S. L. J.

References

1. *Carotenoids: Isolation and Analysis*, Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, 1995; Vol. 1A.
2. *Carotenoids: Spectroscopy*, Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, 1995; Vol. 1B.
3. *Carotenoids: Synthesis*, Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, 1996; Vol. 2.
4. *Carotenoids: Biosynthesis and Metabolism*, Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, 1998; Vol. 3.
5. Liaaen-Jensen, S. Marine carotenoids. *Marine Natural Products, Chemical and Biological Perspectives*; Scheuer, P., Ed.; Academic: New York, 1978; pp 2–73.
6. Bjørnland, T.; Liaaen-Jensen, S. Distribution patterns of carotenoids in relation to chromophyte phylogeny and systematics. *The Chromophyte Algae, Problems and Perspectives*; Green, J. C., Leadbeater, B. S. C., Diver, W. L., Eds.; Clarendon: Oxford, 1989; pp 37–61.
7. Liaaen-Jensen, S.; Egeland, E. S. Microalgal carotenoids. *Chemicals from Microalgae*; Cohen, Z., Ed.; 1999, pp 145–172.
8. Partali, V.; Tangen, K.; Liaaen-Jensen, S. *Comp. Biochem. Physiol.* **1990**, *B92*, 239–246 (Errata **1990**, *B95*, 869).
9. Liaaen-Jensen, S. Allenic and acetylenic carotenoids. *Natural Products Chemistry*; Atta-ur-Rahman, T. I., Ed.; Elsevier: Amsterdam, 1990; pp 133–169.
10. Englert, G.; Aakermann, T.; Liaaen-Jensen, S. *J. Nat. Prod.* **1995**, *58*, 1675–1682.
11. Yamano, Y.; Ito, M. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1599–1610.
12. Yamano, Y.; Tode, C.; Ito, M. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1895–1904.
13. Haugan, J. A.; Liaaen-Jensen, S. *Acta Chem. Scand.* **1992**, *46*, 614–624.
14. Haugan, J. A.; Liaaen-Jensen, S. *Acta Chem. Scand.* **1994**, *48*, 68–75.
15. Egeland, E. S.; Garrido, J. L.; Zapata, M.; Maestro, M. A.; Liaaen-Jensen, S. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1223–1230.
16. Liaaen-Jensen, S. *Pure Appl. Chem.* **1997**, *69*, 2027–2038.
17. Strand, A.; Kvernberg, K.; Karlsen, A. M.; Liaaen-Jensen, S. *Biochem. Syst. Ecol.* **2000**, in press.
18. Yamano, Y.; Sumiya, S.; Suzuki, K.; Kurimoto, Y.; Koyama, Y.; Shimamura, T.; Ito, M. *Tetrahedron Lett.* **1992**, *33*, 2991–2994.
19. Haugan, J. A.; Englert, G.; Aakermann, T.; Glinz, E.; Liaaen-Jensen, S. *Acta Chem. Scand.* **1994**, *48*, 769–779.
20. Haugan, J. A.; Liaaen-Jensen, S. *Tetrahedron Lett.* **1994**, *35*, 2245–2248.
21. Refvem, T.; Strand, A.; Kjeldstad, B.; Haugan, J. A.; Liaaen-Jensen, S. *Acta Chem. Scand.* **1999**, *53*, 114–123.
22. Strand, A.; Liaaen-Jensen, S. *J. Chem. Soc., Perkin Trans. 1* **2000**, 595–598.
23. Aakermann, T.; Liaaen-Jensen, S. *Phytochemistry* **1992**, *31*, 1779–1782.
24. Hertzberg, S.; Ramdahl, T.; Johansen, J. E.; Liaaen-Jensen, S. *Acta Chem. Scand.* **1983**, *B37*, 267–280.
25. Fiksdahl, A.; Bjørnland, T.; Liaaen-Jensen, S. *Phytochemistry* **1984**, *23*, 649–655.
26. Haugan, J. A.; Liaaen-Jensen, S. *Acta Chem. Scand.* **1994**, *48*, 899–904.
27. Haugan, J. A.; Liaaen-Jensen, S. *Tetrahedron: Asymmetry* **1994**, *5*, 1781–1793.
28. Bjørnland, T.; Tangen, K. *J. Phycol.* **1979**, *15*, 457–463.
29. Braarud, T. *Galathea Rep.* **1957**, *1*, 137–138.
30. Fiksdahl, A. PhD Thesis, University of Trondheim-NTH, 1983.
31. Bjørnland, T.; Tangen, K.; Bjerkeng, B.; Pereira, C.; Hegge, K.; Guillard, R. R. L.; Liaaen-Jensen, S. Eighth Intetnational Symposium on Carotenoids; P40, Boston, 1987.
32. Aasen, A. J.; Liaaen-Jensen, S. *Acta Chem. Scand.* **1967**, *21*, 2185–2204.
33. Puntervold, O.; Liaaen-Jensen, S. *Acta Chem. Scand.* **1975**, *B28*, 1096–1097.
34. Chae, C. H.; Song, P.-S.; Johansen, J. E.; Liaaen-Jensen, S. *J. Am. Chem. Soc.* **1977**, *99*, 5609.
35. Weedon, B.; C, L. *Rev. Pure Appl. Chem.* **1970**, *20*, 51–66.
36. Fiksdahl, A.; Tauber, D. L.; Liaaen-Jensen, S.; Saucy, G.; Weber, F. *Acta Chem. Scand.* **1979**, *33*, 192–196.
37. Enzell, C. R.; Bach, S. Mass spectrometry. In *Carotenoids: Spectroscopy*. Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; 1995; Vol. 1B, pp 261–320.
38. Englert, G. NMR spectroscopy. In *Carotenoids: Spectroscopy*. Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, 1995; Vol. 1B, pp 147–260.
39. Kjøsen, H.; Norgård, S.; Liaaen-Jensen, S.; Svec, W. A.; Strain, H. H.; Wegfahrt, P.; Rapoport, H.; Haxo, F. T. *Acta Chem. Scand.* **1976**, *B30*, 157–164.
40. Bjørnland, T.; Liaaen-Jensen, S.; Throndsen, J. *Phytochemistry* **1989**, *28*, 3347–3353.
41. Englert, G.; Bjørnland, T.; Liaaen-Jensen, S. *Magn. Reson. Chem.* **1990**, *28*, 519–528.
42. Haugan, J. A.; Englert, G.; Glinz, E.; Liaaen-Jensen, S. *Acta Chem. Scand.* **1992**, *46*, 389–395.
43. Ke, B.; Imsgard, F.; Kjøsen, H.; Liaaen-Jensen, S. *Biochim. Biophys. Acta* **1970**, *210*, 139–152.
44. Zechmeister, L. *cis–trans* Isomeric Carotenoids and Aryl Polyenes, Springer: Wien, 1962.
45. Sturzenegger, V.; Buchecker, R.; Wagnière, G. *Helv. Chim. Acta* **1980**, *63*, 1074–1092.
46. Baumeler, A.; Eugster, C. H. *Helv. Chim. Acta* **1991**, *74*, 469–486.
47. Buchecker, R.; Noach, K. Circular dichroism. In *Carotenoids: Spectroscopy*. Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, 1995; Vol. 1B, pp 63–116.
48. Baumeler, A.; Zerbe, O.; Kunz, R.; Eugster, C. H. *Helv. Chim. Acta* **1994**, *77*, 909–930.
49. Russel, S. W.; Weedon, B. C. L. *J. Chem. Soc., Chem. Commun.* **1969**, 85–86.
50. Johansen, J. E.; Svec, W. A.; Liaaen-Jensen, S.; Haxo, F. T. *Phytochemistry* **1974**, *13*, 2261–2271.
51. Bjørnland, T.; Aguilar-Martinez, M. *Phytochemistry* **1976**, *15*, 291–296.

52. Repeta, D. J.; Bjørnland, T. Preparation of carotenoid standards. Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods; In *Monographs on Oceanographic Methods*, Jeffrey, S. W., Mantoura, R. F. C., Wright, S. W., Eds.; UNESCO: Paris, 1997; Vol. 10, pp 239–260.

53. Friebolin, H. *Basic One- and Two-Dimensional NMR Spectroscopy*, 2nd ed.; VCH: New York, 1993.

54. Bernhard, K.; Grosjean, M. Infrared spectroscopy. In *Carotenoids: Spectroscopy*. Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, 1995; Vol. 1B, pp 117–134.