Acta Chemica Scandinavica, 1993: 47: 1207–1213
Printed in Belgium – all rights reserved

ACTA
CHEMICA SCANDINAVICA
ISSN 0904–213X

Algal Carotenoids 55. Structure Elucidation of (3*S*,5*R*,6*R*,3'*S*,5'*R*,6'*S*)-13'-*cis*-7',8'-Dihydroneoxanthin-20'-al 3'-β-D-Lactoside (P457). Part 1. Reisolation, Derivatization and Synthesis of Model Compounds

Torunn Aakermann,[a] Robert R. L. Guillard[b] and Synnøve Liaaen-Jensen[a]

[a]Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway and [b]Bigelow Laboratory for Ocean Sciences, McKown Point, West Boothbay Harbor, Maine 0475, USA

Aakerman, T., Guillard, R. R. L. and Liaaen-Jensen, S., 1993. Algal Carotenoids 55. Structure Elucidation of (3*S*,5*R*,6*R*,3'*S*,5'*R*,6'*S*)-13'-*cis*-7',8'-Dihydroneoxanthin-20'-al 3'-β-D-Lactoside (P457). Part 1. Reisolation, Derivatization and Synthesis of Model Compounds. – Acta Chem. Scand. 47: 1207–1213. © Acta Chemica Scandinavica 1993.

The structure of the title compound, a minor glycosidic carotenoid isolated from peridinin-producing dinoflagellates, has been investigated by chemical and spectroscopic methods.

This carotenoid represents one of the most complex carotenoid structures known, containing structural elements such as lactoside (first example), cross-conjugated C_{40}-carotenal, allene, 5,6-epoxide and saturated C-7,8 bond.

By comparison of the ^{1}H NMR data for the *syn*- and *anti*-hydroxy epoxide model compounds prepared here with ^{1}H NMR data for P457, published in detail elsewhere, the position of the epoxide function and the glycosylated C-3' hydroxy group appears to be *anti* in P457.

The polar, minor carotenoid given the preliminary designation P457 (λ_{max} 457 nm in acetone) was first isolated in the early seventies from five different dinoflagellates.[1] The absorption spectrum in visible light (VIS) was compatible with an octaene chromophore conjugated with a carbonyl group. No molecular ion of acetylated P457 could be observed upon electron impact, but a fragment ion at *m/z* 331 was indicative of a tetraacetyl hexoside structure. Silylation of acetylated P457 provided a TMS ether, compatible with the presence of one hydroxy group on a tertiary carbon.

Reisolation of P457 from symbiotic zooxanthellae,[2] revealed, by $LiAlH_4$ reduction, a glycoside structure rather than a glycosyl ester of a carotenoid carboxylic acid. A fragment ion at *m/z* 619.1880 ($C_{26}H_{35}O_{17}$) was consistent with the oxonium ion of a heptaacetyl dihexoside. Structural elements such as hydroxy, allene and conjugated carbonyl were evident from the IR data. The disaccharide involved was considered to be gentiobiose or cellobiose from comparison with known ^{1}H NMR data. Subsequent reisolation and ^{1}H NMR studies including extensive decoupling experiments of acetylated P457 favoured a cross-conjugated carotenal chromophore, and the partial structure **1**, Scheme 1, was suggested.[3]

Improved methodology has now permitted the reisolation and structure elucidation of P457. Detailed NMR studies of P457 itself and its hepta- and octa-acetate, in full agreement with the structure **2**, Scheme 1, will be reported in Part 2.[4] The mass cultivation of dinoflagellates, isolation and purification of P457, chemical derivatizations and the synthesis of relevant model compounds are covered in this paper.

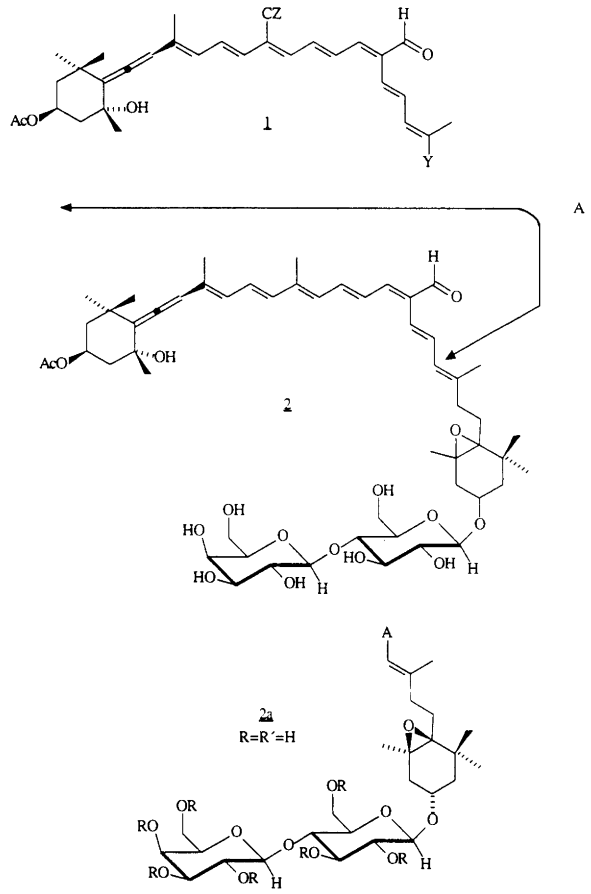
Results and discussion

Reisolation. Several modifications of the isolation procedure were investigated in order to optimize separation from colourless contaminants. The recommended procedure given in Scheme 2 provided 1 mg pure P457 from 30 g lyophilized cells, originating from ca. 200 l of axenic culture of the dinoflagellate *Amphidinium carterae*.

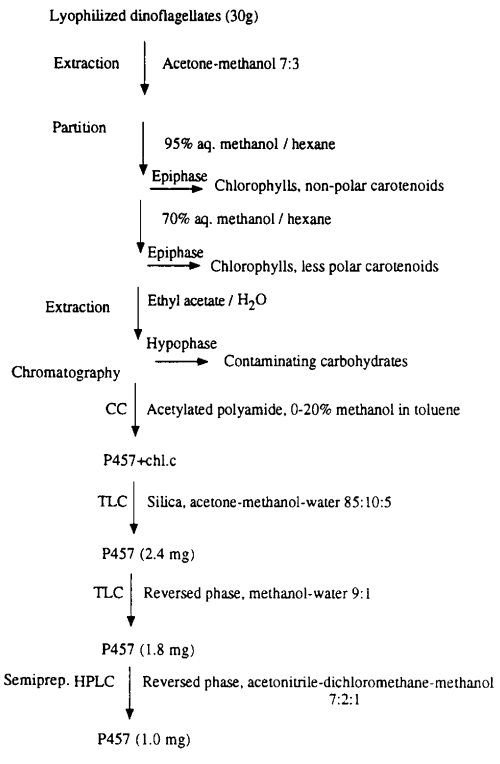
Structural evidence–derivatizations. Evidence in favour of structure **2a**, Scheme 1, for P457, including an *anti* configuration of the lactoside/epoxy function in the primed end group, will be discussed.

In methanol, P457 readily formed a derivative P420 (**3**), Scheme 3, which displayed a 32 nm hypsochromi-

Part 54. *Biochem. Syst. Ecol. In press.*



Scheme 1.



Scheme 2.

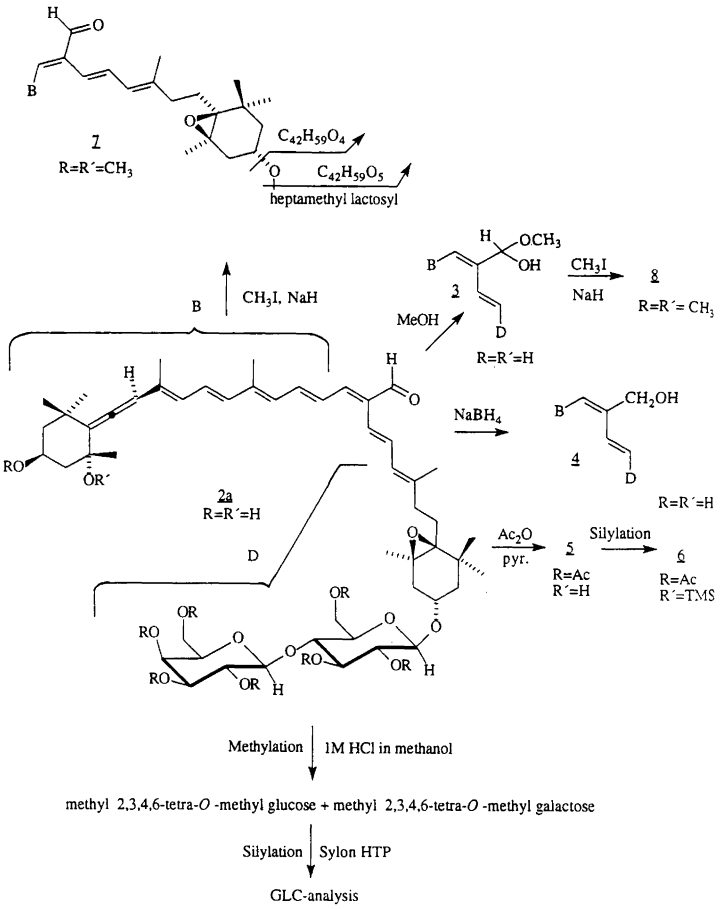
cally shifted VIS spectrum (methanol) and increased spectral fine structure. This result was rationalized as a nucleophilic attack of methanol on the cross-conjugated aldehyde function, resulting in hemiacetal formation. Treatment of P457 with $NaBH_4$ provided a reduction product (**4**) with the same octaene chromophore as in P420. Attempted acid-catalysed furanoid rearrangement gave no chromophoric change, compatible with the absent C-7′,8′ double bond required for 5′,8′-furanoxide formation.

Acetylated P457 (**5**) provided a mono-TMS ether (**6**) without intermediates upon silylation at −30°C, confirming the presence of one tertiary hydroxy group. Acetylated P457 (**5**) and its TMS ether **6** failed to provide molecular ions upon electron impact, a problem which was circumvented by methylation. Methylation of P457 (**2a**) with CH_3I and NaH[5] also resulted in methylation of the tertiary hydroxy group and provided a final nonamethyl ether **7**. Upon electron impact a molecular ion was obtained (*m/z* 1066, corresponding to $C_{61}H_{94}O_{15}$) with $M+1 = 67\%$ of M, as calculated for a C_{61}-compound.[6] In a model experiment neoxanthin (**9**), Scheme 4, also possessing a hydroxy group on a tertiary carbon, afforded a trimethyl ether. The molecular ion of P457 nonamethyl ether (**7**) was further confirmed by the presence of an $M-1$ peak, compatible with α-cleavage of an aldehyde, and an $M-32$ ion (loss of methanol). Peak matching of the fragment ions *m/z* 643.4354 ($C_{42}H_{59}O_5$) and 627.4405 ($C_{42}H_{59}O_4$) was consistent with the composition $C_{42}H_{60}O_5$ for the aglycone, see Scheme 3. Carotenoids with in-chain substituted methyl groups and adjacent *cis* double bonds easily eliminate benzaldehyde (106 mass units),[7] and a relatively strong fragment ion *m/z* 960 (10%) was observed for **7**. A weak fragment ion, *m/z* 423 (1%), was compatible with the oxonium ion of a heptamethylated dihexoside. The oxonium ion for a tetramethylated hexoside was observed at *m/z* 219 (41%), confirmed by loss of two methanol units (*m/z* 187, base peak). The methyl hemiacetal P420 (**3**) also provided what is presumed to be the nonamethyl ether (**8**), upon methylation, according to MS data.

The mass spectra of both P457 octaacetate (**5**), and its TMS ether **6** exhibited *m/z* 619.1910 ($C_{26}H_{35}O_7$) and *m/z* 331 ions, compatible with oxonium ions of a heptaacetylated dihexoside and tetraacetylated hexoside, respectively. Consequently MS data for the P457 derivatives **5–8** were all consistent with the disaccharide structure.

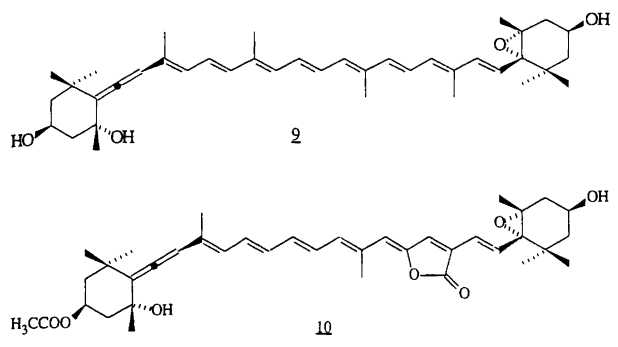
An aliquot of P457 was subjected to acidic methanolysis, and the resulting methylated sugar silylated for GLC analysis. The presence of glucose and galactose in a 1:1 ratio was confirmed for the disaccharide present in P457 (**2a**), making lactose a strong candidate, as proved by the NMR data.[4]

The FT-IR spectrum confirmed the presence of an allene (1931 cm^{-1}) and a cross-conjugated aldehyde (1654 cm^{-1}), as well as hydroxy (3350 cm^{-1}) and tertiary C–O functions (1153 cm^{-1}). Mass spectral data for the



Scheme 3.

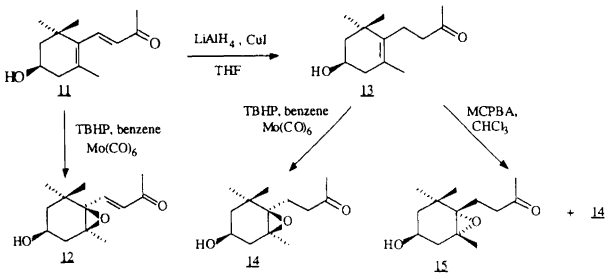
methylated derivatives **7** and **8** and evidence for the dihexoside provided the clue to the molecular formula $C_{40}H_{56}O_5$ for the aglycone present in P457 (**2a**), a structure corresponding to 13 double-bond equivalents. From the chemical derivatizations and spectroscopic evidence (IR, MS, ^{1}H NMR) the oxygen functions were accounted for as an aldehyde, one tertiary hydroxy group, one secondary hydroxy group, one glycosidically bound hydroxy group and one inert oxygen. Simple 1D ^{1}H NMR spectra were compatible with the presence of the allenic end group and the chromophore assigned. By inference the second end group was formulated as the so far unknown 7′,8′-dihydro-5′,6′-epoxide, carrying the glycosidic substituent (**2**). This structure could be verified by detailed NMR studies.[4]



Scheme 4.

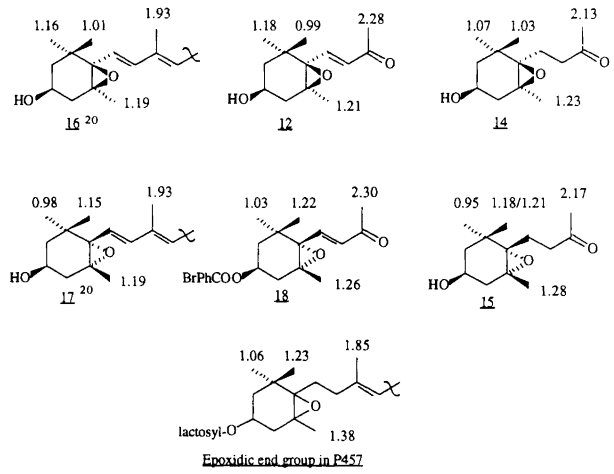
The chirality aspect remains to be considered. The relative stereochemistry of the allenic end group was proved by NMR spectroscopy.[4] Since the CD spectrum of P457 exhibited a negative Cotton effect and resembled that of peridinin (**10**, Scheme 4) of known absolute configuration and similar VIS spectrum/chromophoric length, the chirality of the allenic end group is considered documented. However, the relative stereochemistry of the second end group, not expected to influence the Cotton effect owing to the absence of conjugation, could not be clearly proved by NMR spectral studies on P457 and its acetates.[4] By biosynthetic analogy the *anti*-3′-hydroxy/5′,6′-epoxy structure of the epoxidic end group is favoured for the carotenoid aglycone, as in common carotenoid 5,6-epoxides, cf. neoxanthin (**9**) and peridinin (**10**), Scheme 4. Experimental support was sought by synthesis of relevant models of *syn* and *anti* hydroxy epoxides.

Model compounds. (3*R*)-3-Hydroxy-β-ionone (**11**), Scheme 5, was prepared[8] in several steps from optically active actinol by known procedures.[9,10] Stereoselective epoxidation of **11** with TBHP (*tert*-butyl hydroperoxide)



Scheme 5.

and $Mo(CO)_6$[11] to the *syn*-hydroxy epoxide **12**, followed by attempted selective reduction of the carbon–carbon double bond with copper iodide modified $LiAlH_4$[12] resulted in product mixtures.[8] Selective reduction of the carbon–carbon double bond[12] in (3*R*)-3-hydroxy-β-ionone (**11**) was therefore effected to give the non-conjugated ketone **13** prior to the epoxidation. Stereoselective epoxidation[11] of **13** with TBHP and $Mo(CO)_6$ provided the *syn*-hydroxy epoxide **14**. The NMR spectral characterization included 1D ^1H NMR, 2D ^1H, ^1H-COSY and difference NOE experiments. Epoxidation of **13** with MCPBA[13] yielded a mixture of the *syn*-hydroxy epoxide **14** and the *anti*-hydroxy epoxide **15** in a ca. 3:1 ratio. ^1H NMR assignments of the *syn*-(**14**) and the *anti*-(**15**) hydroxy epoxides are given in Scheme 6 together with relevant model compounds.[8]



Scheme 6.

Since glycosidation of carotenoids with 3-hydroxylated β-rings does not influence the chemical shifts of the Me-16, Me-17 and Me-18 groups,[14] the *syn*- and *anti*-hydroxy epoxides **14** and **15** were considered as suitable ^1H NMR models. The chemical shift for the ring methyl protons in the *syn*-hydroxy epoxides **12** and **16** were quite similar. Also for the *anti*-models **17** and **18** the correlation was sufficient for the methyl ketones **14** and **15** to be considered as useful models for a carotenoid polyene chain in this context. In the *anti*-hydroxy epoxide **17**, Me-16 resonated at higher field than Me-17, while the opposite was observed for the *syn*-hydroxy epoxide **16**.

The difference in chemical shift between Me-16 and Me-17 (using carotenoid numbering also for the β-ionone derivatives) in the *syn*-hydroxy epoxide enone **12** was 0.19 ppm, while the reduced compound **14** revealed a chemical shift difference of only 0.04 ppm. A similar effect was not observed for the *anti*-compounds **18** and **15**. The chemical-shift difference $\Delta = 0.17$ ppm between Me-16′ and Me-17′ in P457 suggests that the position of the epoxide function and the glycosylated C-3′ hydroxy group are in *anti*-positions.

In conclusion it is suggested in conjunction with the detailed NMR evidence presented in Part 2[4] that P457 has the configuration (3*S*,5*R*,6*R*,3′*S*,5′*R*,6′*S*)-13′-*cis*-7′,8′-dihydroneoxanthin-20′-al 3′-β-D-lactoside, compatible with biosynthetic analogues, cf. neoxanthin (**9**) and peridinin (**10**), Scheme 4. P457 is the first structurally elucidated carotenoid lactoside and one of the structurally most complex carotenoids encountered. It represents the first carotenoid diglycoside isolated from algae, the first carotenoid glycoside encountered in eukaryotic algae, the first cross-conjugated carotenal in algae and the first 7,8-dihydroxanthophyll discovered in dinoflagellates. Its complex structure confirms the versatility of dinoflagellates as regards the biosynthesis of carotenoids with special structural variations.

Experimental

Biological material. The dinoflagellate *Amphidinium carterae* Hulburt (clone 'Amphi': CCMP 1314) was obtained from the Center for Culture of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME 04575, USA, and cultivated at the Bigelow Laboratory. Axenic stocks were maintained in seawater of ca. 32‰ salinity enriched as 'f/2' (without added silicate) in volumes of 50–1000 ml as inoculum for carboy cultures of 8–20 l.[15] For carboy cultures, the nutrients were added aseptically to seawater autoclaved separately and cooled. Filtered air was provided as necessary by bubbling. Growth was in a chamber at 18–20 °C under 14 h/day of fluorescent illumination provided by 'cool white' (Sylvania Co.) lamps yielding a flux of $2–4 \times 10^{16}$ photons $s^{-1}\mu m^{-2}$ at the surface of carboys (ca. 1×10^{16} for stock cultures). Cells were harvested in a Sharples continuous-flow centrifuge, frozen at ca. −20°C at once, then lyophilized and stored at −20°C. Yields are exemplified by batch 1 (2 × 17 l), which provided 4.43 g lyophilized cells, and batch 2 (4 × 15 l), which provided 9.66 g lyophilized cells. Several batches were cultivated, the yield totalling ca. 80 g of lyophilized cells.

Isolation procedure. General precautions for work with carotenoids were taken.[16] Distilled or analytical-grade solvents and reagents were used.

Several isolation procedures were investigated. In the recommended procedure extraction of lyophilized, ground cells (30 g) was carried out on a glass-sinter filter with acetone–methanol 7:3, followed by pure methanol

until the extract was colourless. The extract was evaporated and the residue partitioned between (i) hexane and 95% aq. methanol and (ii) hexane and 70% aq. methanol. The hypophasic pigments were transferred to ethyl acetate, washed repeatedly with water and submitted to column chromatography, followed by thin layer chromatography (TLC), Scheme 2.

Chromatography. Column chromatography (CC) was carried out on an acetylated polyamide column (MN-Polyamide SC 6-AC, Mackerey–Nagel) using toluene with 0–20% methanol as the eluent.

Preparative TLC was performed on 0.5 mm silica plates using acetone–methanol–water 85:10:5 as the eluent (yield of P457 ca. 2.4 mg), and on Whatman KC 18F reversed-phase plates, using methanol–water 9:1 as the eluent. Yield ca. 1.8 mg P457.

Semipreparative HPLC was carried out on a Perkin Elmer Series 2 Liquid Chromatograph with a Pye Unicam PU 4021 multichannel detector. Conditions: reversed-phase column (Hypersil ODS, $l = 25$ cm, $d = 4.6$ mm, particle size $= 5$ μm), solvent acetonitrile–dichloromethane–methanol 7:2:1, flow $= 0.8$ ml min^{-1}. Yield ca. 1.0 mg P457.

Analysis of the silylated methyl glycosides was carried out on a Carlo Erba GC 6000 Vega Series 2 gas chromatograph, using a DB5 (30 m) column, a split ratio of 1:10 and with H_2 as the carrier gas (40 cm s^{-1}). The temperature program was 140–180°C with steps of 2°C min^{-1}.

Spectroscopy. VIS spectra were recorded on a Perkin Elmer 552 UV–VIS spectrophotometer in methanol. $E^{1\%}_{1cm} = 2500$ was used. Spectral fine structure is expressed as % III/II.[17]

Mass spectra were recorded on an AE1 MS 901 instrument with a direct inlet system at 230°C. Only prominent or diagnostically useful peaks are cited.

CD spectra were recorded on a Jobin Yvon Auto Dichrograph Mark IV in methanol at room temperature.

IR spectra were recorded on a Nicolet 20 SXC FT-IR spectrometer with the carotenoid in a KBr-disc (0.5 mg carotenoid in 0.2 g KBr).

Chemical derivatizations. General procedures were used.[18] Methylation was carried out in dry THF for 17 h, using NaH and methyl iodide.[5]

Acetylation was carried out in dry pyridine with acetic anhydride for 24 h.

Attempts at furanoid rearrangement were carried out on acetylated P457 in methanol-free $CDCl_3$ upon addition of HCl in $CDCl_3$ to a final concentration of 0.003 M.

Silylation of P457 acetate was effected in dry pyridine using hexamethyldisilane and trimethylsilyl chloride as silylating agents at −30°C (1 h), then at room temperature (20 h). The reaction was monitored by TLC.

$NaBH_4$ reduction of P457 in abs. ethanol was effected in a suspension of $NaBH_4$ in abs. ethanol.

P420 was formed upon storage of P457 in methanol at −20°C.

Methanolysis of P457 (ca. 50 μg) was performed in methanolic HCl (1 M, 0.5 ml) at 80°C for 24 h. The reaction mixture was evaporated to dryness with a stream of N_2. The residue was dissolved and redissolved in dry methanol, evaporated to dryness three times and finally dried in a vacuum desiccator over P_2O_5 and KOH for 24 h. The methyl glycosides were silylated with the Sylon HTP reagent (25 μl) for 1 min, and the silylated methyl glycosides analysed by GLC. Silylated methyl glycosides of glucose, galactose and mannose were used as reference compounds. Myoinositol was used as an internal standard.

P457 [(3S,5R,6R,3'S,5'R,6'S)-13'-cis-7',8'-dihydroneoxanthin-20'-al 3'-β-D-lactoside] (**2a**). Available ca. 1.0 mg; $R_f = 0.88$ (silica; acetone–methanol–water 85:10:5); VIS λ_{max} (424) 452 (480) nm; FT-IR cm^{-1} 3350 (OH), 2957, 2926, 2871, 2855 (C–H, sp^3), 1931 (C=C=C), 1717 (sat. C=O), 1654 (cross-conjugated C=O), 1409 and 1379 (*gem* CH_3), 1153 (*tert* C–O), 965 (*trans* CH=CH); MS not informative; CD λ/nm (Δε) 220 (−6), 229 (−8), 260 (0.4), 278 (−1.2), 310 (−0.2); ^1H NMR, ^{13}C NMR; cf. Englert *et al.*[4]

P457 methyl hemiacetal (**3**). $R_f = 0.84$ (silica; acetone–methanol–water 85:10:5); VIS λ_{max} 397, *418*, 442 nm, % III/II = 94.

$NaBH_4$-reduced P457 (**4**). $R_f = 0.84$ (silica; acetone–methanol–water 85:10:5); VIS λ_{max} 395, *418*, 442 nm % III/II = 42.

P457 octaacetate (**5**). $R_f = 0.49$ (silica, 45% acetone in hexane); VIS λ_{max} (424), *450* nm; MS [IP 70 eV, *m/z* (% rel. int.)]: 619 (3, [$C_{26}H_{35}O_{17}$, oxonium ion heptaacetylated dihexoside]), 331 (100, [oxonium ion tetraacetylated hexoside]).

P457 octaacetate TMS ether (**6**). $R_f = 0.74$ (silica, 45% acetone in hexane); VIS λ_{max} (420) *448* nm; MS [IP 70 eV, *m/z* (% rel. int.)]: 619 (8, [$C_{26}H_{35}O_{17}$, oxonium ion heptaacetylated dihexoside]), 331 (100, [oxonium ion tetraacetylated hexoside]).

P457 nonamethyl ether (**7**). $R_f = 0.89$ (45% acetone in hexane), 0.38 (silica; 35% acetone in hexane): VIS λ_{max} 450 nm; MS [IP 70 eV, *m/z* (% rel. int.)]: 1067 (1, [*M* + 1]), 1066 (2, [*M*]), 1065 (0.5, [*M* − 1]), 1064 (1, [*M* − 2]), 1034 (1, [*M* − 32]), 960 (10, [*M* − 106]), 928 (1, [*M* − 106–32]), 847 (1, [*M*-oxonium ion tetramethylated hexoside], 815 (1, [847 − 32]), 643.4354 (1, [*M* − 423]), 627.4405 (2, [*M* − 423 − 16]), 611.4110 (2, [643 − 32]), 595 (3, [627 − 32]), 579 (1, [643 − 32 − 32]), 563 (2, [627 − 32 − 32]), 537.3938 (6, [643–106]), 521 (2, [627–106]), 489.3727 (4, [627 − 106 − 32]), 423 (1, [oxonium ion hepta-

methylated dihexoside]), 359 (2, [423 – 32 – 32]), 327 (6, [423 – 32 – 32 – 32]), 263 (8, [423 – 32 – 32 – 32 – 32 – 32]), 219 (42, [oxonium ion tetramethylated hexoside]), 187 (100, [219–32]), 155 (32, [219–32–32]).

P457 methyl hemiacetal nonamethyl ether (**8**). $R_f = 0.92$ (silica, 45% acetone in hexane), 0.42 (silica, 35% acetone in hexane); VIS λ_{max} 395, *419*, 445 nm, % III/II = 72; MS [IP 70 eV, *m/z* (% rel. int.)]: 974 (1, [*M* – 92 – 32]), 960 (14, [*M* – 106 – 32]), 928 (1, [*M* – 106 – 32 – 32]), 643.4354 (1, [*M* – 423 – 32]), 627.4405 (1, [*M* – 439 – 32 – 16]), 611.4110 (1, [643–32]), 595 (3, [627 – 32 – 32]), 579 (3, [643–32–32–32]), 551 (2, [643 – 92 – 32]), 537.3938 (6, [643 – 106 – 32]), 521 (2, [627 – 106 – 32]), 505 (1, [643 – 106 – 32 – 32]), 489.3727 (3, [627 – 106 – 32 – 32]), 423 (1, [oxonium ion heptamethylated dihexoside]), 359 (2, [423 – 32 – 32]), 327 (6, [423 – 32 – 32 – 32]), 263 (7, [423 – 32 – 32 – 32 – 32 – 32]), 219 (41, [oxonium ion tetramethylated hexoside]), 187 (100, [219–32]), 155 (32, [219 – 32 – 32]).

Synthesis of model compounds. IUPAC nomenclature is used. However, carotenoid numbering of the carbon skeleton is used in the ^{1}H NMR assignments for comparative purposes.

Optically active actinol was silylated[9] and converted in a Grignard reaction into 4-[(4*R*)-1-hydroxy-4-trimethylsilyloxy-2,6,6-trimethylcyclohexyl]but-3-yn-2-ol.[10] The TMS ether was hydrolysed with methanolic KOH (5%) and the resulting triol acetylated to 2-acetoxy-4-[(4*R*)-4-acetoxy-2,6,6-trimethylcyclohex-1-enyl]but-3-yn-2-ol, which was dehydrated to *trans*-4-[(4*R*)-4-hydroxy-2,6,6-trimethylcyclohex-1-enyl]but-3-en-2-ol.[10] The allylic hydroxy group was oxidized by MnO_2 to give *trans*-4-[(4*R*)-4-hydroxy-2,6,6-trimethylcyclohex-1-enyl]but-3-en-2-one [(3*R*)-3-hydroxy-β-ionone, **11**],[10] yield 1.23 g. Additional **11** was supplied from F. Hoffmann-La Roche, Basel.

4-[(1R,2S,4R)-1,2-Epoxy-4-hydroxy-2,6,6-trimethylcyclohexyl]but-3-en-2-one (**12**). 4-[(4*R*)-4-Hydroxy-2,6,6-trimethylcyclohexyl]but-3-en-2-one [(3*R*)-3-hydroxy-β-ionone, **11**] was epoxidized selectively to the *syn*-hydroxy epoxide **12** by the following procedure.[11] To a solution of **11** (0.45 g, 2.16 mmol) in benzene (5 ml) was added $Mo(CO)_6$ (ca. 4 mg) and the mixture heated to reflux. The oil-bath was removed and TBHP in benzene (ca. 2.35 M,[11] 1.20 ml, 2.82 mmol) was added. The resulting mixture was refluxed for 7 h and stirred at room temp. for 15 h. Aqueous Na_2SO_3 (10%, 1 ml) was added dropwise and the two-phase mixture stirred well for 1 h. The product was extracted with ether and the ether extract washed repeatedly with aqueous NaCl. Yield of **12** 0.40 g, 1.79 mmol, 83%.

4-[(4R)-4-Hydroxy-2,6,6-trimethylcyclohex-1-enyl]-butan-2-one (**13**). 4-[(4*R*)-4-Hydroxy-2,6,6-trimethylcyclohexyl]but-3-en-2-one ((3*R*)-3-hydroxy-β-ionone, **11**) was reduced selectively by the following procedure.[12] CuI (0.37 g, 1.92 mmol) in dry THF (4 ml) was cooled in an ice-bath. $LiAlH_4$ in THF (1.52 M, 0.32 ml, 0.48 mmol) was added under an N_2-atmosphere. A brownish-black colour appeared immediately. The mixture was stirred at 0°C for 15 min. (3*R*)-3-Hydroxy-β-ionone (**11**, 0.10 g, 0.48 mmol) in dry THF (1.5 ml) was added dropwise. The resulting mixture was stirred at 0°C for 2 h 45 min and at room temp. for 20 min. Aqueous $NaHCO_3$ was added, the product extracted with ether and the ether extracts washed repeatedly with aqueous $NaHCO_3$ and aqueous NaCl. The product was purified by TLC (silica; 20% ether in chloroform). Yield of **13** 35 mg, 0.17 mmol, 35%; MS [IP 70 eV, *m/z* (% rel. int.)], 210 (5, [*M*]), 192 (25, [*M* – 18]), 119 (100), 43 (66, [CH_3CO]); ^{1}H NMR (400 MHz, $CDCl_3$) 1.015 (s, 3 H, Me-16/17), 1.029 (s, 3 H, Me-17/16), 1.413 (t, $J_{2,2} = J_{2,3} = 11.9$ Hz, 1 H, H-2_{ax}), 1.588 (s, 3 H, Me-18), ca. 1.71 (m, 1 H, H-2_{eq}), ca. 1.95 (m, 2 H, H-4_{ax} + H-7), 2.140 (s, 3 H, Me-19), ca. 2.20 (m, 1 H, H-4_{eq}/H-7), ca. 2.27 (m, 1 H, H-7/H-4_{eq}), ca. 2.48 (m, 2 H, H-8).

4-[(1R,2S,4R)-1,2-Epoxy-4-hydroxy-2,6,6-trimethylcyclohexyl]butan-2-one (**14**). 4-[(4*R*)-4-Hydroxy-2,6,6-trimethylcyclohex-1-enyl)butan-2-one (**13**) was epoxidized selectively to the *syn*-hydroxy epoxide **14** by the following procedure.[11] To a solution of **13** (40 mg, 0.19 mmol) in benzene (1 ml) was added $Mo(CO)_6$ (ca. 1 mg) and the mixture was heated to reflux. The oil-bath was removed and TBHP in benzene (ca. 2.35 M,[11] 0.09 ml, 0.21 mmol) was added. The resulting mixture was refluxed for 15 min. Aqueous Na_2SO_3 (10%, 1 ml) was added dropwise and the two-phase mixture stirred well for 15 min. The product was extracted with ether and the ether extract washed repeatedly with aqueous NaCl. Yield of **14**, 25 mg, 0.11 mmol, 58%; MS [IP 70 eV, *m/z* (% rel. int.)], 226 (5, [*M*]), 208 (5, [*M* – 18]), 43 (100); ^{1}H NMR (400 MHz, $CDCl_3$), 1.036 (s, 3 H, Me-16/17), 1.069 (s, 3 H, Me-16), ca. 1.23 (m, 1H, H-2_{eq}), 1.236 (s, 3 H, Me-18), 1.516 (t, $J_{2,2} = J_{2,3} = 11.9$ Hz, 1 H, H-2_{ax}), 1.786 (dd, $J_{4,4} = 15.1$ Hz, $J_{4,3} = 8.8$ Hz, 1 H, H-4_{ax}), ca. 1.93 (m, 2 H, H-7), ca. 2.13 (m, 1 H, H-4_{eq}), 2.134 (s, 3 H, Me-19), ca. 2.51 (m, 2 H, H-8).

4-[(1R,2S,4R)-1,2-Epoxy-4-hydroxy-2,6,6-trimethylcyclohexyl]butan-2-one (**14**) *and 4-[(1S,2R,4R)-1,2-epoxy-4-hydroxy-2,6,6-trimethylcyclohexyl]butan-2-one* (**15**). 4-[(4*R*)-4-hydroxy-2,6,6-trimethylcyclohex-1-enyl]butan-2-one (**13**) was epoxidized with MCPBA to yield a mixture of the *syn*- and *anti*-hydroxy epoxides **14** and **15**.[13] To a cooled (4°C) solution of **13** (60 mg, 0.29 mmol) in chloroform (2 ml) was added dropwise MCPBA (64 mg, 0.36 mmol) in chloroform (5 ml). The mixture was stirred at 4°C for 3 h. Aqueous $NaHCO_3$ was added, the product extracted with ether and the ether extract washed repeatedly with aqueous $NaHCO_3$ and aqueous NaCl. Yield of **14** + **15** 40 mg, 0.18 mmol, 61%.

^{1}H NMR (400 MHz, $CDCl_3$) **14**: 1.042 (s, Me-16), 1.073 (s, Me-17), 1.243 (s, Me-18), 2.141 (s, Me-19). **15** (tentatively): 0.953 (s, Me-16/17), 1.175/1.208 (s, Me-17/16), 1.283 (s, Me-18), 2.173 (s, Me-19); ratio 14:15 ca. 3:1.

*trans-4-[(1*S*,2*R*,4*R*)-4-(*p*-Bromobenzoyloxy)-1,2-epoxy-2,6,6-trimethylcyclohexyl)but-3-en-2-one* (**18**). **18** was obtained previously by ozonolysis of peridinin (**10**) bromoacetate[19] and is characterized here by 400 MHz ^{1}H NMR spectroscopy, see Scheme 6.

Acknowledgments. Gifts of optically active actinol and additional (*R*)-hydroxy-β-ionone from Dr. H. Mayer, F. Hoffmann-La Roche, Basel, are greatfully acknowledged. We thank Dr. Bjørn Erik Christensen for assistance with the GLC analysis. T. Aa. held a fellowship from The Norwegian Research Council for Scientific and Industrial Research and was subsequently supported by a research grant from F. Hoffmann-La Roche to S.L.J. R.R.L.G. was supported in part by NSF Grant OCE-87-16342.

References

1. Johansen, J. E., Svec, W. A., Liaaen-Jensen, S. and Haxo, F. T. *Phytochemistry 13* (1974) 2261.
2. Skjetne, T., Haxo, F. T. and Liaaen-Jensen, S. *Biochem. Syst. Ecol. 12* (1984) 149.
3. Andrewes, A. G. and Liaaen-Jensen, S. *Abstr. 7th Int. IUPAC Carotenoid Symp.*, München, 1984, P39.
4. Englert, G., Aakermann, T., Schiedt, K. and Liaaen-Jensen, S. *Magn. Reson. Chem. In preparation.*
5. Nybraaten, G. and Liaaen-Jensen, S. *Acta Chem. Scand., Ser. B 28* (1974) 1219.
6. Silverstein, R. M., Bassler, G. C. and Morrill, T. C. *'Spectrometric Identification of Organic Compounds'*, 4th ed., Wiley, New York 1981, p. 10.
7. Francis, G. W. and Liaaen-Jensen, S. *Acta Chem. Scand. 24* (1970) 2705.
8. Aakermann, T. Dr. ing. thesis, University of Trondheim-NTH, Trondheim, Norway 1992.
9. Kienzle, F. and Mayer, H. *Helv. Chim. Acta 61* (1978) 2609.
10. Loeber, D. E., Russel, S. W., Toube, T. P., Weedon, B. C. L. and Diment, J. *J. Chem. Soc. C* (1971) 404.
11. Sharpless, K. B. and Michaelson, R. C. *J. Am. Chem. Soc. 95* (1973) 6136.
12. Ashby, E. C., Lin, J. J. and Kovar, R. *J. Org. Chem. 41* (1976) 1939.
13. Buchecker, R., Marti, U. and Eugster, C. H. *Helv. Chim. Acta 67* (1984) 2043.
14. Hundle, B. S., Beyer, P., Kleinig, H., Eńglert, G. and Hearst, J. E. *Phytochem. Photobiol. 54* (1991) 89.
15. Guillard, R. R. L. In: Berg, C. J., Ed., *Culture of Marine Invertebrates, Selected Readings*, Hutchinson Ross, Stroudsberg 1983, p. 108.
16. Schiedt, K. and Liaaen-Jensen, S. In: Britton, G., Pfander, H. and Liaaen-Jensen, S., Eds. *Carotenoids, Vol. 1. Isolation and Analysis*, Birkhäuser, Basel. *In press.*
17. Ke, B., Kjøsen, H. and Liaaen-Jensen, S. *Biochim. Biophys. Acta 210* (1970) 139.
18. Liaaen-Jensen, S. and Jensen, A. *Methods Enzymol. 23* (1971) 586.
19. Johansen, J. E., Borch, G. and Liaaen-Jensen, S. *Phytochemistry 19* (1980) 441.
20. Englert, G. In: Britton, G., Pfander, H. and Liaaen-Jensen, S., Eds., *Carotenoids Vol. 1B. Spectroscopy*, Birkhäuser, Basel. *In press.*

Received March 29, 1993.