Acta Chemica Scandinavica B 30 (1976) 109—120

Algal Carotenoids. XIV.* Structural Studies on Peridinin. Part 1. Structure Elucidation

H. H. STRAIN,[a] W. A. SVEC,[a,**] P. WEGFAHRT,[b] H. RAPOPORT,[b,**] F. T. HAXO,[c,**] S. NORGÅRD,[d] H. KJØSEN[d] and S. LIAAEN-JENSEN[d,**]

[a]Chemical Division, Argonne National Laboratory, Argonne, Illinois 61440, U.S.A.,[b] Department of Chemistry, University of California, Berkeley, California 94720, U.S.A., [c]Scripps Institution of Oceanography, University of California, La Jolla, California 92037, U.S.A. and [d]Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

The characteristic pigment of the dinoflagellates, peridinin, has been studied as a joint project in our four laboratories. Based on detailed spectroscopic and chemical evidence peridinin has been assigned the structure 5′,6′-epoxy-3,5,3′-trihydroxy-6,7-didehydro-5,6,5′,6′-tetrahydro-12,13,20-trinor-β,β-caroten-19′,11′-olide 3-acetate (*1*).

Dinoflagellates (class, Dinophyceae; division, Pyrrophyta) together with the diatoms are the major producers of organic matter in the sea.[1] These planktonic algae are the most frequent cause of "red tide", natural blooms in which the cells are sufficiently abundant to confer a distinct reddish tinge to the water.[2] The dinoflagellate chloroplast is typically brown in colour and contains relatively large amounts of carotenoid pigments. The major one, peridinin, constitutes 70 – 80 % of the total [3] and functions as an auxiliary light harvesting pigment for photosynthesis.[4,5]

Peridinin was first isolated and named by Schütt in 1890.[6] The source of this crude preparation was a harvest of mixed marine dinoflagellates, principally *Ceratium tripos*, and its name was derived from Peridineen (peridinians), a collective term for the dinoflagellates. Peridinin, as a homogeneous pigment, has since been isolated from several different dinoflagellate sources, [7–10] and it is now recognized as an important taxonomic marker for this class of algae.[11]

A pigment obtained from the sea anemone *Anemonia sulcata*, and named sulcatoxanthin,[12] has since been shown to be identical to peridinin and the actual source of the pigment to be the endozoic dinoflagellate symbionts, zooxanthellae, of the sea anemone.[13] Peridinin has also been obtained from other marine sources, namely the zooxanthellae of various corals, clams [3] and sea anemones.[13]

Together with fucoxanthin (*2*), the major xanthophyll of brown algae and diatoms,[8] peridinin is one of the dominant carotenoid pigments in Nature.

Previous investigations of peridinin have disclosed some of its physical and chemical properties. Thus combustion analysis indicated a molecular composition of $C_{40}H_{52}O_8$ (Mw = 660),[12] whereas high resolution mass spectrometry [14] gave a composition $C_{39}H_{50}O_7$ (Mw = 630) for the highest observable ion.

The visible light absorption spectrum exhibited pronounced fine-structure in non-polar solvent (λ_{max} 454, 484 nm, hexane)[10] but a broad round-shaped spectrum in polar solvents (λ_{max} 472 nm, ethanol, E(1 cm, 1 %) = 1325).[3] This dramatic loss of fine-structure is somewhat unusual and the observed extinction coefficient is exceptionally low.

In the presence of alkali peridinin is rapidly decolourized [10,13] and reduction with $LiAlH_4$ gives a mixture of products which have essentially pentaene chromophores.[10]

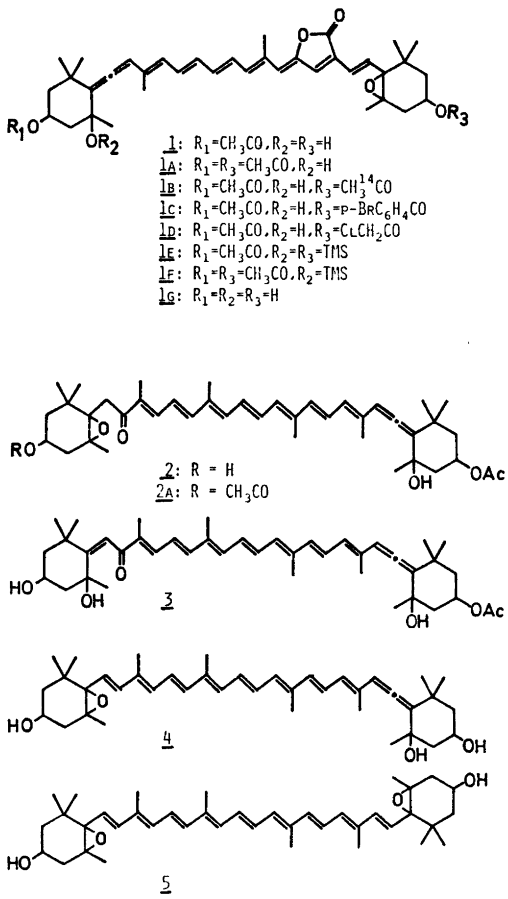
* Part XIII. *Acta Chem. Scand. B 28* (1974) 949.
** To whom correspondence should be addressed.

With acid peridinin is slowly transformed, giving products with visible light absorption maxima shifted hypsochromically by 20 nm,[10] suggesting a 5,6-epoxide to 5,8-furanoxide rearrangement.[10,15]

The present co-operative investigation in our four laboratories led to a proposed structure for peridinin (*1*), communicated briefly in a priority note.[16] More detailed evidence is now presented.

The main cultivations were carried out at La Jolla; isolation and physical characterization at Argonne, Berkeley and La Jolla; chemical and physical studies at Berkeley and Trondheim.

For convenience these studies will be presented in two separate parts. The present paper contains the necessary data for the structure elucidation, whereas the second part [17] gives supporting evidence in terms of the proposed structure.



Scheme 1.

RESULTS AND DISCUSSION

Peridinin (*1*) was obtained from several different sources, namely from a natural bloom containing more than 95 % *Gonyaulax polyedra*, from the zooxanthellae of the Pacific coast sea anemone *Anthopleura* (previously *Bunodactis*) *xanthogrammica* and from unialgal cultures of *Cachonina niei*.

The isolation involved extraction of the fresh, frozen, or freeze-dried algal material with acetone or methanol and chromatography on sucrose columns or reversed phase chromatography on polyethylene columns, followed by further purification on calcium carbonate, alumina or silica. Homogeneous fractions were then crystallized from suitable solvents.

Peridinin (*1*, Scheme 1) was obtained as purple crystals of m.p. 128 – 132 °C from ether-hexane. Analysis of a carefully purified sample gave C = 73.15 % and H = 7.68 % fitting best for $C_{41}H_{52}O_8$ (Mw = 672), requiring C = 73.18 % and H = 7.79 %. The high resolution mass spectrum on the other hand had the highest observable ion at *m/e* 630.3553 corresponding to $C_{39}H_{50}O_7$ (calc. 630.3555). These results differ by C_2H_2O which could be an element of ketene. This raises some doubt as to whether the molecular ion is observed on electron impact. However, loss of ketene from acetates is usually not favoured over the loss of acetic acid.[18,19]

Attempts to distinguish between the two alternatives by measuring the specific activity of peridinin ^{14}C-acetate (*1b*), prepared by acetylation with ^{14}C-acetic anhydride of known activity, were unsuccessful.

The presence of at least one secondary acetoxy function follows from the IR absorptions at 1745 and 1250 cm^{-1}, a methyl singlet at δ 2.01 and a methine multiplet at δ 5.4 in the ^{1}H NMR spectrum and hydrolysis to peridininol (*1g*).

Attempts to determine the number of acetate functions by acid hydrolysis of peridinin ^{14}C-acetate (*1b*) and determination of the specific activity of the liberated acetic acid as acetanilide did not give reliable results.

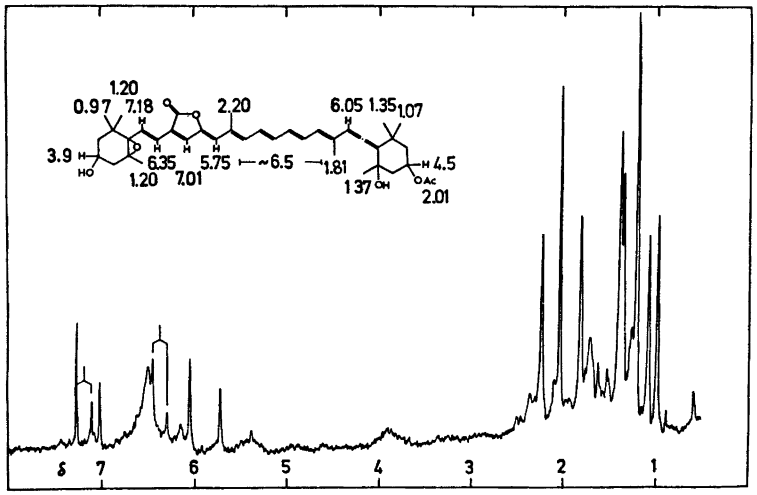


Fig. 1. ^{1}H NMR spectrum (100 MHz) of peridinin (*1*) in $CDCl_3$ solution.

Unspecific determination of ester functions by colourimetry of the hydroxamic acid-ferric ion complex gave 1.7 ester functions per molecule of peridinin. Under the same conditions fucoxanthin (*2*) and fucoxanthin acetate (*2a*) gave 0.9 and 1.7 ester groups, respectively. Moreover, the ^{13}C NMR spectrum of peridinin *p*-bromobenzoate (*1c*, Fig. 2) exhibited three signals at -42.0, -40.1 and -36.2 ppm relative to benzene, in the region where ester and lactone carbonyls normally resonate.[20]

In an attempt to determine the carbon skeleton, peridinin was hydrogenated, the reaction mixture reduced with KBH_4, and the products converted to the hydrocarbons by the method of Cope *et al.*[21] Mass spectrometry gave the highest molecular ion at *m/e* 510, corresponding to $C_{37}H_{66}$, suggesting that peridinin has a C_{37}-skeleton with five rings. However, similar treatment of fucoxanthin (*2*) gave a compound with the molecular ion at 6 mass units lower than that expected for the fully saturated compound, thus allowing no conclusions as to the number of rings in peridinin.

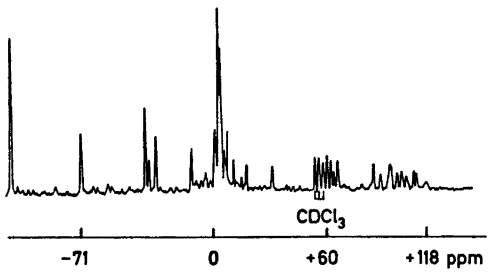


Fig. 2. ^{13}C NMR spectrum of peridinin *p*-bromobenzoate (*1c*) in $CDCl_3$.

Since all derivatives prepared from peridinin gave presumed molecular ions consistent with *m/e* 630 being the molecular ion for peridinin, it may now be concluded that peridinin has a C_{37}-skeleton, is a natural monoacetate ($C_{39}H_{50}O_7$), and in addition contains an unknown ester function.

Examination of the peridinin ^{1}H NMR spectrum (Fig. 1) shows that only nine methyl groups are present, six of which are in saturated environments. Of the remaining three, one is at an unusually low field (δ 2.20) compatible with a methyl ketone or an aromatic methyl.[22] However, no support for these assignments is found in the ^{13}C NMR spectrum of peridinin *p*-bromobenzoate (*1c*, Fig. 2)[20] or in the IR spectrum (Fig. 3) of peridinin itself.

Under standard conditions peridinin readily forms a monoacetate (*1a*) a di-TMS(=trimethylsilyl) ether (*1e*) and a monoacetate-mono-TMS ether (*1f*) as well as a chloroacetate (*1d*) and a *p*-bromobenzoate (*1c*). This suggests that peridinin has two free hydroxy functions. One of these is secondary as supported by a one proton multiplet at δ 3.9 and the other is tertiary.

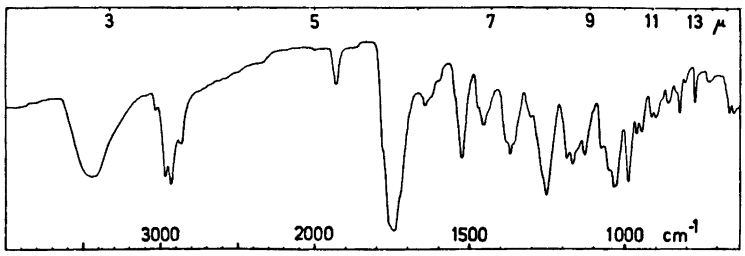


Fig. 3. IR spectrum (KBr) of peridinin (*1*).

The IR spectra of peridinin (Fig. 3) and its derivatives show a medium strength, characteristic absorption at 1930 cm^{-1} indicating that peridinin is an allenic compound.[19] Supporting evidence is found in the ^{13}C NMR spectrum of the *p*-bromobenzoate (*1c*) which has a signal at −74.6 ppm relative to benzene, assigned to the central allenic carbon,[23] and also in the mass spectrum of peridinin itself where an abundant hydrocarbon fragment ion at *m/e* 197.1325 ($C_{15}H_{17}$) is observed. Similar fragments (by composition) are observed in the mass spectra of both fucoxanthin (*2*) [24] and isofucoxanthin (*3*),[25] where they are most likely derived from the allenic end groups.

The ^1H NMR spectrum of peridinin exhibits signals which coincide with all the signals assigned to the allenic end group A of fucoxanthin (*2*).[24] These include the acetate at δ 2.01 and the methyl group next to the allene at δ 1.81 and the allenic proton at δ 6.05, Table 1.

In parallel experiments with fucoxanthin (*2*), addition of the $Eu(dpm)_3$ shift reagent [26,27] to the ^1H NMR samples caused, in both spectra, virtually parallel shifts of all the signals which coincide in the original spectra, Table 2. Also the induced shifts of the other saturated methyl signals of peridinin agree fairly well with those of the epoxy end group signals of fucoxanthin (*2*), Table 2, indicating that peridinin and fucoxanthin also have the other end group in common. The probable presence of an epoxidic end group B, such as in neoxanthin (*4*) and violaxanthin (*5*), is substantiated by the ^1H NMR data for *4* and *5*, Table 1.

Ozonolysis of peridinin *p*-bromobenzoate (*1c*) led to the isolation, by TLC, of an allenic methyl ketone (*6*) with properties identical to that obtained on ozonolysis of fucoxanthin (*2*).[24,25]

Further evidence for the second end group B is found in the peridinin mass spectrum with the base peak at *m/e* 181.1218 ($C_{11}H_{17}O_2$), Scheme 3. Abundant ions of this composition are usually encountered for hydroxylated 5,6-epoxidic or 5,8-furanoxide carotenoids.[27] This fragment ion suggests that peridinin is a 5,6-epoxide as already indicated by comparative ^1H NMR data, Tables 1 and 2.

Additional evidence for end group B comes

Table 1. ^1H NMR signals of peridinin and some model carotenoids.

Compound	Methyl groups at C-1	C-5	C-9	C-13	C-13′	C-9′	C-5′	C-1′	Other acetate	H-8′
Fucoxanthin *(2)*	9.03 8.97	8.80	8.08	8.04	8.04	8.21	8.63	8.93 8.67	8.00	3.95
Peridinin *(1)*	9.03 8.80	8.80		7.80		8.19	8.63	8.93 8.65	7.99	3.95
Neoxanthin *(4)*	9.04 8.86	8.83	8.09	8.06	8.06	8.21	8.67	8.94 8.63		
Violaxanthin *(5)*	9.05 8.88	8.84	8.11	8.08	8.08	8.11	8.84	9.05 8.88		

Table 2. Induced chemical shifts observed for peridinin *(1)* and fucoxanthin *(2)* with 0.0 and 1.0 molar ratios of $Eu(dpm)_3$.

A. Signals of methyl groups.

Molar ratio substr./ $Eu(dpm)_3$	Carbon No. 1	1	1′	1′	5	5′	9	9′	13	13′
Peridinin *(1)*										
0	9.03	8.80	8.93	8.65	8.80	8.63	—	8.19	7.80	—
1	8.00	8.30	8.30	8.30	7.86	7.68	—	8.05	7.57	—
Δ	1.03	0.50	0.73	0.35	0.94	0.95	—	0.14	0.23	—
Fucoxanthin *(2)*										
0	9.03	8.97	8.93	8.67	8.80	8.63	8.08	8.21	8.04	8.04
1	7.96	8.30	8.30	8.30	7.86	7.72	7.72	8.05	7.86	7.92
Δ	1.07	0.67	0.73	0.37	0.94	0.91	0.40	0.16	0.18	0.12

B. Signals of other hydrogens.

Molar ratio substr./ $Eu(dpm)_3$	Carbon No. 7	8	8′	10	12	14—10′	Acetate
Peridinin *(1)*							
0	2.82	3.65	3.95	2.99	4.25	3.5	7.99
1	1.95	3.25	3.85	2.86	4.17	3.45	7.57
Δ	0.87	0.40	0.10	0.13	0.08	0.05	0.42
Fucoxanthin *(2)*							
0	—	—	3.95	—	—	—	7.99
1	—	—	3.85	—	—	—	7.57
Δ	—	—	0.10	—	—	—	0.42

from the behaviour of peridinin towards acids. Under mild acidic conditions peridinin and peridinin acetate *(1d)* are both slowly isomerized to two new products (Part 2 [17]) with identical visible light absorptions shifted 20 nm hypsochromically and less polar than the starting compounds, compatible with the formation of two epimeric (at C-8) 5,8-furanoxides.[29] Epimers of this type are normally readily separated.[28] The low rate of reaction is, however, unusual.[30]

The position of the hydroxy substituent in this presumed six-membered end group is evident from the ^1H NMR multiplet at δ 3.9, assigned to a methine proton at C-3 rather than C-2 or C-4, since otherwise distinct triplet coupling patterns could have been observed.[31]

It is inferred therefore, that peridinin has the second end group in common with fucoxanthin *(2)*, neoxanthin *(4)* and violaxanthin *(5)*. End group B explains the acid catalysed rearrangement of peridinin.

Assignment of the two end groups A and B, Scheme 2, leaves a $C_{13}H_{11}O_2$ central structural element unaccounted for.

The visible light absorption spectrum of peridinin in hexane (Fig. 4) exhibits maxima at 454, 484 nm with a very pronounced fine-structure (% III/II [32] = 80). However, on changing the solvent to methanol only one broad maximum at 468 nm is observed. This extreme behaviour indicates that peridinin is a conjugated carbonyl compound.[19] In agreement with cross-conjugation, the low-temperature electronic spectrum (Fig. 4) exhibits pronounced fine-structure in the first overtone band (~300 nm), as observed for some cross-conjugated

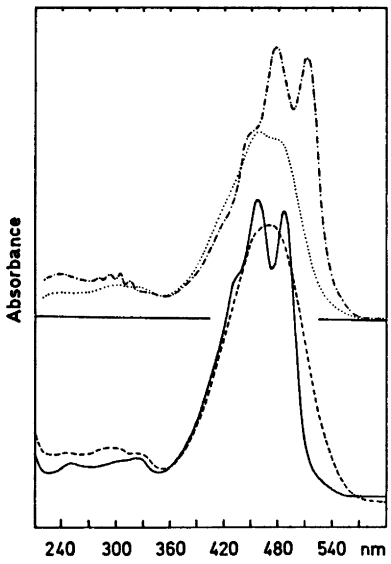


Fig. 4. Electronic spectra of peridinin (*1*) in hexane (——), methanol (– – –) and EPA at room temperature (···) and liquid nitrogen temperature (– · –).

carotenoids.[33] From the ^{13}C NMR data of the *p*-bromobenzoate (*1c*) the conjugated carbonyl function must be of an ester type, since only two of the three ester carbonyl signals at −42.0, −40.1 and −36.2 ppm are accounted for by the acetate and the *p*-bromobenzoate carbonyls and there is no signal in the region for keto carbonyls (*ca.* −50 ppm).[20] No aldehyde signal in the ^1H NMR spectrum and no IR absorption in the usual 1600−1730 cm^{-1} region rule out the presence of keto or aldehyde functions.

With complex metal hydrides peridinin is reduced to a number of compounds [17] which have essentially aliphatic pentaene chromophores. The large hypsochromic shifts in the visible light absorptions upon reduction, *ca.* 110 nm, cannot be explained by simple reduction of the conjugated carbonyl group and must represent a break of conjugation.

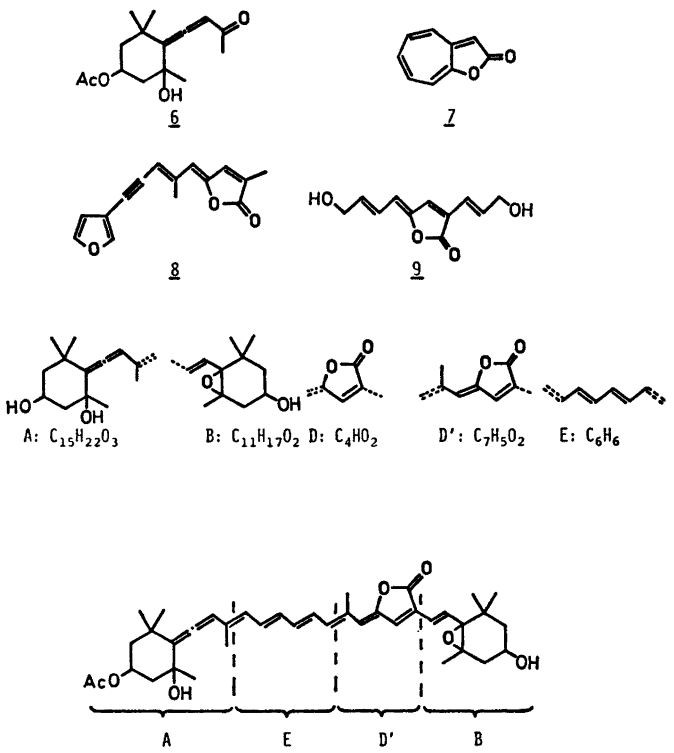
The olefinic region of the peridinin ^1H NMR spectrum is unusually simple, consisting of three singlets at δ 5.75, 6.05 and 7.01, the one at δ 6.05 being ascribed to the allenic proton, Table 1, Fig. 1. Two doublets at δ 6.35 and 7.18 with *trans*, $J = 16$ Hz, couplings were made apparent upon addition of $Eu(dpm)_3$. Approximately six protons resonate at *ca.* δ 6.5.

An unprecedented loss of CO_2 on electron impact and IR absorptions at 1745 (broad) and 1525 (sharp) cm^{-1} may be explained by a conjugated lactone, assuming that the lactone carbonyl vibration is superimposed on that of the acetate. Lactones are, however, so far unknown among carotenoids, and their properties must therefore be extrapolated from model compounds. Normally substituted α-pyrones have carbonyl IR frequencies in the 1725−1735 cm^{-1} region and seven-membered lactones at even lower frequencies.[33] Butenolides, however, have carbonyl absorptions in the 1740−1775 cm^{-1} region,[33] for example, the model compound *7* absorbs at 1740 cm^{-1}.[34] It is therefore implied that the lactone moiety represents a butenolide in conjugation with the main chromophore of the peridinin molecule. The unusual IR absorption at 1525 cm^{-1} is also attributed to the butenolide moiety D, since in other butenolides similar absorptions have been ascribed to a vibration of the butenolide ring.[35,36]

A conjugate butenolide moiety explains adequately both the reduction data [17] and the remaining ester carbonyl signal in the ^{13}C NMR spectrum of the *p*-bromobenzoate *1c*.[20] Furthermore, a ^{13}C NMR signal at −17.5 ppm may be assigned to an enolic carbon.[20]

The remaining C_9H_{10} element must contain a vinylic methyl group in unusual magnetic surroundings to account for the ^1H NMR signal at δ 2.20, and it is reasonable to assume that the strong deshielding is caused by the close proximity to the lactone. For example, the sesquiterpene lactone freelingyne (*8*) has a central methyl group which resonates at δ 2.33 and the endocyclic and one exocyclic olefinic proton give rise to signals at δ 7.02 and 5.62, respectively,[37] in good agreement with the olefinic singlets at δ 7.01 and 5.75 in the peridinin spectrum. It is therefore likely, also considering the biogenetic isoprene rule,[38] that peridinin contains a structural element of the same type as freelingyne (*8*).

Assuming that the structural element D′ (Scheme 2) is present, the remaining C_6H_6, as a conjugated polyene system (E, Scheme 2), would then account for the rest of the mole-



Scheme 2.

cule, supported by the six proton ^1H NMR signal at δ 6.5 already mentioned.

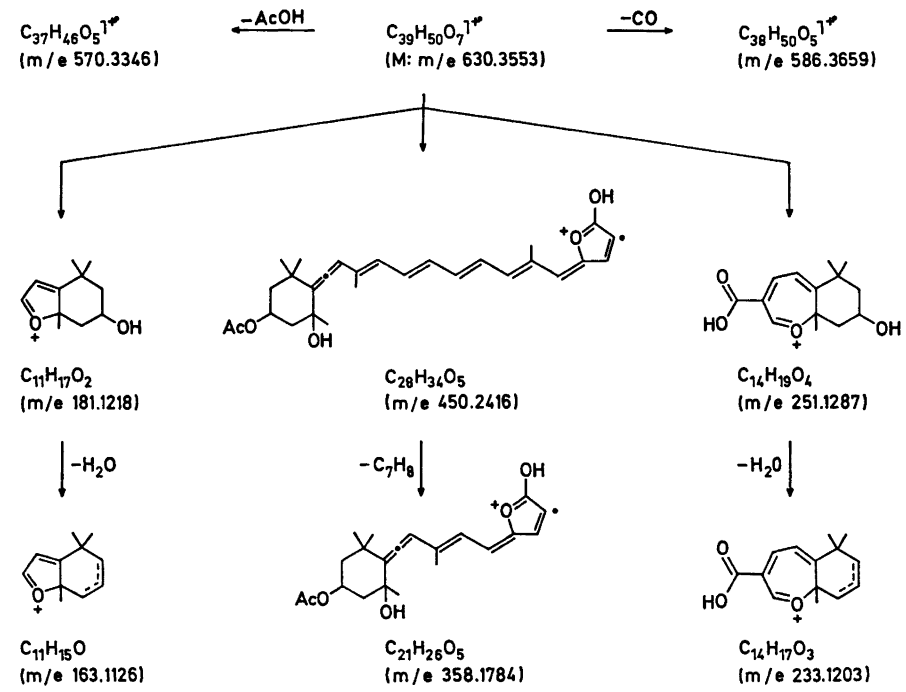
It now remains to combine the various structural elements A,B,D′,E in a way which adequately explains the properties of peridinin.

Examination of the high resolution mass spectrum [17,43] (Scheme 3) shows that the smallest O_5-fragment has a composition $C_{21}H_{26}O_5$ (*m/e* 358.1784). Another O_5-fragment, $C_{28}H_{34}O_5$ (*m/e* 450.2416, differs from the first by C_7H_8, which may be an element of toluene containing E, Scheme 2. Since toluene (C_7H_8) is also lost from the molecular ion and other fragments, it is reasonable to assume that the two fragments are interconnected by C_7H_8 and that the larger one represents the true molecular boundaries for the five oxygen atoms. The fragment $C_{28}H_{34}O_5$ must, by necessity, contain the allenic end group A plus E, Scheme 2, to fulfill the carbon and oxygen requirements.

On the other hand, the smallest O_4-fragment, $C_{14}H_{19}O_4$ (*m/e* 251.1287), cannot be derived from the allenic end group, since ozonolysis revealed only three oxygen atoms within a C_{15} element. Still disregarding the possibility of gross skeletal rearrangements, this fragment must therefore be derived from the epoxidic end of the molecule including the lactone oxygens. A similar fragment, $C_{14}H_{17}O_3$ (*m/e* 233.1203), has obviously lost an element of water, whereas no corresponding fragment representing a loss of acetic acid was observed

One may consequently assume that the lactone moiety is located within fourteen carbons containing the epoxidic end group and within twenty-six carbons containing the allenic end group, excluding the acetate. One then arrives at structure *1* which satisfies all the relevant data. Taking biosynthetic considerations into account, this implies that the methyl group at C-9′ of a traditional C_{40}-skeleton is oxidized to give the lactone, and the C_3-element lost from the skeleton must include the methyl group at C-13.

Regarding the visible light absorption of peridinin, extrapolation of the absorption



Scheme 3.

maximum of the antibiotic lactone tetrenolin (*9*), λ_{max} 340 nm in methanol,[39] to that of peridinin, λ_{max} 468 nm in methanol, is not successful. However, the unique chromophore of the proposed structure contains eight spectroscopically efficient double bonds, expected λ_{max} *ca.* 425 nm in hexane,[19] cross-conjugated with the lactone carbonyl. Cross-conjugation is known to cause substantial bathochromic shifts in related polyenes.[40,41]

Tetrenolin (*9*) also has an exocyclic double bond in a similar position to peridinin, the olefinic protons of which give rise to signals at δ 6.90 and 7.38 [39] in fair agreement with the two doublets at δ 6.35 and 7.18 in the peridinin ^{1}H NMR spectrum.

Additional information which supports the proposed structure is presented in the following paper.[17]

Regarding the biosynthesis of peridinin, hypotheses regarding the formation of the lactone and the loss of the C_3-unit have already been advanced.[16] A somewhat modified mechanism for the expulsion of the C_3-unit, taking into account the principle of conservation of orbital symmetry,[42] proceeding *via* a cyclobutene derivative and scission of the C_3-unit as an acetylenic element, has subsequently been proposed.[43] Coupling of the two geranylgeranyl residues [44] in an unusual way facilitating the expulsion of the C_3-element, or even a totally independent biosynthesis, may also be considered. Since, however, dinoflagellates also contain normal C_{40}-carotenoids, it seems likely that peridinin would have a normal carotenoid precursor, although no obvious precursor has been found so far.[45]

The peridinin structure contains six chiral centers, so far of undetermined configuration. Work on the stereochemistry of peridinin (*1*) will be pursued.

The currently used definition of carotenoid [46a] has recently been changed [46b] in order to include peridinin (*1*) and related nor-carotenoids.[45]

EXPERIMENTAL

Materials and methods employed in the Norwegian laboratory were as described elsewhere.[47]

Biological material. Gonyaulax polyedra was harvested at La Jolla during a "red tide" in which this species represented about 99 % of the phytoplankton enumerated by microscopic examination. The harvests were freeze-dried and held at −10 °C.

Anthopleura xanthogrammica was collected at Halt Moon Bay (Pillar Point), California.

Cachonina niei (Indiana Culture Collection No. 1564) provided the major starting material used in the isolation of peridinin. Large scale culture of the alga was grown in 10 l bottles or in 180 l polyethylene drums at 18−20 °C in an enriched sea-water medium.[48,49] Illumination was provided by "cool white" fluorescent lamps at a light intensity of 500−800 cd. The cultures were bubbled lightly with air during growth and were harvested either directly by centrifugation or after initially floculating the cells with alum. Yields ranged from 0.2−0.4 g wet weight/l after 10 d growth. Harvests were freeze-dried and held at −10 °C.

Isolation. Peridinin was isolated at La Jolla as described previously,[3] and at Argonne by the following procedure:

The sea anemones (2 kg wet weight) were cut into pieces and extracted with methanol and ether until the extracts were colourless. The pigments were transferred into ether by dilution with aq. NaCl.

Freeze-dried *Cachonina niei* (10−20 g) was extracted alternately with methanol and ether in a column containing a layer of Celite. The filtrate was washed with aq. NaCl solution and evaporated.

The pigments were dissolved in ether-petroleum ether (1:4) and chromatographed on columns of powdered sugar using 1.5 % propanol in petroleum ether as developer. The major zone (peridinin) was cut out and eluted with ethanol-petroleum ether. Peridinin was rechromatographed on sugar columns developed with 2, 2.25, and 2.5 % propanol in petroleum ether as above. The eluate was washed with water, dried and evaporated.

Homogeneous peridinin was crystallized from chloroform-lipopure hexane (1:15) and the crystals collected by centrifugation; approximate yield 1.5 mg peridinin/g cells.

Recrystallization was effected from the same solvent system or methanol-water.

Peridinin (*1*). [43,50] Crystallization of chromatographically homogeneous fractions from ether-hexane afforded peridinin as purple crystals; m.p. 128−132 °C. Found: C 73.15; H 7.68. $C_{41}H_{52}O_8$ (Mw = 672) requires C 73.18; H 7.79; $C_{39}H_{50}O_7$ (Mw = 630) requires C 74.25; H 7.99. λ_{max} (hexane) 454, 484 nm; % III/II [32] = 80; λ_{max} (acetone) 466 nm; λ_{max} (ethanol) 475 [E(1 cm, 1 %) = 1350] nm; ν_{max} (KBr) 3450 (bonded OH), 3040−2800 (CH), 1930 (C=C=C), 1745 (broad, C=O), 1525 (C=C), 1450 (CH_2), 1365 (CH_3), 1250 (C−O−, acetate), 1190-1110 (C−O−), 1080−1010 (C−O−), 985 (*trans*-CH=CH−), 960, 942, 913, 900, 860, 820 and 770 cm^{-1}; δ ($CDCl_3$) 0.97 (s, 3 H, CH_3 at C-1′), 1.07 (s, 3 H, CH_3 at C-1), 1.20 (s, 2 × 3 H, CH_3 at C-1′ and C-5′), 1.35 (s, 3 H, CH_3 at C-1), 1.37 (s, 3 H, CH_3 at C-5), 1.81 (s, 3 H, CH_3 at C-9), 2.01 (s, 3 H, CH_3, acetate), 2.20 (s, 3 H, CH_3 at C-13′), 3.9 (m, 1 H, H-3′), 5.4 (m, 1 H, H-3), 5.75 (s, 1 H, H-12′), 6.05 (s, 1 H, H-11), 6.35 (d, 1 H, J = 16 Hz, H-8′), *ca.* 6.5 (m, 6 H, olefinic), 7.01 (s, 1 H, H-10′), and 7.18 (d, 1 H, J = 16 Hz, H-7′); for Eu(dpm)$_3$ shift experiments [43] see Table 2. *m/e* (high resolution) 630.3553 (M = $C_{39}H_{50}O_7$, calc. 630.3555), 612 (M − H_2O), 594 (M − 2 × H_2O), 586 (M − CO_2), 570 (M − AcOH, m*), 568 (M − H_2O − CO_2), 552 (612 − AcOH, *m**), 538 (M − C_7H_8), 534 (M − 2H_2O − AcOH), 520 (M − H_2O − C_7H_8), 508 (M − H_2O − AcOH − CO_2), 478 (538 − AcOH, *m**), 450 ($C_{28}H_{34}O_5$), 397 ($C_{28}H_{29}O_3$), 358 ($C_{21}H_{26}O_5$), 257 ($C_{17}H_{23}O_3$), 251 ($C_{14}H_{19}O_4$), 234 ($C_{14}H_{18}O_3$), 233 ($C_{14}H_{17}O_3$), 223 ($C_{17}H_{19}$), 212 ($C_{16}H_{20}$), 197 ($C_{15}H_{17}$, 75 %), 181 ($C_{11}H_{17}O_2$, 100 %), 167 ($C_{10}H_{15}O_2$), 163 ($C_{11}H_{15}O$), 149 ($C_{10}H_{13}O$), and 125 ($C_7H_9O_2$).

Peridinin acetate (*1a*).[51] Peridinin (*1*, 25 mg) and acetic anhydride (0.5 ml) in dry pyridine (5 ml) were reacted at room temp. for 16 h. Ether (50 ml) was added and the reaction mixture extracted twice with water, then with 0.05 N HCl, 10 % $NaHCO_3$ and dried over Na_2SO_4. The solvent was evaporated and the residue crystallized from boiling hexane; yield 19 mg (71 %); λ_{max} (hexane) 455, 486 nm, λ_{max} (methanol) 472 nm; ν_{max} (CCl_4) 1930 (C=C=C), 1760, 1740 (C=O) cm^{-1}; *m/e* 672 (M, consistent with $C_{41}H_{52}O_5$), 610 538, 223, 216 and 197.

Peridinin ^{14}*C-acetate* (*1b*).[51] ^{14}C-acetic anhydride was prepared from sodium ^{14}C-acetate and unlabelled acetic anhydride by exchange and distillation of the product. The specific activity was 3.83×10^5 dpm/mmol counted as acetanilide.

Peridinin (*1*, 10 mg) and ^{14}C-acetic anhydride (100 µl) was treated as for peridinin acetate (*1a*) above. The product was purified by chromatography on $CaCO_3$ and sucrose columns. Crystallization twice from ether-hexane gave 5 mg (47 %) peridinin ^{14}C-acetate (*1b*).

Two samples (1 mg each) were dissolved in scintillation grade toluene, ozonized at −78 °C until colourless, scintillation solution added and the samples counted to 549 and 551 dpm/mg, respectively, corresponding to a molecular weight of 696 for *1b*.

Acetate estimation by dilution of activity.[51] Peridinin ^{14}C-acetate (*1b*, 20 mg), prepared with ^{14}C-acetic anhydride of acyl activity 3.071×10^6 dpm/mmol as above, was hydrolyzed in 12 N H_2SO_4:dioxane (1:1,10 ml) and refluxed for 16 h. Steam distillation liberated 2.24 mg (1.25 equiv.) as estimated by titration. The neutralized liberated acid was freeze-dried and the residue suspended in ether (10 ml). The *m*-iodoanilide was prepared by addition of oxalyl chloride (0.1 ml), stirring for 1 h and addition

of *m*-iodoaniline (0.5 ml). After 1 h at room temperature ether was added, the suspension filtered and the filtrate washed with dilute HCl and 10 % $NaHCO_3$, dried over Na_2SO_4 and the solvent evaporated. Isolation by TLC and sublimation twice gave 2 mg *m*-iodoacetanilide, homogeneous by TLC. Two samples were counted giving specific activities of 3.70×10^3 and 4.12×10^3 dpm/mg corresponding to dilutions of the original activity by 3.2:1 and 2.9:1, respectively.

Peridinin p-*bromobenzoate* (*1c*).[51] Peridinin (*1*, 50 mg) in dry pyridine (5 ml) and *p*-bromobenzoyl chloride (200 mg) in dry pyridine (5 ml) were pooled at 0 °C and reacted at room temperature for 20 h. The reaction mixture was diluted with ether, washed with water, 0.2 N HCl and 10 % $NaHCO_3$. Evaporation of the solvent and crystallization of the residue twice from ether-hexane gave 53 mg (82 %) *p*-bromobenzoate (*1c*).

Peridinin chloroacetate (*1d*).[51] Peridinin (*1*, 5 mg) in acetone (10 ml) and chloroacetyl chloride (50 µl) in dry pyridine (1 ml) were pooled at 0 °C and reacted at room temp. for 12 h. The reaction mixture was diluted with ether (25 ml), washed with water, dilute HCl and finally with 10 % $NaHCO_3$. The solvent was removed and the residue crystallized twice from ether-hexane. The chloroacetate (4 mg, 71 %), homogeneous on TLC, had *m/e* 706 ($M = C_{41}H_{51}{}^{35}ClO_8$).

Peridinin di-TMS ether (*1e*).[50] Peridinin (*1*, 1 mg) was treated with hexamethyldisilazane (0.2 ml) and trimethylchlorosilane (0.1 ml) in dry pyridine (1 ml) at room temp. for 2 h. Excess reagents were removed by evaporation and the residue chromatographed on $CaCO_3$ and eluted with 4 % acetone in hexane. The di-TMS ether (0.6 mg, 60 %), homogeneous on TLC, had λ_{max} (hexane) 454, 484 nm, λ_{max} (methanol) 472 nm; *m/e* 774 ($C_{45}H_{66}O_7Si_2$).

Peridinin acetate-TMS ether (*1f*).[50] Peridinin acetate (*1a*, 0.3 mg) was silylated as above for 5 h and the product chromatographed on silica plates developed with 30 % acetone in petroleum ether. The homogeneous TMS ether (0.28 mg, 93 %) had λ_{max} (petroleum ether) 457, 487 nm; % $III/II^{32} = 60$; *m/e* 744 ($M = C_{44}H_{60}O_8Si$).

Peridininol (*1g*).[50] Peridinin (*1*) was saponified as described in the following paper.[17] Peridininol (3.4 mg, 6.5 %) had λ_{max} (acetone) 464 nm; *m/e* 588 ($M = C_{37}H_{48}O_6$), 570 ($M - H_2O$), 552 ($M - 2H_2O$).

Upon acetylation peridininol (*1g*) gave a diacetate (M = 672), inseparable from peridinin acetate (1a), and two monoacetates (M = 630), one of which could not be separated from peridinin (*1*).

Acid treatment of peridinin (*1*).[50] See a following paper.[17]

Acid treatment of peridinin acetate (*1a*).[51] Peridinin acetate (*1a*, 1 mg) in CH_2Cl_2 (5 ml) was treated with 2 drops of CH_2Cl_2 saturated with HCl gas at room temperature for 2 h during which period the visible light absorption shifted from λ_{max} 467, 483 nm to λ_{max} 446, (463) nm. Two products, both less polar than *1a* on TLC (silica), were observed.

Hydrogenation of peridinin.[51] Peridinin (*1*, 25 mg) in ethanol (96 %, 10 ml) was hydrogenated at atmospheric pressure over PtO_2 catalyst (10 mg) for 4 h. The H_2-uptake was 9.8 equivalents per mol peridinin. Filtration and evaporation of the solvent gave 23 mg (93 %) crude hydrogenation product; λ_{max} (hexane) 267, 273 nm; ν_{max} (liq) 1750–1730 (C=O) cm^{-1}; δ (60 MHz, $CDCl_3$) 0.8–1.1 (*ca.* 15 H), 1.1–1.4 (*ca.* 30 H), 1.4–1.8 (*ca.* 20 H), 2.02 (s, 3 H), 2.5 (m, 1 H) and 5.2 (m, 1 H).

TLC (silica-$CHCl_3$) revealed the presence of at least six products.

Perhydrogenation of peridinin.[51] The crude hydrogenation product (22 mg) in methanol (25 ml) was reduced with KBH_4 (100 mg) by refluxing for 1 h. Excess KBH_4 was destroyed with 1 N HCl, the solvent evaporated and the residue redissolved in ether. The solution was washed with 10 % $NaHCO_3$, dried over Na_2SO_4 and evaporated to give 19 mg (83 %) of crude reduction product which had no detectable UV absorption and no carbonyl absorption in IR.

The above crude product (18 mg), freshly distilled HI (55 %, 3 ml) and red phosphorus (100 mg) in glacial acetic acid (9 ml) were refluxed for 24 h. The acetic acid was removed *in vacuo*, the aqueous phase extracted with ether, the extract washed with 10 % $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated to give 14 mg (75 %) of a complex mixture of products as demonstrated by TLC (silica-CCl_4). The non-polar material ($R_F = 0.8 - 0.95$) had no significant C=C absorption in the IR. Mass spectrometry of this fraction gave the highest observable molecular ion at *m/e* 510 corresponding to a hydrocarbon $C_{37}H_{66}$.

After similar treatment fucoxanthin (*2*) gave the highest molecular ion at *m/e* 552 ($C_{40}H_{72}$), 6 mass units less than expected (*m/e* 558) for $C_{40}H_{78}$. Similar treatment of β-carotene gave one fraction with the molecular ion at the expected *m/e* 558.

Hydroxamic acid test for ester functions.[51] Peridinin (*1*, 2.5 mg) in ethanol (96 %, 2 ml), 7.0 % hydroxylamine hydrochloride (1 ml), and 10 % NaOH (1 ml) were incubated at 37 °C for 15 min. 0.1 M Citrate buffer (pH 1.4, 1 ml) and water (2 ml) were added, the solution extracted with benzene (3 × 3 ml) and the benzene phase removed. A 0.3 M solution of $Fe(NH_4)_3(SO_4)_4.24H_2O$ (1 ml) was added to the water phase and the volume adjusted to 10 ml in a volumetric flask. An absorption of 0.59 at 495 nm, as read against a blank and standards of isopropyl acetate (0.002 and 0.004 mM, absorbance 0.18 and 0.36, respectively), corresponded to 1.7 ester functions per molecule of *1* (for Mw = 630).

Similar treatment of fucoxanthin (*2*, 4.5 mg) and fucoxanthin acetate (*2a*, 3.0 mg) gave absorbances of 0.54 and 0.68, corresponding to 0.9 and 1.7 ester functions per molecule of *2* and *2a*, respectively.

Ozonolysis of peridinin p-*bromobenzoate* (*1c*).[51] Peridinin *p*-bromobenzoate (*1c*, 25 mg) in methanol-chloroform (3:1, 50 ml) was ozonized at −78 °C until the solution had a light yellow colour. Dimethyl sulfide (2 ml) was added and the solution allowed to reach ambient temp. within 2 h. Evaporation of the solvent and TLC (silica, benzene-acetone 4:1) gave one homogeneous fraction ($R_F = 0.3$). Purification on an alumina (grade 3) [52] column, eluting with 1 % acetone in benzene, gave a colourless viscous oil (*6*, 3 mg) with λ_{max} (hexane) 225 nm, λ_{max} (ethanol) 233 nm; ν_{max} (liq) 3450 (OH), 1945 (C=C=C), 1730 (C=O, acetate) and 1675 (C=O, ketone) cm^{-1}; δ (CCl_4) 1.14 (3 H), 1.41 (2 × 3 H), 1.96 (3 H), 2.10 (3 H) and 5.76 (1 H); *m/e* 266.1514 ($M = C_{15}H_{22}O_4$, calc. 266.1518), 223 ($M - C_2H_3O$), 206 ($M - C_2H_4O_2$).

The other TLC fractions all contained *p*-bromobenzoic acid when checked by mass spectrometry.

Acknowledgements. Dr. B. Ke, Kettering Research Laboratory, Yellow Springs, Ohio, kindly recorded the low temperature spectrum of peridinin.

The Argonne group acknowledges the participation of Miss M. C. Grandolfo and Dr. K. Aitzetmüller in the introductory phase of this work and the kind interest of Dr. J. J. Katz.

FTH acknowledges the assistance of Susan Wang in the isolation of peridinin from *Gonyaulax polyedra*, the assistance of A. R. Loeblich III and J. R. Lance in culturing *Cachonina niei*, and research support from The Marine Life Research Program of the California Cooperative Fisheries Program.

SLJ acknowledges research grants from the Norwegian Council of Science and Humanities and Hoffmann-La Roche, Basel, used for the support of HK and technical assistance, respectively. SN was supported by a grant from The Norwegian Institute of Technology, University of Trondheim.

REFERENCES

1. Graham, H. W. In Smith, G. M. *Manual of Phycology*, Chronica Botanica, Waltham 1951, p. 104.
2. Ryther, J. H. In Johnson, F. H., Ed., *The Luminescence of Biological Systems*, American Association for the Advancement of Science, Washington 1955, p. 387.
3. Jeffrey, S. W. and Haxo, F. T. *Biol. Bull. 135* (1968) 149.
4. Haxo, F. T. In Allen, M. B., Ed., *Comparative Biochemistry of Photoreactive Systems*, Academic, New York 1960, p. 339.
5. Halldal, P. *Biol. Bull. 134* (1968) 411.
6. Schütt, F. *Ber. Deut. Bot. Ges. 8* (1890) 9.
7. Pinckard, J. H., Kittredge, J. S., Fox, D. L., Haxo, F. T. and Zechmeister, L. *Arch. Biochem. Biophys. 44* (1953) 189.
8. Jeffrey, S. W. *Biochem. J. 80* (1961) 336.
9. Jeffrey, S. W. *Biochim. Biophys. Acta 162* (1968) 271.
10. Loeblich, A. R. and Smith, V. E. *Lipids 3* (1968) 5.
11. Goodwin, T. W. In Goodwin, T. W., Ed., *Aspects of Terpenoid Chemistry and Biochemistry*, Academic, London 1971, Chapter 11.
12. Heilbron, I. M., Jackson, H. and Jones, R. N. *Biochem. J. 29* (1935) 1384.
13. Strain, H. H., Manning, W. M. and Hardin, G. *Biol. Bull. 86* (1944) 169.
14. Strain, H. H. and Svec, W. A. *Advan. Chromatogr. 8* (1969) 119, 172.
15. Karrer, P. and Jucker, E. *Helv. Chim. Acta 29* (1946) 229.
16. Strain, H. H., Svec, W. A., Aitzetmüller, K., Grandolfo, M. C., Katz, J. J., Kjøsen, H., Norgård, S., Liaaen-Jensen, S., Haxo, F. T., Wegfahrt, P. and Rapoport, H. *J. Amer. Chem. Soc. 93* (1971) 1823.
17. Kjøsen, H., Norgård, S., Liaaen-Jensen, S., Svec, W. A., Strain, H. H., Wegfahrt, P., Rapoport, H. and Haxo, F. T. *Acta Chem. Scand. B 30* (1976) 157.
18. Budzikiewicz, H., Djerassi, C. and Williams, D. H. *Structure Elucidation of Natural Products by Mass Spectrometry*, Holden Day, San Francisco 1964, Vol I, p. 9.
19. Vetter, W., Englert, G., Rigassi, N. and Schwieter, U. In Isler, O., Ed., *Carotenoids*, Birkhäuser, Basel 1971, p. 189.
20. Stothers, J. B. *Quart. Rev. Chem. Soc. 19* (1965) 144.
21. Cope, A. C., Bly, R. K., Burrows, E. P., Ceder, O. J., Ciganek, E., Gillis, B. T., Porter, R. F. and Johnson, H. E. *J. Amer. Chem. Soc. 84* (1962) 2170.
22. Jackman, L. M. and Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, Pergamon, London 1969, Chapter 3-2.
23. Savitsky, G. and Namikawa, K. *J. Phys. Chem. 68* (1964) 1956.
24. Bonnet, R., Mallams, A. K., Spark, A. A., Tee, J. L., Weedon, B. C. L. and McCormick, A. *J. Chem. Soc. C* (1969) 429.
25. Jensen, A. *Norwegian Institute Seaweed Research Report No. 31*, Tapir, Trondheim 1966.
26. Sanders, J. K. M. and Williams, D. H. *J. Amer. Chem. Soc. 93* (1971) 641.
27. Kjøsen, H. and Liaaen-Jensen, S. *Acta Chem. Scand. 26* (1972) 2185.

28. Baldas, J., Porter, Q. N., Cholnoky, L., Szabolcs, J. and Weedon, B. C. L. *Chem. Commun.* (1966) 852.
29. Strain, H. H. *Arch. Biochem. Biophys. 48* (1954) 458.
30. Weedon, B. C. L. in Isler, O., Ed., *Carotenoids*, Birkhäuser, Basel 1971, p. 267.
31. Kjøsen, H., Arpin, N. and Liaaen-Jensen, S. *Acta Chem. Scand. 26* (1972) 3053.
32. Ke, B., Imsgard, F., Kjøsen, H. and Liaaen-Jensen, S. *Biochim. Biophys. Acta 210* (1970) 139.
33. Bellamy, L. J. *The Infra-red Spectra of Complex Molecules*, 2nd ed., Methuen, London 1964, p. 185.
34. Matsumara, S. *Bull. Chem. Soc. Jap. 34* (1961) 361.
35. Bohlmann, F., Herbst, P., Arndt, C., Schönowsky, H. and Gleinig, H. *Chem. Ber. 94* (1961) 3193.
36. Hauge, K. *Thesis*, Norwegian Institute of Technology, Univ. Trondheim 1972, p. 158.
37. Massy-Westropp, R. A., Reynolds, G. D. and Spotswood, T. M. *Tetrahedron Lett.* (1966) 1939.
38. Ruzicka, L. *Experientia 9* (1953) 357.
39. Gallo, G. G., Coronelli, C., Vigevani, A. and Lancini, G. C. *Tetrahedron 25* (1969) 5677.
40. Haeck, H. H. and Kralt, T. *Rec. Trav. Chim. Pays-Bas 85* (1966) 343.
41. Aasen, A. J. and Liaaen-Jensen, S. *Acta Chem. Scand. 21* (1967) 2185.
42. Woodward, R. B. and Hoffmann, R. *The Conservation of Orbital Symmetry*, Verlag Chemie, Weinheim 1970, pp. 45, 73.
43. Kjøsen, H. *Thesis*, Norwegian Institute of Technology, Univ. Trondheim 1972.
44. Goodwin, T. W. In Isler, O., Ed., *Carotenoids*, Birkhäuser, Basel 1971, p. 577.
45. Johansen, J. E., Svec, W. A., Liaaen-Jensen, S. and Haxo, F. T. *Phytochemistry 13* (1974) 2261.
46. IUPAC Commission on the Nomenclature of Organic Chemistry and IUPAC-IUB Commission on Biochemical Nomenclature: a. Tentative Rules for the Nomenclature of Carotenoids. *Biochemistry 10* (1971) 4827; b. *Nomenclature of Carotenoids* (Rules approved 1974), Butterworths, London. *In press.*
47. Kjøsen, H. and Liaaen-Jensen, S. *Acta Chem. Scand. 26* (1972) 4121.
48. Siegelman, H. W. and Guillard, R. R. *Methods Enzymol. 23* (1971) 110.
49. Loeblich, A. R. *Thesis*, Univ. of California, San Diego 1971, p. 55.
50. Norgård, S. *Thesis*, Norwegian Institute of Technology, University of Trondheim, 1972.
51. Wegfahrt, P. *Thesis*, Univ. California, Berkeley 1970.
52. Brockmann, H. and Schodder, H. *Ber. Deut. Chem. Ges. 74* (1941) 73.

Received June 6, 1975.