Phytochemistry, 1974, Vol. 13, pp. 2261 to 2271. Pergamon Press. Printed in England.

CAROTENOIDS OF THE DINOPHYCEAE*

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(*Received* 3 *February* 1974)

Key Word Index—*Amphidinium*, etc.; Dinophyceae; Pyrrophyta; carotenoids; peridinin; carotenoid glycosides; dinoxanthin.

Abstract—The carotenoids of the photosynthetic dinoflagellates *Amphidinium carterae* (two strains), *Glenodinium* sp., *Gymnodinium splendens*, *G. nelsoni* and *Gyrodinium dorsum* have been investigated, quantitatively and qualitatively. Peridinin is the principal carotenoid in all species; also present are β-carotene, diadinoxanthin, dinoxanthin, pyrrhoxanthin, astaxanthin, peridininol, diatoxanthin and pyrrhoxanthinol. New structures have been assigned to dinoxanthin and pyrrhoxanthin while peridininol and pyrrhoxanthinol are new carotenoids not previously reported. A carotenoid glycoside, P-457, found in four species, is a hexoside. Dinoxanthin is the only plausible biosynthetic precursor of peridinin that could be detected.

INTRODUCTION

DINOPHYCEAE are members of an algal class (division Pyrrophyta), carotenoids of which, except for peridinin (**1**), have not been adequately investigated by modern spectroscopic methods. Previous investigations on the carotenoids of dinoflagellates, including zooxanthellae (symbiotic dinoflagellates) are listed in Table 1. There is general agreement that β-carotene (**2**) is the only hydrocarbon present,[2,6,9,11] although a γ-carotene-like pigment also has been reported.[10] The principal carotenoid is peridinin (**1**). Peridinin (**1**), first

* Part X in the series "Algal Carotenoids"; for Part IX see *Acta Chem. Scand.* In press.
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[1] (a) PARSONS, T. R. (1961) *J. Fish. Res. Board Canada* **18**, 1017; (b) TAYLOR, D. L. (1973) *Ann. Rev. Microbiol.* **27**, 171.
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isolated in 1890 by Schütt,[16] is considered identical with sulcatoxanthin isolated from the sea anemone *Anemonia sulcata*.[14] It is a unique bicyclic nor-carotenoid with a C_{37}-skeleton.[17] Two dinoflagellate species (*Glenodinium foliaceum* and *Gymnodinium veneficum*) are reported to contain fucoxanthin (**3**) instead of peridinin (**1**) as the major carotenoid.[3,10]

TABLE 1. CAROTENOID COMPOSITION OF SOME DINOPHYCEAE, IN PER CENT OF TOTAL CAROTENOIDS

Species[13]	Ref.	Carotenes	Dinoxanthin	Diadinoxanthin	Peridinin	Fucoxanthin	Pyrrhoxanthin	Other xanthophylls	mg/g dry weight
Prorocentrales (order):									
Prorocentraceae (family)									
Exuviaella sp.	1a	7	24		38			31	3·4
Prorocentrum micans	2	5			53			42	
Prorocentrum micans	3	4		24	66			6	
Peridiniales:									
Gymnodiniaceae									
Amphidinium carterae	1,4	8	26		52			14	5·0
Amphidinium klebsii	5	-			+			+	
Amphidinium sp.	6	2·5	11·1		84·0			2·4	
Amphidinium sp.	7	6	9	12	70			3	
Gymnodinium veneficum	3	4		19		64		13	
Gymnodinium sp.	8	11	24		65				0·80
Gyrodinium resplendens	9	+	+	+	+		+	+	
Glenodiniaceae									
Glenodinium foliaceum	10	+		+		+		+	
Peridiniaceae									
Peridinium cinctum	11	+	+	+	+			+	
Peridinium trochoidium	12	+	+		+				
Gonyaulacaceae									
Gonyaulax polyedra	13				+			+	
Zoozanthellae*									
Tridacna crocea (clam)†	6	3·0	15·7		77·0			4·3	
Pocillipora sp. (coral)†	6	+	+	+	+			+	
Anthopleura xanthogrammica (sea anemone)‡	11	+	+	+	+			+	
Anemonia sulcata (sea anemone)	14				+§				

* Pigments from the symbiotic dinoflagellate.

† Five species of *Tridacnid* clams and eight species of *Zooantharian* and *Alcyonyarian* corals and one *Hydrozoan* coral were found to have an identical pigment pattern.

‡ Previously named *Bunodactis xanthogrammica*.[11,15]

§ Named sulcatoxanthin.

Associated with peridinin (**1**) are diadinoxanthin (**4**) and dinoxanthin and certain carotenoids in smaller quantities, some of which are believed to be *cis*-isomers.[11,15] Structure **4** for diadinoxanthin has been independently confirmed.[18,19] Most workers (Table 1) report dinoxanthin as a member of the Dinophyceae carotenoids, but no structure has been proposed. Strain *et al.*[11] described its spectral properties in visible light [λ_{max} (ethanol) 441·5 and 471 nm], relative polarity on magnesia and sugar columns and colour reaction with concentrated hydrochloric acid. Loeblich and Smith[9] described dinoxanthin in its unesterified form as a dihydroxy-5,6-monoepoxide with a nonaene chromophore and

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[17] 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. (1971) *J. Am. Chem. Soc.* **93**, 1823.

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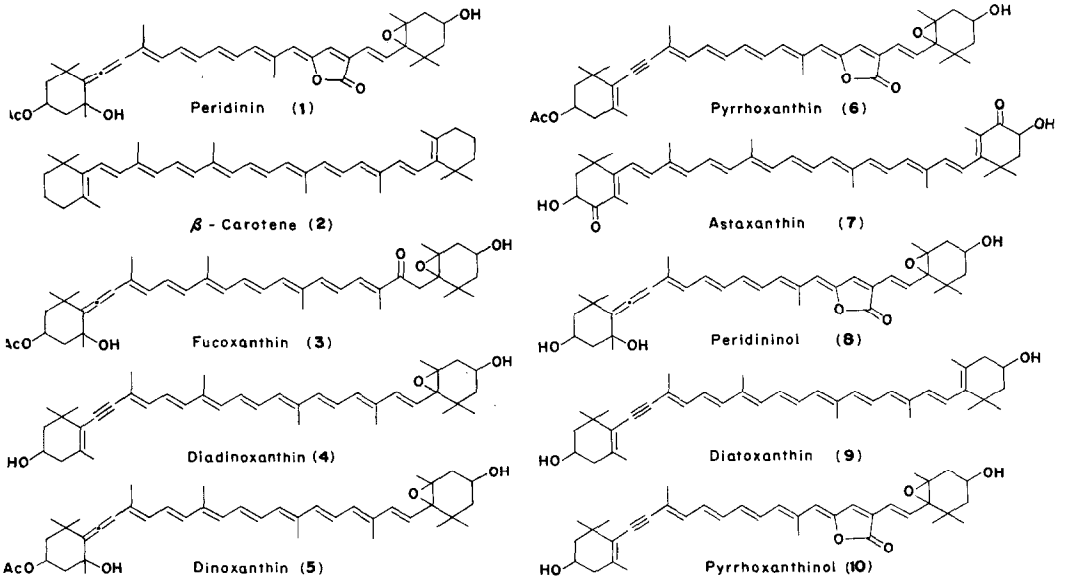
non-allylic hydroxy groups. The occurrence of dinoxanthin may not be restricted to dinoflagellates since it has been reported to be present in a Chrysophyte (*Isochrysis galbana*).[12]



The presence of unidentified xanthophylls has been frequently reported in dinoflagellates.[2,5–7,9–11] Loeblich and Smith[9] isolated and described several minor xanthophylls from the marine dinoflagellate *Gyrodinium resplendens*. One of these, pyrrhoxanthin [λ_{max} (hexane) 459 and 487 nm; λ_{max} (ethanol) 471 nm], possesses a conjugated carbonyl group like peridinin (**1**) and was considered to be a 5,6-monoepoxide.

The structure of peridinin (**1**) suggests that dinoflagellates possess enzyme systems capable of modifying the polyene chain. In other organisms structural diversification usually occurs in the carotenoid end group. A hypothetical scheme for the formation of the peridinin (**1**) skeleton has been presented by Strain *et al.* (Scheme 1a).[17] Taking into account the fact that peridinin-containing dinoflagellates are photosynthetic, Kjøsen[20] suggested that the expulsion of the C_3-unit may be a photosensitized reaction sequence, although enzymatically controlled (Scheme 1b).

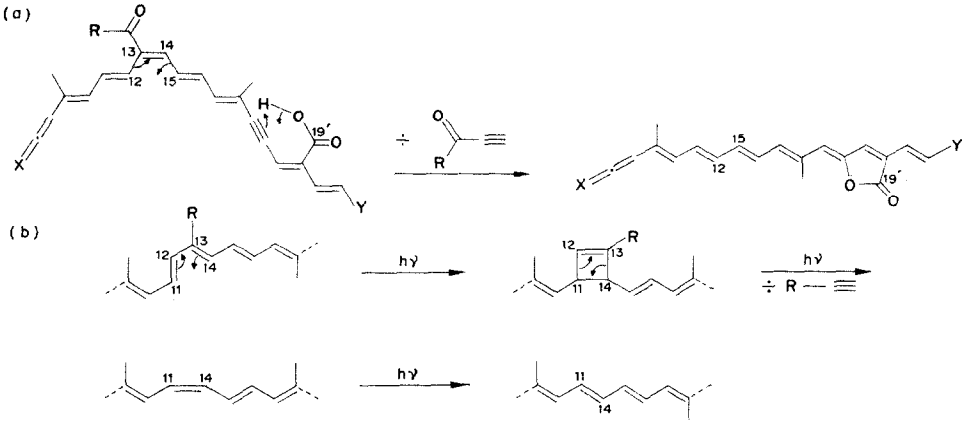
The aim of the present work was to analyze the carotenoids of selected dinoflagellates with special attention to the minor carotenoids, in order to gain knowledge of the biosynthesis of the unique C_{37}-skeleton of peridinin (**1**). A further goal was to provide a more secure basis for evaluating phyletic relationships among the brownish coloured algae.

RESULTS

Cultures of *Amphidinium carterae* (Plymouth No. 450 and Provasoli strain), *Gymnodinium nelsoni* strain GSBL, *G. splendens*, *Gyrodinium dorsum* (all of order Peridiniales, family Gymnodiniaceae[21]) and *Glenodinium* sp. (order Peridiniales, family Glenodiniaceae[21]) were grown under conditions described below. The carotenoid composition of each

[20] KJØSEN, H. (1972) *Synthetic and Spectroscopic Studies of Carotenoids*, The University of Trondheim, Trondheim.

[21] PARKE, M. and DIXON, P. S. (1968) *J. Mar. Biol. Ass. U.K.* **48**, 783.



alga was analyzed (Table 3); judging from the appearance on initial TLC plates, the six cultures contained the same pigments (Table 2). However, subsequent purifications of the individual carotenoids suggest that there may be some species differences in minor carotenoids. The chromatographic separation was complicated by furanoid rearrangement of 5,6-epoxides, probably due to traces of acid derived from the biological material. The pigments from each species were investigated separately, and similar fractions were pooled when considered identical, judged by electronic and MS and co-chromatography.

Identified pigments

The carotenoids are treated in order of increasing adsorption (Table 2). β-Carotene (**2**) which comprised from 0·4 to 3·7% of the total carotenoids, was the only carotene present

TABLE 2. APPEARANCE OF AN EXTRACT FROM *Gyrodinium dorsum* ON TLC (KIESELGEL G, DEVELOPER 30% ACETONE IN PETROL.), TYPICAL FOR THE DINOFLAGELLATES INVESTIGATED

Zone no.	R_f	Colour of zone	λ_{max} (Me_2CO)	Carotenoid	Solvent used on rechrom.
1	0·96	Yellow	(428), 452, 477	β-Carotene	100% p.
2	0·94–0·66	Tr. of yellow, orange, red		Unidentified	15% a.p.
3	0·67–0·50	Grey, green, tr. of yellow		Unidentified	20% a.p., s.
4	0·47	Yellow	418, 442, 470 457·5 452, 479	Dinoxanthin Pyrrhoxanthin Diatoxanthin	30% a.p.
5	0·41	Yellow	426, 447·5, 478	Diadinoxanthin	30% a.p.
6	0·33	Red	466	Peridinin	
6A			457·5	Pyrrhoxanthinol	35% a.p.
7	0·07	Orange–red	466	Peridininol	50% a.p.
8	0·00	Brownish-green	457	P-457	30% b.m.

a.p. = % Me_2CO in petrol.
b.m. = % C_6H_6 in MeOH.
tr. = Traces.
s. = Saponified before rechromatography.

and was identified by its spectral properties (electronic and MS) and by co-chromatography with synthetic material. The next four pigments (one zone on TLC, cfr. zone 4; Table 2) were separated after acetylation. They comprised from 3·1 to 9·4% of the total carotenoids. Diatoxanthin (**9**) was identified on the basis of the electronic, IR, and MS properties of the diacetate (**12**, formulae numbers of structures not given refer to the Experimental) and from the MS of the unacetylated mixture. Authentic diatoxanthin (**9**) or its diacetate (**12**) were not available for comparison.

TABLE 3. CAROTENOID COMPONENTS IN ORDER OF POLARITY OF SIX DINOFLAGELLATES

	Order:	Peridiniales											
	Family:	Gymnodiniaceae										Glenodiniaceae	
	Species:	*Amphidinium carterae*		*Amphidinium carterae* Plymouth 450		*Gymnodinium nelsoni*		*Gymnodinium splendens*		*Gyrodinium dorsum*		*Glenodinium* sp.	
Pigment	$E^{1\%}_{1cm}$ used in acetone	(mg)	(% of total)	(mg)	(% of total)	(mg)	(% of total)	(mg)	(% of total)	(mg)	(% of total)	(mg)	(% of total)
β-Carotene	2500	0·30	0·4	0·14	0·9	0·06	3·7	0·12	0·9	0·08	0·4	0·20	0·4
Unknown	2500	0·18	0·2	0·04	0·3	0·01	0·6	0·03	0·2	0·40	1·9	0·14	0·3
Diatoxanthin	2100	3·28	4·6	1·09§	7·3	0·05‖	3·1			2·02	9·4	2·25	4·9
Pyrrhoxanthin													
Dinoxanthin				0·08	0·5			0·51	4·0				
Astaxanthin	2500	–	–	–	–	–	–	–	–	–	–	0·15	0·3
Diadinoxanthin	2250†	18·42	25·8	0·56	3·8	0·22	13·4	2·87	22·2	4·87	22·6	4·34	9·4
Peridinin	1350*	48·50	67·9	12·75	85·5	1·30	79·2	9·18	70·9	13·67	63·6	38·13	82·9
Pyrrhoxanthinol	1350	–	–	–	–	–	–			0·10	0·5	–	–
Peridininol	1350	0·09	0·1	0·25	1·7	tr.		0·12	0·9	0·12	0·6	0·28	0·6
P-457	2500	0·70	1·0	–	–			0·11	0·9	0·21	1·0	0·53	1·2
Total (mg)		71·47		14·91		1·64		12·94		21·47		46·02	
Total sample dry weight (g)		19·90		25·00¶				12·65		12·67		11·70	
mg/g Dry weight		3·59		2·91				1·02		1·70		3·93	

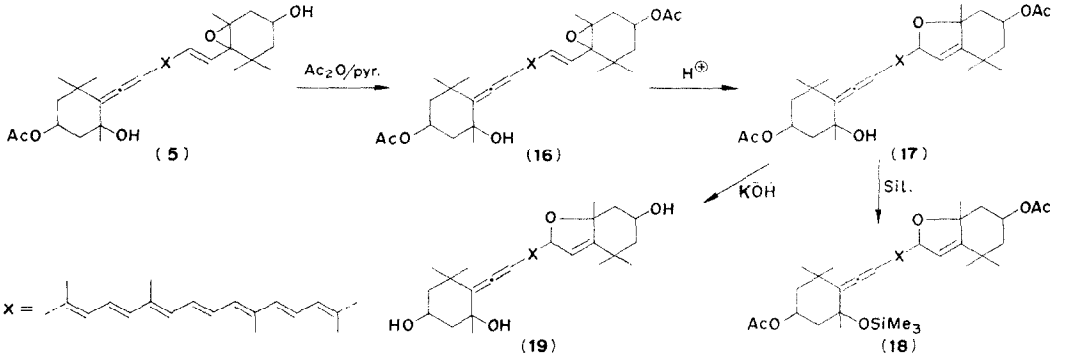
* Reference 6.
† Present study.
‡ The $E^{1\%}_{1cm}$ of dinoxanthin (= neoxanthin-3-acetate) is used.[22]
§ Includes some diadinochrome (rearranged diadinoxanthin).
‖ Presence of diatoxanthin and pyrrhoxanthin are uncertain.
¶ Only 1/5 of the extract was investigated.

Pyrrhoxanthin (**6**) gave a monoacetate **13**. A parent peak in the MS of the unacetylated mixture at *m/e* 612·3454 ($C_{39}H_{48}O_6$) indicated that pyrrhoxanthin (**10**) is closely related to peridinin (**1**, $C_{39}H_{50}O_7$). The IR spectrum of pyrrhoxanthin acetate (**13**) showed a weak absorption at 2170 cm^{-1}, characteristic of an acetylenic group. Upon treatment of peridinin acetate (**14**) with $POCl_3$, a mixture of the allenic anhydroperidinin acetate (**15**) and the acetylenic pyrrhoxanthin acetate (**13**) was formed, as reported separately.[22] The electronic spectra of semisynthetic and natural pyrrhoxanthin acetate (**13**) were identical and the compounds could not be separated on kieselguhr or alumina paper. The acetoxy end group of pyrrhoxanthin (**10**) was assigned to the acetylenic end group, tentatively C-3, for the same reasons mentioned below for dinoxanthin (**5**). Previous data reported for pyrrhoxanthin[9] are consistent with this formulation.

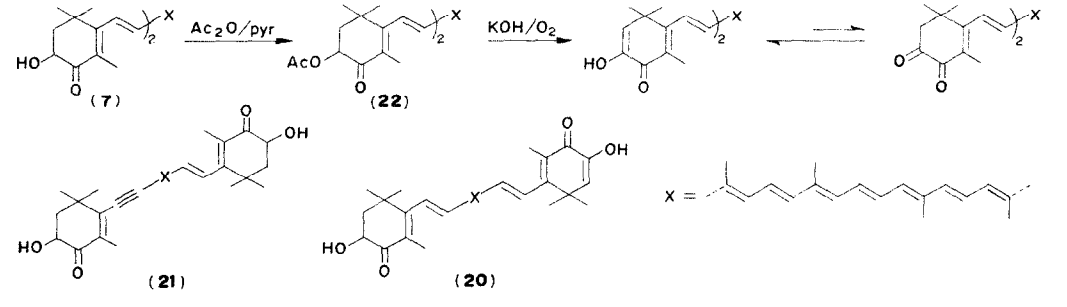
Dinoxanthin (**5**), the major component of the above mixture (zone 4; Table 2), gave a monoacetate (**16**). A molecular ion at *m/e* 642·4284 ($C_{42}H_{58}O_5$) before acetylation was consistent with the monoacetate of a C_{40}-carotenoid. Dinoxanthin acetate (**16**) gave a

[22] JOHANSEN, J. E. and LIAAEN-JENSEN, S., *Acta Chem. Scand.* In press.

hypsochromic shift (18 nm) in visible light on treatment with dilute hydrogen chloride in acetone, as expected for furanoid rearrangement of a 5,6-epoxide.[23] Dinochrome acetate (**17** = furanoid rearranged **16**) gave a mono(trimethyl)silyl ether **18** on silylation and neochrome (**19**) upon saponification. Dinoxanthin acetate (**16**) exhibited IR absorption at 1925 cm^{-1}, ascribable to an allenic group. The acetoxy function was assigned to the allenic end-group, tentatively C-3, from MS data. The MS of the unacetylated mixture containing dinoxanthin (**5**, a natural acetate) had prominent peaks at *m/e* 181 and 221, typical of a 3-hydroxy-5,6-epoxide end-group,[24] whereas no peaks at *m/e* 223 and 263, characteristic of 3-acetoxy-5,6-epoxides were observed. Dinoxanthin (**5**) is thus considered to be neoxanthin-3-acetate. Previous data reported for dinoxanthin[7,9] are consistent with this formulation.



Astaxanthin (**7**) was found only in *Glenodinium* sp. (0·3% of total carotenoids). Identification of the crystalline sample as astaxanthin (**7**) was based upon comparison of the electronic spectra and co-chromatography with authentic material. The MS showed the molecular ion at *m/e* 596·3866 ($C_{40}H_{52}O_4$). An *m/e* 594·3704 ($C_{40}H_{50}O_4$) peak could either represent an M-2 peak, the molecular ion of partly oxidized astaxanthin (**20**) or the molecular ion of monoacetylenic astaxanthin (e.g. **21**). The absence of a corresponding peak (*m/e* 678) beside the molecular ion *m/e* 680 in the diacetate (**22**) obtained after acetylation of the mixture (zone 4; Table 2) argues against the co-existence of the acetylenic derivative (e.g. **21**) of astaxanthin (**7**). Astaxanthin diacetate (**22**) co-chromatographed with an authentic sample. On saponification in the presence of air astaxanthin diacetate (**22**) gave a product which was strongly retained on alumina paper, a typical property of carotenoid diosphenols[23] and as expected for astacene.



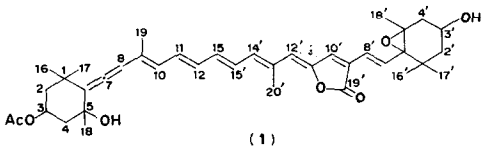
[23] LIAAEN-JENSEN, S. (1971) *Carotenoids* (ISLER, O., ed.), Birkhäuser, Stuttgart.
[24] BALDAS, J., PORTER, Q. N., CHOLNOKY, L., SZABOLCS J. and WEEDON, B. C. L. (1966) *Chem. Commun.*, 852.

Diadinoxanthin (**4**) m.p. 172–175°C (uncorrect, previously reported 158–162°C[18]) was the second most abundant carotenoid (from 9·4 to 25·8%). It was identified on the basis of spectral properties (electronic, IR, MS and NMR spectra), and by chemical reactions. Diadinoxanthin (**4**) gave a diacetate (**23**) on acetylation and furanoid rearrangement to diadinochrome (**24**) on treatment with dilute hydrochloric acid in acetone. Co-chromatography with authentic diadinoxanthin (**4**) gave no separation.

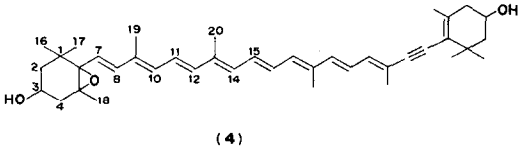
TABLE 4. ABSORPTION MAXIMA OF ALL-*trans* DIADINOXANTHIN (**4**)

Solvent	λ_{max} (nm)	$E^{1\%}_{1\,cm}$	% D_B/D_{II}	% III/II
Hexane and petrol.	278, 338, (424), 445·5, 474·5	2110	7	57
MeOH	338, 444·5, 474	2250	6	54
Me_2CO	340, 426, 447·5, 478	2230	9	61

Peridinin (**1**) was the most abundant carotenoid in all species and comprised from 63·6 to 85·5% of the total carotenoids. Its spectral properties (electronic, IR, MS and NMR spectrum) were consistent with reported data.[17] Upon co-chromatography with authentic peridinin (**1**) no separation was obtained.



(**1**)



(**4**)

Pyrrhoxanthinol (**10**), found in *Gyrodinium dorsum* (0·5% of total carotenoids), was identified from its spectral (electronic and MS) properties only. Its electronic spectrum was identical to that of pyrrhoxanthin (**6**) indicating the presence of the same chromophore. A molecular ion at *m/e* 570·3351 ($C_{37}H_{46}O_5$) indicated that this pigment was pyrrhoxanthin (**6**) lacking the acetoxy group at C-3. Pyrrhoxanthinol (**10**) was slightly more polar than peridinin (**1**), and was only observed during rechromatography of the pooled peridinin (**1**) extracts.

Peridininol (**8**) comprised from trace amounts to 1·7% of the total carotenoids. Peridininol (**8**) and peridinin (**1**) had identical electronic spectra, but peridininol (**8**) was more polar and had a molecular ion at *m/e* 588·3460 ($C_{37}H_{48}O_6$) consistent with a deactylated peridinin (**1**). Peridininol (**8**) gave peridinin acetate (**14**) on acetylation, and peridinin (**1**) gave peridininol (**8**) on careful treatment with alkali.

Unidentified pigments

P-457 (**11**) was the most polar carotenoid found. Attempts to record IR- and MS were unsuccessful. The electronic spectrum of P-457 (**11**) indicated an octaene conjugated with a carbonyl group. The MS of its acetate, P-457-ac. 1 (**25**) exhibited peaks at *m/e* 331, 211, 169 and 109, strongly indicating that P-457 (**11**) was a hexoside.[25] Exact mass measurement of the prominent peaks in the higher mass region gave no further structural information. Silylation of P-457-ac. 1 (**25**), monitored by PC, indicated that a mono(trimethyl)silyl ether (**26**) was formed, thus revealing the presence of one tertiary hydroxy group in P-457 (**11**).

[25] BIEMANN, K., DE JONGH, D. C. and SCHNOES, H. K. (1963) *J. Am. Chem. Soc.* **85**, 1763.

The reaction products formed upon treatment of P-457 (**7**) with dilute acidic methanol, or diazomethane and of P-457-ac. 4 (**27**) with $LiAlH_4$ were compared. All had octaene-type chromophore (hypsochromic shift of 25–26 nm in acetone and methanol and increased spectral fine structure).

Several minor carotenoid pigments were observed on the initial TLC-plate between β-carotene (**2**) and the mixture of dinoxanthin (**5**), pyrrhoxanthin (**6**), diatoxanthin (**9**) and astaxanthin (**7**). Some of these were partly masked by large amounts of pheophytin and chlorophyll. The latter were removed by saponification prior to rechromatography of the mixed carotenoid fraction. Data for these minor carotenoids are summarized in the Experimental. Identification of the trace pigments requires further investigations with larger amounts of starting material.

DISCUSSION

It may be concluded from the previous and present investigations that peridinin (**1**) is the major carotenoid of dinoflagellates. Two exceptions have been reported,[3,10] where fucoxanthin (**3**) replaces peridinin (**1**). Two *Woloszynska* spp. also appear to contain fucoxanthin (**3**) rather than peridinin (**1**).[26] In a recent screening of another twenty dinoflagellates from uni-algal cultures, seventeen contained peridinin and three (including *Glenodinium foliaceum* = *Peridinium foliaceum*) showed replacement of peridinin by fucoxanthin.[27]

From the present study, β-carotene (**2**), diatoxanthin (**9**), pyrrhoxanthin (**6**), dinoxanthin (**5**) and peridininol (**8**) seem to be regularly present. Pyrrhoxanthinol (**10**) was encountered only in one species, but might have been overlooked in other species examined. A glycosidic carotenoid was found in four species, the first report of a glycosidic carotenoid in dinoflagellates. Because of the extreme polarity of this pigment it may have been overlooked in other cases. Astaxanthin (**7**) does not seem to occur generally, and was only found in the species belonging to the family Glenodiniaceae. The presence of astaxanthin (**7**) is surprising since its structure is not closely related to other known dinoflagellates carotenoids, except β-carotene (**2**).

Dinoxanthin (**5**) represents a likely C_{40}-precursor of peridinin (**1**) with both the peridinin end groups pre-made. No minor carotenoids were present which could enlighten the postulated structural modifications of the polyene chain leading to peridinin (**1**). However, we here report the occurrence of peridininol (**8**), pyrrhoxanthin (**6**) and pyrrhoxanthinol (**10**) which represent structural variations of peridinin (**1**). The possibility of the alcohols **8** and **10** representing deacetylated artefacts of the naturally occurring acetates (**1** and **6**) is not likely considering the isolation procedure used.

EXPERIMENTAL

Biological material. The dinoflagellate cultures examined and their origins were as follows: *Amphidinium carterae* Hulb. from L. Provasoli (original designation *A. klebsii* Kof. et Swezy*); *Amphidinium carterae* Hulb., Plymouth 450 (from L. Provasoli through D. L. Taylor and originally designated *A. hoefleri*)*; *Glenodinium* sp. from L. Provasoli through M. Bernhard; *Gymnodinium splendens* Lebour, Haxo Py-14; *Gyrodinium dorsum* Kofoid, Haxo Py-18, through D. Davenport; *Gymnodinium nelsoni* strain GSBL from R. Guillard. The dinoflagellates

[26] WHITTLE, S. J. and CASSELTON, P. J. (1968) *Brit. Phycol. Bull.* **3**, 602.
[27] JEFFREY, S. W., SIELICKI, M. and HAXO, F. T., To be published.

* Clarification of species designations by personal communications from L. Provasoli and D. L. Taylor; see also TAYLOR, D. L. (1971) *Brit. Phycol. J.* **6**, 129.

were grown as uni-algal cultures on Loeblich's modification (GPM) of *Gonyaulax polyedra* medium.[9,13,28] Large scale culture was at 18–20°C in 180 l. polyethylene drums,[29] omitting soil extract and with a 1/2 supplementation of nutrients during the growth period. Illumination was continuous at an intensity of 3300–6600 lx, provided by cool white fluorescent lamps. The cultures were bubbled lightly with air through glass tubes, keeping the algae in suspension during growth. Algae were harvested near the end of log phase growth (10–19 days) on a Sharples continuous flow centrifuge, yielding 42–100 g fr. wt of cells/1721. The packed cells were suspended in 0·05 M Tris buffer at pH 8·0, frozen, lyophylized and stored at 5°.

Materials and methods. Unless otherwise specified these were as described elsewhere.[30] IR spectra were recorded in KBr, PMR spectra in $CDCl_3$ and MS on an AEI MS 902 mass spectrometer with direct inlet system at 70 eV, 190–270°C. Exact masses were measured using PFK and $(C_4F_9)_3N$ as standards.

Isolation. Freeze-dried cells were extracted with MeOH–Me_2CO (3:2). The crude extracts were centrifuged and taken to dryness under vacuum and dissolved in ether. The pigments were separated initially by TLC on silicagel G (1 mm thickness, 30% Me_2CO in petrol.). A brownish–green ppt., insoluble in ether, was dissolved in MeOH prior to chromatography. The carotenoids from each zone were rechromatographed in appropriate TLC systems, described in detail from *Gyrodinium dorsum* in Table 2, and the individual components characterized (R_f-value, co-chromatography, visible and MS). The carotenoids obtained from *Amphidinium carterae*, *Gymnodinium splendens*. *Gymnodinium dorsum* and *Glenodinium* sp. were mixed when considered to be identical. The native carotenoids are considered below in order of increasing adsorption (Table 2). The carotenoids of zone 4 were acetylated to achieve separation.

β-Carotene (2) had λ_{max} (Me_2CO) (428), 452 and 477 nm: λ_{max} (petrol.) (425), 446·5 and 472 nm; *m/e* 536 (M, 83·4%), 444 (M-92, 8·3%), 430 (M-106, 3·7%) and 378 (M-158, 0·9%). Chromatography on alumina paper (SS288, 1% ether in petrol.) gave two zones, shown to be isomers by iodine catalyzed stereomutation. The all-*trans* isomer could not be separated from synthetic β-carotene (**2**) in the same system (R_f 0·50).

Diatoxanthin (**9**) exhibited a molecular ion in the MS of the zone 4 pigments (Table 2) at *m/e* 566·4127 (calc. 566·4124 for $C_{40}H_{54}O_2$). The zone 4 pool from four species on acetylation yielded 0·64 mg *diatoxanthin diacetate* (**12**), R_f 0·84 (SS288, 5% Me_2CO in petrol.), had λ_{max} (Me_2CO) 452 and 479 nm; λ_{max} (hexane) 447 and 474·5 nm; ν_{max} (KBr) 3020, 2955, 2920 and 2850 (CH), 2170 (–C≡C–), 1737 (C=O),1625, 1565 (C=C), 1453 (CH_2), 1363 (Me), 1242 (C–O), 1188, 1147, 1125, 1073, 1030 (C–O), 967 and 958 (*trans* –CH=CH–), 890, 830 (>C=CH–), 746 and 720 cm^{-1}; *m/e* 650 (M, 59·1%), 608 (M-42, 0·8%), 590 (M-60, 4·1%) and 558 (M-92, 0·9%).

Pyrrhoxanthin (**6**) exhibited molecular ion in the MS of zone 4 at *m/e* 612·3454 (calc. 612·3451 for $C_{39}H_{48}O_6$). The pooled fractions of zone 4 from 4 species on acetylation yielded 1·31 mg *pyrrhoxanthin acetate* (**13**) λ_{max} (Me_2CO) 457·5 nm; λ_{max} (MeOH) 457 nm; ν_{max} (KBr) 3020, 2955, 2925 and 2855 (CH), 2170 (–C≡C–), 1756 and 1740 (C=O), 1630, 1520 (C=C), 1460 (CH_2), 1379 and 1366 (Me), 1244, 1231, 1127, 1043, 1030 (C–O), 986 (*trans* –CH=CH–), 943, 905, 820 (>C=CH–), 768, 725 and 643 cm^{-1}; *m/e* 654 (M, 21·2%), 526 (M-28, 0·7%), 612 (M-42, 1·2%), 594 (M-60, 5·0%), 579 (M-75, 2·3%), 574 (M-80, 1·0%, 562 (M-92, 1·2%), 478 (5·7%), 450 (1·8%), 303 (10·7%), 285 (10·6%), 233 (7·6%) and 223 (15·3%). PC (SS287, 5% Me_2CO in petrol.) gave two isomers: neo A:R_f 0·50 (orange); λ_{max} (hexane) 267, 336, 455 and 484 nm, % D_B/D_{II} = 24,[31] % III/II = 23[31] and *trans*:R_f 0·41 (red); λ_{max} (hexane) 268, (336), 459 and 489 nm, % D_B/D_{II} = 14, % III/II = 32. These were interconverible by iodine catalyzed stereomutation. The *trans* isomer could not be separated (SS287, 5% Me_2CO in petrol.) from semisynthetic pyrrhoxanthin acetate (**13**) obtained from peridinin acetate (**14**).[22]

Dinoxanthin (**5**) had a molecular ion in the MS of the zone 4 pigments (Table 2) at *m/e* 642·4287 calc. 642·4284 $C_{42}H_{58}O_5$). On acetylation of the pigments of zone 4 (Table 2) from four species 2·66 mg *dinoxanthin acetate* (**16**) was obtained; R_f 0·57 (SS287, 10% Me_2CO in petrol.); λ_{max} (Me_2CO) 418, 442 and 470 nm; ν_{max} (KBr) 3430 (OH), 3030, 2960, 2920 and 2853 (CH), 1930 (allene), 1738 (C=O), 1628, 1447 (CH_2), 1366 (Me), 1245, 1160, 1120, 1073, 1031 (C–O), 967 (*trans* –CH=CH–), 888, 885, 837 (>C=CH–) and 821 cm^{-1}; *m/e* 684 (M, 66·7%), 666 (M-18, 14·5%), 624 (M-60, 16·1%), 604 (M-80, 10·6%), 592 (M-92, 5·5%), 586 (35·3%), 544 (5·1%), 263 (58·8%), 223 (35·2%) and 221 (27·4%). The diacetate (**16**) on standing rearranged to dinochrome acetate (**17**)[22]; λ_{max} (acetone) 401, 424 and 450 nm. Dinochrome acetate (**17**, 0·10 mg) was saponified (1 hr). Yield 0·10 mg (100%) neochrome (**19**); λ_{max} (acetone) 401, 424 and 450 nm; *m/e* 600 (M). Dinochrome acetate (**17**, 0·10 mg) was silylated (1 hr, room temperature), yield 0·02 mg (13%) **17**-mono(trimethyl)silyl ether (**18**); R_f 0·91 (SS287, 5% acetone in petroleum ether); λ_{max} (acetone) 400, 423 and 448·5 nm; *m/e* 756 (M, 53·0%), 714 (M-42, 2·9%), 696 (M-60, 2·9%), 676 (M-80, 10·3%), 666 (M-90, 25·8%), 664 (M-92, 13·2%) and 586 (M-80–90, 50·0%).

Astaxanthin (**7**). An acetone soln of zone 4 (Table 2) from *Glenodinium* sp. yielded red crystals of **7** (0·04 mg) before acetylation; λ_{max} (acetone) 472 nm; *m/e* 596·3856 (calc. 596·3866 for $C_{40}H_{52}O_4$, M_1, 71·8%), 594·3704 (calc. 594·3709 for $C_{40}H_{50}O_4$, M_2, 75·0%), 504 (M_1-92, 13·3%) and 502 (M_2-92, 16·7%). Co-chromatography with authentic astaxanthin (**7**) gave no separation, R_f 0·44 (SS287, 10% Me_2CO in petrol.). Astaxanthin diacetate (**22**,

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0·11 mg) was obtained on acetylation of the zone 4 pigments (Table 2) from *Glenodinium* sp.; λ_{max} (acetone) 474 nm; *m/e* 680 (M, 10·7%), 638 (M-42, 0·4%), 622 (M-58, 1·8%), 620 (M-60, 0·4%), 588 (M-92, 0·8%) and 564 (M-116?, 1·4%). Co-chromatography with authentic astaxanthin diacetate (**22**) gave one zone, R_f 0·73 (SS287, 10% Me_2CO in petrol.). Astaxanthin diacetate (**22**) treated with 1% KOH in MeOH (5 ml) for 1 hr gave a product which was completely retained on alumina paper (100% acetone). The product exhibited λ_{max} (acetone) 475 nm.

Diadinoxanthin (**4**) from 4 species was purified by TLC on kieselgel G; yield 19·7 mg. PC (SS287, 10% Me_2CO in petrol.) gave two zones: major (80%); R_f 0·49; λ_{max} (ethanol) 277·5, 338, (426), 447 and 476 nm, % D_B/D_{II} = 11, % III/II = 30 and minor (20%); R_f 0·32; λ_{max} (ethanol) (276), 325, 337·5, 421, 441·5 and 470·5 nm, % D_B/D_{II} = 48, % III/II = 34. The iodine catalyzed-equilibrium mixture of each isomer had identical electronic spectra. PC of the 2 mixtures gave the same 2 bands. Co-chromatography with authentic diadinoxanthin (**4**)[18] gave no separation from the least polar zone. Diadinoxanthin (**4**) was crystallized from Me_2CO, yield 7·6 mg and from Me_2CO/hexane, yield 1·3 mg. The latter crystals (all-*trans*) had m.p. 172–175°C (uncorr., reported 158–162°C[18]); λ_{max} (Table 4); ν_{max} (KBr) 3420 (OH), 3030, 2960, 2915 and 2855 (CH), 2175 (–C≡C–), 1650, 1565 (C=C), 1450 (CH_2), 1385 and 1365 (Me), 1295, 1265, 1170, 1125, 1050, 1030 (C–O), 965 (*trans* –CH=CH–), 915, 835 (>C=CH–) and 705 cm^{-1}; *m/e* 582 (M). The first crystals (9-*cis* + all-*trans*?) showed λ_{max} (hexane) 278, 338, (424), 445·5 and 475 nm, % D_B/D_{II} = 12, % III/II = 51; δ ($CDCl_3$; cf. numbering) 0·98 s (Me-1, 3H), 1·15, 1·19 and 1·20 (Me-1', 1',5, 12H), 1·27 s (imp.), 1·93 (Me-5'), 1·96 (Me-9,13,13'), 2·00 (Me-9'), 1·93–2·00 (15H), 2·25 H-4,4', 4H), 3·92 (H-3·3', 2H), 5·98 s (H-7), 6·22 s (H-8), 6·45 and 6·56, 5·98–6·70 (12H; *m/e* 582·4078 (calc. 582·4073 for $C_{40}H_{54}O_3$, M, 55·0%), 566 (M-16, 2·8%), 564 (M-18, 2·9%), 548 (M-16-18, 2·3%), 502 (M-80, 11·5%), 490 (M-92, 5·2%), 352 (21·0%), 221 (81·4%) and 181 (12·4%). Diadinoxanthin (**4**) rearranged to diadinochrome (**24**) upon addition of dil HCl gas in Me_2CO; λ_{max} (acetone) 409, 430 and 458 nm. Diadinoxanthin diacetate (**23**) was obtained on standard acetylation of diadinoxanthin (**4**); λ_{max} (acetone) 426, 447·5 and 478 nm; *m/e* 666 (M).

Peridinin (**1**) from 4 species was purified as above, with a yield 99·6 mg. It had R_f = 0·57 (SS287, 15% Me_2CO in petrol.), was inseparable from authentic peridinin (**1**);[19] ν_{max} (KBr) 3420 (OH), 3020, 2955, 2928 and 2863 (CH), 1929 (allene), 1745 (C=O), 1520, 1453 (CH_3), 1365 (Me), 1248, 1181, 1163, 1126, 1030 (C–O), 982 (*trans*–CH=CH–), 956, 940, 910, 896, 857, 820 (>C–CH–), 769, 718, 655 and 643 cm^{-1}; δ ($CDCl_3$) 0·98 s (Me-1', 3H), 1·08 s (Me-1, 3H), 1·20 s (Me-1',5', 6H), 1·26 s (imp.), 1·36 s (Me-1, 3H), 1·39 s (Me-5, 3H), 1·82 s (Me-9, 3H), 2·04 s (Me in –OAc, 3H), 2·22 s (Me-13', 3H), 3·95 (H-3', 1H), 5·39 (H-3', 1H), 5·73 s (H-12', 1H), 6·06 s (H-8, 1H), 6·37 d (H-8', $J_{7,8}$ 16 Hz) and 7·17 d (H-7', $J_{7,8}$ 16 Hz); *m/e* 630 (M, 13·9%), 612 (M-18, 66·7%), 586 (M-44, 0·8%), 570 (M-60, 1·2%), 552 (M-78, 30·0%), 538 (M-92, 6·7%), 221 (17·8%) and 181 (100%). Peridinin (**1**, 8·54 mg) was acetylated (6 hr), yield 7·44 mg (87%) *peridinin acetate* (**14**); λ_{max} (acetone) 466 nm and *m/e* 672 (*M*). To peridinin (**1**, 6 mg) dissolved in methanol (3 ml) was added K_2CO_3 (15 mg dissolved in 0·2 ml H_2O). The product was transferred to ether after 18 hr, washed, dried and purified by TLC on kieselgel G. The most polar zone showed *m/e* 588 (*M*). Co-chromatography with peridininol (**8**) on kieselguhr paper gave no separation.

Pyrrhoxanthinol (**10**) had R_f 0·45 (SS287, 15% acetone on petrol.); λ_{max} (acetone) 457·5 nm; λ_{max} (hexane) 456·5 and (484) nm; λ_{max} (MeOH) 459 nm; λ_{max} (C_6H_6) 468·5 nm; *m/e* 570·3351 (calc. 570·3345) for $C_{37}H_{46}O_5$, M, 11·6%), 478 (M-92, 7·9%) and 181 (100%).

Peridininol (**8**) had R_f 0·43 (SS287, 20% acetone in petrol.); R_f 0·54 (SS288, 6% methanol in C_6H_6); λ_{max} (acetone) 466 nm; λ_{max} (methanol) 464 nm; λ_{max} (benzene) 467·5 and 493·5 nm; *m/e* 588·3460 (calc. 588·3451 for $C_{37}H_{48}O_6$, M, 6·2%), 570 (M-18, 32·3%), 552 (M-18-18, 19·1%), 544 (M-44, 2·9%), 526 (M-62, 4·1%), 508 (M-80, 3·5%), 496 (M-92, 7·4%), 478 (M-110, 4·4%) and 181 (100%). Peridininol (**8**) gave peridinin acetate (**14**) on acetylation, λ_{max} (acetone) 466 nm; *m/e* 672 (M), 654 (M-18), 628 (M-44), 612 (M-60), 610 (M-62), 594 (M-78) and 580 (M-92).

P-457 (**11**) had R_f 0·62 (SS287, 15% MeOH in C_6H_6); R_f 0·00 (SS288, 100% acetone) and R_f 0·29 (SS288, 100% MeOH), exhibited λ_{max} (Me_2CO) 457 nm; λ_{max} (MeOH) 454·5 nm λ_{max} (benzene) 462 nm; λ_{max} ($CHCl_3$) 464 and 488 nm. P-457 (**11**) was insoluble in ether and hexane, slightly soluble in C_6H_6 and $CHCl_3$, relatively soluble in acetone and readily soluble in methanol and pyridine. IR and MS of P-457 (**11**) could not be obtained. To P-457 (7, 0·19 mg) in methanol (4 ml) was added fresh CH_2N_2 in ether.[32] The soln was stirred at room temp. for 3 hr and worked up, yield 0·09 mg (47%) of a product slightly less polar than P-457 (**11**). The product had λ_{max} (acetone) 400, 421·5 and 448 nm. The MS could not be obtained. Addition of 0·025 N HCl (4 drops) in methanol to P-457 (**11**) in MeOH was followed by a change in the visible spectrum to λ_{max} (MeOH) 303, 314, 397·5, 418·5 and 444·5 nm. P-457 (**11**) in acetone, treated in the same way, did not undergo a spectral change. Similarly no spectral change was observed upon treatment of a methanolic solution of P-457 (**11**) with KOH in methanol.

P-457-ac. 1(**25**) *and P-457-ac. 4* (**27**). P-457 (**11**, 0·89 mg) was acetylated (4 hr), yield 0·48 mg (54%) mixed product. Purification by TLC on kieselgel G gave 5 red products, two of which were further investigated. P-457-ac. 1 (**25**, 0·33 mg, 69%, least polar) gave two zones by PC: R_f 0·59 (25%) and R_f 0·37 (75%, SS288, 50% acetone in petrol.). P-457-ac. 1 (**25**) had λ_{max} (acetone) 457 nm; λ_{max} (C_6H_6) 462 and 486 nm, % III/II = 6; *m/e* (270°) 931·4424 (0·6%), 889 (931-42, 0·4%), 847 (931-84, 0·1%), 839 (931-92, 0·1%); 735·2334 (931-186?, 1·5%), 693 (735-42, 1·4%), 661 (0·7%), 651 (735-84, 0·7%), 643·3693 (735-92, 1·7%), 619·3123, 619·1883 (735-116?, 3·1%), 601 (931-330, 0·8%), 577·5184, 577·1763 (619-42, 2·4%), 535 (619-84, 0·9%), 527·3211 (619-92, 1·7%), 457 (1·5%), 447·1501 (5·9%), 405 (735-330, 447-42, 5·2%), 363 (619-330, 447-84, 2·7%), 355 (447-92, 18·1%), 331 (100%), 289 (43·2%), 229 (38·3%),

[32] DE BOER, TH. J. and BACKER, H. J. (1954) *Recueil* **73**, 229.

211 (7·2%), 169 (88·0%), 159 (81·0%) and 109 (88%), P-457-ac. 4 (**25**, 0·08 mg, 17%) gave 2 zones by PC; R_f = 0·61 (25%) and R_f = 0·49 (75%, SS287, 100% acetone). P-457-ac. 4 (**25**) had λ_{max} (acetone) 457 nm and *m/e* values as P-457-ac. 1 (**27**), but no peaks above *m/e* 735 were observed. P-457-ac. 1 (**25**) was silylated for 1 hr at temp. increasing from −35° to +20°C. PC showed no reaction intermediates. The product, R_f 0·78 and 0·62 (2 zones on SS287, 20% acetone in petrol.) had after purification λ_{max} (acetone) 457 nm; *m/e* 1020 (?) *and* 331. P-457-ac. 4 (**27**) was reduced ($LiAlH_4$, 30 min) at 0° and gave recovered P-457-ac. 4 (**27**, traces. tentatively from colour and R_f), P-457-ac. 4-red. 1 (**28**); R_f 0·4 (on TLC, kieselgel G, 50% acetone in petrol.); λ_{max} (acetone) 339, 398, 420 and 445 nm and P-457-ac.-4-red. 2 (**29**); R_f 0·0 (on TLC); λ_{max} (methanol) 398, 416 and 440·5 nm. MS of P-457-ac. 4-red. 1 (**28**) and red. 2 (**29**) could not be obtained.

Unidentified xanthophylls. AMC-445·5 (**30**, 0·18 mg), isolated after saponification of zone 3 of *Amphidinium carterae* (Provasoli strain) had R_f 0·87 (15% acetone in petrrol.); λ_{max} (acetone) 422, 445·5 and 478 nm; *m/e* 582 (M), 502 (M-80) and 490 (M-92).

GLS-442 (**31**, 0·09 mg), isolated from *Glenodinium* sp. after saponification of zone 3, exhibited λ_{max} (acetone) 422, 446 and 475 nm; λ_{max} (hexane) 418, 442 and 470 nm; *m/e* 582 (M) and 564 (M-18).

GYD-479·5 (**32**, 0·03 mg) obtained from *Gyrodinium dorsum* (zone 2), showed λ_{max} (acetone) 483 nm and λ_{max} (hexane) 456, 479·5 and 511 nm.

GYD-439·5 (**33**, 0·13 mg) isolated after saponification of zone 3 of *Gyrodinium dorsum* had λ_{max} (acetone) 421, 443·5 and 473·5 nm; λ_{max} (hexane) 416, 439·5 and 468·5 nm; *m/e* 580·3919 (calc. 580·3917 for $C_{40}H_{52}O_3$, M) and 488 (M-92).

Acknowledgements—The *Gymnodinium nelsoni* GSBL was cultured by Dr. R. Guillard, Woods Hole Oceanographic Institution. F.T.H. acknowledges the assistance of Mr. J. R. Lance in culturing and research support from the Marine Life Research Program of the California Cooperative Fisheries Program. J.E.J. and W.A.S. were supported by a research grant from Hoffmann-La Roche, Basel, to S.L.J.