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The Structure of Peridinin, the Characteristic Dinoflagellate Carotenoid

Sir:

The principal carotenoid pigment of dinoflagellates (class, Dinophyceae; division, Pyrrophyta) is an important substance in the carbon economy and ecology of the world.[1] First isolated and named peridinin by Schütt in 1890,[2] it has since been isolated from various marine and fresh-water dinoflagellates[3,4] including endozooic symbionts (zooxanthellae) of marine corals, clams,[5] and sea anemones.[4,6,7] A preparation from an anemone (*Anemonia sulcata*) was once called sulcatoxanthin.[6]

Peridinin, mp 107–109°, is an orange-red pigment (λ_{max} (hexane) 455, 485 nm; (ethanol) 475 nm),[7] readily separable from other carotenoids by chromatography. It is decolorized by alkalies[4,7] and reacts slowly with acids, the absorption maxima being shifted hypsochromically 20 nm.[4] Reduction with $LiAlH_4$ shifts the maxima to 329, 345, 364, and 390 nm in methanol.[4] Earlier analyses indicated a molecular formula $C_{40}H_{52}O_8$, mol wt 660,[6] but high-resolution mass spectrometry established[8] the molecular formula as $C_{39}H_{50}O_7$ (630.3529).

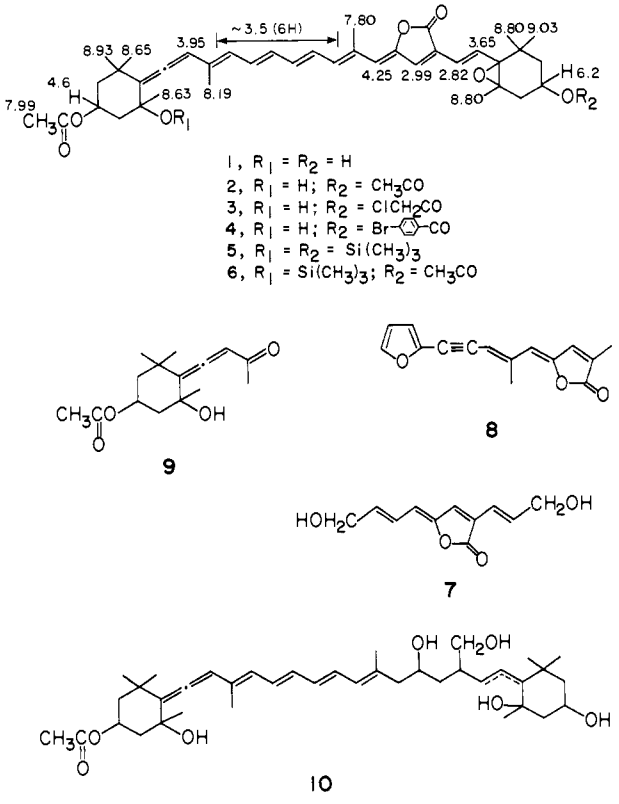
Current studies were carried out cooperatively in our four laboratories. For these studies, peridinin with identical properties was isolated from the zooxanthellae of the Pacific Coast sea anemone,[7] *Bunodactis* (now *Anthopleura*) *xanthogrammica*, from a natural bloom or red tide of dinoflagellates, greater than 98% *Gonyaulax polyedra*, and from cultures of *Cachonina niei* and *Amphidinium operculatum*.

On the basis of detailed physical and chemical evidence, we now assign structure **1** to peridinin, a unique tricyclic carotenoid with a C_{37} skeleton. The terminal rings are linked by a chain differing from the usual carotenoid structure by the absence of two in-chain carbons and two branching methyl groups, one of the latter being replaced by a carboxylic function in the form of a butenolide unit which forms part of the chromophore.

Peridinin (**1**), itself a monoacetate, on esterification yielded one further acetate (**2**), chloroacetate (**3**), or *p*-bromobenzoate (**4**). On silylation, it gave a di(trimethylsilyl) ether (**5**). The diacetate (**2**), on silylation, gave a mono(trimethylsilyl) ether (**6**). These results establish the function of four of the oxygen atoms: one acetate, one tertiary hydroxyl, and one esterifiable hydroxyl. The three remaining oxygen functions were assigned to one epoxy group and a butenolide group of the type encountered in tetrenolin (**7**)[9] and freelingyne (**8**).[10]

The location of three oxygen atoms in a terminal ring of known structure was established by ozonolysis of peridinin *p*-bromobenzoate (**4**). The resulting allenic ketone (**9**) was identical (uv, ir, nmr, mass spectra) with the ketone obtained on cleavage of fucoxanthin.[11,12] This result defined one end of the peridinin molecule and accounted for three oxygen functions: one acetate and one tertiary hydroxyl.

The pmr signal assignments of peridinin (recorded in $CDCl_3$ at 220 MHz) are given on structure **1** and



agree with previous assignments for the allenic end group of fucoxanthin[11,12] and the epoxidic end group of violaxanthin[13] and neoxanthin.[14] The olefinic region

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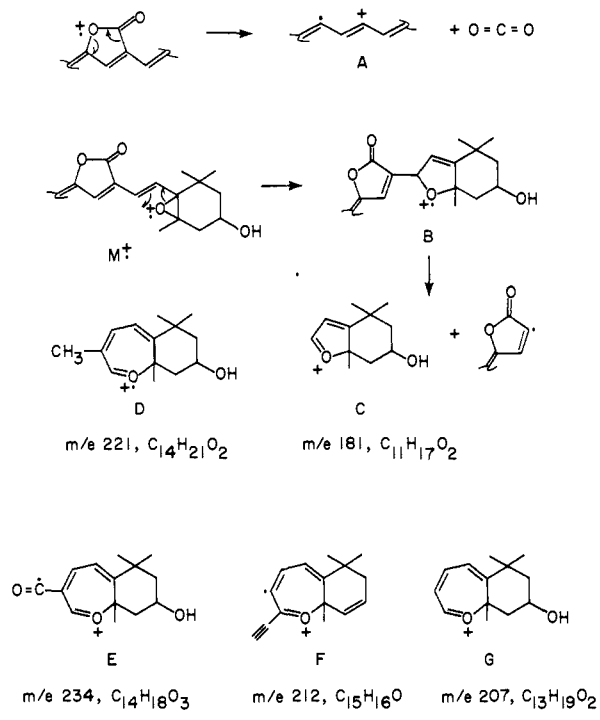
comprises three singlets assigned to the allenic proton and the exocyclic and endocyclic butenolide olefinic protons. An AB doublet, not distinguishable in the spectrum of peridinin itself, was made apparent with increasing concentrations of $Eu(dpm)_3$,[15] causing a paramagnetic shift at equimolar (20 μM) concentration of these doublets to τ 1.85 (J = 15 Hz) and 2.98 (J = 15 Hz) and further demonstrating their proximity to oxygen functions in the molecule. The signal assignments of the butenolide structural element and the neighboring olefinic protons and in chain methyl group are in good agreement with the assignments for tetrenolin (**7**) and freelingyne (**8**). Other $Eu(dpm)_3$ induced chemical shifts of peridinin (**1**) paralleled those observed for similar protons in fucoxanthin.

The ^{13}C-resonance spectrum of peridinin *p*-bromobenzoate (**4**) defined the central allene carbon[16] by a −74-ppm signal (relative to benzene), and the three signals attributable to carbonyl carbon (−43, −40, and −36 ppm) were in agreement with the three different types of carbonyl carbon[17,18] present. Assignment of a −17 ppm signal to the enol carbon is in agreement with data on related structures.

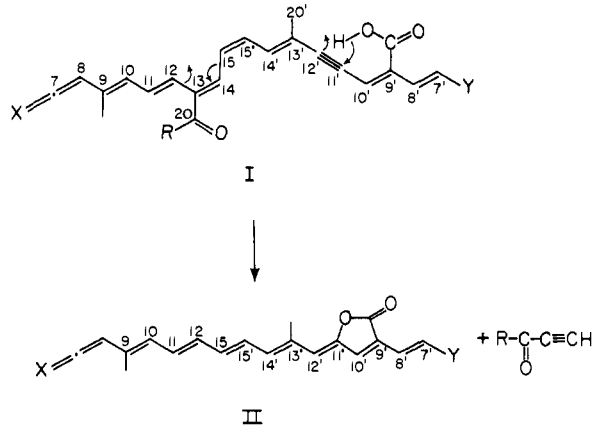
The mass spectrum of peridinin is consistent with and supports structure **1**. Expected losses of acetic acid, water, and toluene are observed. Fragments due to the loss of xylene, characteristic of most carotenoids,[19] were not found, in agreement with the location of the in-chain methyl groups. Loss of CO_2, previously not observed in carotenoid mass spectra, is readily explained from the lactone structure *via* fragment A. The assignment of diagnostically useful ions in the mass spectrum of **1** at *m/e* 181 ($C_{11}H_{17}O_2$) as C and *m/e* 234 ($C_{14}H_{18}O_3$) as E were supported by the expected mass shift in spectra of the diacetate **2**, chloroacetate **3**, and silyl ether derivative **5**. Further evidence for the absence of the usual in-chain methyl at C-9′ and its replacement by the carbonyl of the butenolide is seen in the absence of the usually abundant homopyryllium ion D, *m/e* 221 ($C_{14}H_{21}O_2$).[20] In its place were observed three other homopyryllium ions E, F, and G, reflecting this structural change.

The electronic spectrum of peridinin (**1**, λ_{max}^{hexane} 455 nm) is satisfactorily explained by allowing a *ca.* 28 nm (hexane or acetone) contribution for the cross-conjugated carbonyl group in the butenolide arrangement; *cf.* similar increment for other cross-conjugated carotenoids.[21]

In further support of structure **1** for peridinin, carefully controlled sodium borohydride reduction afforded a mixture of products with the pentaene chromophore. For the most saturated product, with eight additional hydrogen atoms (M = 638), acetylation and silylation experiments demonstrated the presence of two additional hydroxy groups accessible for acetylation and one additional tertiary hydroxyl compatible with structure **10**. In other products only the butenolide group or epoxy group was reduced.



Peridinin (**1**) occurs together with carotenoids possessing the ordinary isoprenoid C_{40}-type skeleton. The biosynthetic formation of its unusual C_{37} skeleton could be rationalized in terms of oxidation of the 20-methyl group, which by analogy with carotenoids of the rhodopinal series[21] would result in inversion of the adjacent double bond to the cis configuration, thereby facilitating rearrangement and elimination of a C_3-acetylenic moiety. The butenolide group could be formed by oxidation of the 19′-methyl group to a carboxylic acid followed by lactonization *via* addition to a carbon–carbon triple bond,[22] according to the reaction I → II. Alternatively, lactonization could occur by addi-



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tion to a double bond, followed by reoxidation. Full details will be presented in a future publication.

(23) Supported by the U. S. Atomic Energy Commission.
(24) S. N. was supported by a grant from the University of Trondheim and H. K. by a grant from the Norwegian Research Council for Science and Humanities to S. L. J.
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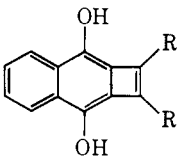
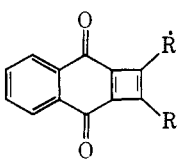
On the Antiaromaticity of the Cyclobutadiene Ring

Sir:

It has been shown recently[1] by polarography measurements that the hydroquinone I (R = CH_3 or C_6H_5), as well as its dianion, was more difficult to oxidize



I



II

than is naphthohydroquinone or its dianion, the authors stating: "the bulk of the effect is due to the antiaromaticity of the cyclobutadiene ring in II. Such antiaromaticity should raise the energy of I as well as of II, although to a lesser extent."

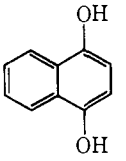
The effects observed can be easily rationalized in a very simple manner, using the theoretical indices which have been shown[2] to be correlated with the red-ox properties of quinone systems, in particular the loss in resonance energy accompanying the oxidation[3] and also the energies of the molecular orbitals involved in the electron transfer.[4] (Although the indices used are defined in the Hückel approximation, we consider that a correlation of the type mentioned may be useful as long as it has not been proven nonexistent. A new case fitting into the correlation here supports its usefulness.) Table I gives the appropriate values for a series of quinones of relevance.

It is seen that the oxidation of naphthohydroquinone proceeds against a loss of resonance energy of 0.9 β and that this loss is strongly *increased* in the system I/II, making oxidation more difficult. But still more interesting is the fact that the lowest empty molecular orbital (LEMO) of the quinone II has become *bonding*, a strong indication of an exceptional high tendency to accept electrons. This feature results from the presence of the cyclobutadiene ring, whose degenerate nonbonding orbitals both become bonding in the quinone II, one being filled and the other empty. The occurrence of a bonding LEMO in a quinone is reminiscent of the situation in diphenoquinone (XIV), which indeed is known as a powerful oxidizing agent. Both I and II see their resonance energies increased with respect to the naphthoquinones III and IV, but the increase is larger in the reduced form than in the oxidized form. The number of π electrons in I and III (or II and IV) being different, their relative *thermodynamic* stabilities are not properly measured by R but rather by R divided by the number of π electrons. Then, I and III are practically the same, whereas II appears destabilized. This clearly comes from the fact that, in I, the square cycle can assume, at least partly, a dimethylenecyclobutene structure, which is less, if at all, antiaromatic than a pure cyclobutene.

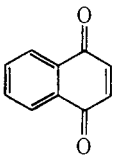
Table I. Energy Indices Related to Oxidation-Reduction[a]

hq/q	R_{hq}	R_q	ΔR	$HOMO_{hq}$	$LEMO_q$
I/II	4.73	3.44	1.29	+0.28	+0.11
III/IV	4.19	3.28	0.91	+0.41	−0.33
V/VI	5.37	4.50	0.87	+0.32	−0.24
VII/VIII	2.88	2.17	0.70	+0.17	−0.42
IX/X	2.49	1.39	1.10	+0.67	−0.21
XI/XII	2.90	1.69	1.21	+0.21	+0.08
XII/XIV	4.89	3.44	1.45	+0.53	+0.04

[a] In Hückel β units. R, resonance energy; ΔR, loss of resonance energy upon oxidation; $HOMO_{hq}$, highest occupied orbital of the hydroquinone; HOMO's are usually *bonding* (+ sign); $LEMO_q$, lowest empty orbital of the quinone; LEMO's are usually *antibonding* (− sign).



III



IV

It may be observed that a stabilization (or at least an absence of destabilization) of I from the point of view just developed is not at all incompatible with an increased instability as measured by various properties; for instance, the HOMO value is smaller than in III, making the reduction *per se* easier in I than in III.

The conclusion that no strong destabilization is expected when the square cycle can assume a dimethylenecyclobutene structure could be examined on compounds V and VI; there, ΔR is very similar to its value for III/IV, the quinone appearing as an acceptor only slightly better than IV.

On the other hand, it would seem worthwhile to find a case where the antiaromaticity of the cyclobutene ring would result in an effect opposite to the one observed in ref 1, namely an increased ease of oxidation of a hydroquinone. This should occur in a system where the dimethylenecyclobutene structure is imposed

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