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Algal Carotenoids. XV.* Structural Studies on Peridinin. Part 2. Supporting Evidence

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The chemical reactions of peridinin (*1*) towards acids, base and complex metal hydride ($NaBH_4$) have been investigated. In all cases complex mixtures of products were obtained. The physical and chemical properties of these products provide strong support for the structure proposed for peridinin (*1*).

The fragmentation pattern of peridinin (*1*) on electron impact is rationalized in terms of this structure.

The structure of peridinin (*1*), the principal carotenoid pigment of the dinoflagellates, has recently been elucidated.[1,2] With its seven oxygen functions, including a butenolide moiety as part of the chromophore and a C_{37} skeleton, peridinin is a most remarkable carotenoid.

In its chemical behaviour peridinin exhibits some uncommon properties. Simple derivative formations, *e.g.* silylation and ester formation under standard conditions, follow the expected courses and give well defined products.[2] However, previous reports have stated that peridinin is rapidly decolourized by alkali,[3,4] reacts slowly with acids resulting in a 20 nm hypsochromic shift of the visible light absorption spectrum [4] and that reduction with $LiAlH_4$ results in a mixture of products with electronic spectra consistent with pentaene chromophores.[4]

In the present work, a detailed examination of the complex reaction mixtures obtained from peridinin on treatment with acids, base and complex metal hydride ($NaBH_4$), are reported. Preliminary observations on the dehydration with phosphorus oxychloride are also presented.

The physical and chemical properties of these reaction products support the structure (*1*) proposed for peridinin.

The fragmentation pattern on electron impact is further rationalized in terms of the proposed structure.

RESULTS AND DISCUSSION

Acid treatment. Direct comparison of the acid lability of peridinin (*1*) with that of β-carotene diepoxide (*2* = 5,6:5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene) towards 1 % citric acid in methanol showed that *2* was completely isomerized (Δ nm = 40) within 10 h. Peridinin (*1*) was unaffected during the same period. With 1 % HCl in methanol *2* was totally isomerized in 3 min whereas *1* required 1.5 h for complete conversion (Δ nm = 20). Peridinin (*1*) is thus clearly much more stable towards acids than common carotenoid expoxides.[5]

Treatment of peridinin (*1*) with 1 % HCl-methanol resulted in four new products of which the two major ones, while readily separated by TLC, had identical spectral characteristics (λ_{max} 446 nm in acetone; *m/e* 630 = M, 197, 181). The magnitude of the hypsochromic shift in their visible light absorptions relative to peridinin (*1*, λ_{max} 466 nm in acetone) is as

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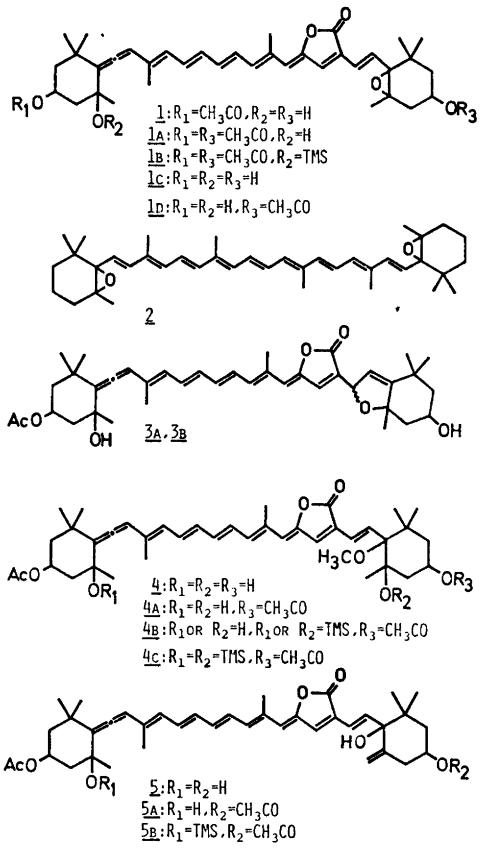


Scheme 1.

expected for a 5,6-epoxide to 5,8-furanoxide rearrangement,[5] and these two products were therefore identified as the epimeric furanoxides *3a* and *3b*.

The two minor products *4* and *5* had identical adsorptive properties and visible light absorptions (λ_{max} 459, (474) nm in acetone) and could only be separated after acetylation. The major acetate (*4a*, *m/e* 704 = M) gave a mono-TMS ether (*4b*, *m/e* 776 = M) and a di-TMS ether (*4c*, *m/e* 848 = M) on silylation, all products having unchanged visible light absorptions. Addition of methanol to peridinin (*1*, Mw = 630) would account for the formation of product *4*. Protonation of the epoxy function followed by substitution (S_N1) at C-6′ with methanol would explain the formation of the new tertiary hydroxy function giving a TMS ether upon silylation, see below. Structure *4* is therefore a likely representation of this product.

The minor acetate (*5a*, *m/e* 672 = M) exhibited the same absorption as the major acetate *4a* in visible light. Upon silylation it formed a mono-TMS ether (*5b*, *m/e* 744 = M) with unchanged spectral properties in visible light. Neither of these products (*5a* and *5b*) co-chromatographed with peridinin acetate (*1a*, *m/e* 672 = M)[2] or the TMS ether of peridinin acetate (*1b*, *m/e* 744 = M).[2] Product *5* is thus an isomer of peridinin (*1*). Acid catalyzed opening of the epoxy group of peridinin followed by elimination of a proton may account for this product, tentatively represented by structure *5* with a hydroxy function at C-6′. Tertiary hydroxy groups in this sterically hindered position are known to be inert towards standard acetylation and silylation conditions.[6] Since no allylic methyl ether was formed, *cf.* lutein,[7] the new double bond is tentatively assigned the exocyclic position.

Base treatment. Reaction of peridinin (*1*) with 0.5 % K_2CO_3 in water-methanol (0.03:1) under conditions similar to those used for fucoxanthin,[8] resulted in severe decomposition of the pigment. After 16 h all peridinin had reacted and recovery of coloured products was only 10 %.

The major product (*1c*, *m/e* 588 = M) had the same visible light absorption spectrum as peridinin (*1*), gave two monoacetates (*1* and *1d*, *m/e* 630 = M) one of which co-chromatographed with peridinin (*1*), and a diacetate (*1a*, *m/e* 672 = M) which co-chromatographed with peridinin acetate (*1a*). Product *1c* was therefore identified as deacetylated peridinin (*1c*), here called peridininol, in analogy with deacetylated fucoxanthin, fucoxanthinol.[8]

One minor product (*6*, *m/e* 620 = M) had λ_{max} 391 nm in acetone solution and gave a diacetate (*6a*, *m/e* 704 = M) on acetylation with unchanged visible light absorption. On silylation the diacetate *6a* gave a di-TMS ether (*6b*, *m/e* 848 = M) with unchanged spectral properties in the visible region. Product *6* thus possesses two primary or secondary and two tertiary hydroxy groups. Assuming that peridinin (*1*) is first deacetylated, formal addition of methanol (630 − 42 + 32 = 620) would account for the molecular weight of 620. However, from the available data no obvious structure may be proposed for this product.

A third product *7*, with λ_{max} 372 nm in acetone, gave upon acetylation an acetate (*7a*, *m/e* 684=M) with unchanged visible light absorption. Assuming that the acetylated product *7a* is a diacetate, the original product *6* must have had a molecular weight of 600 (600+42+42=684). No structural conclusions may be drawn from these data.

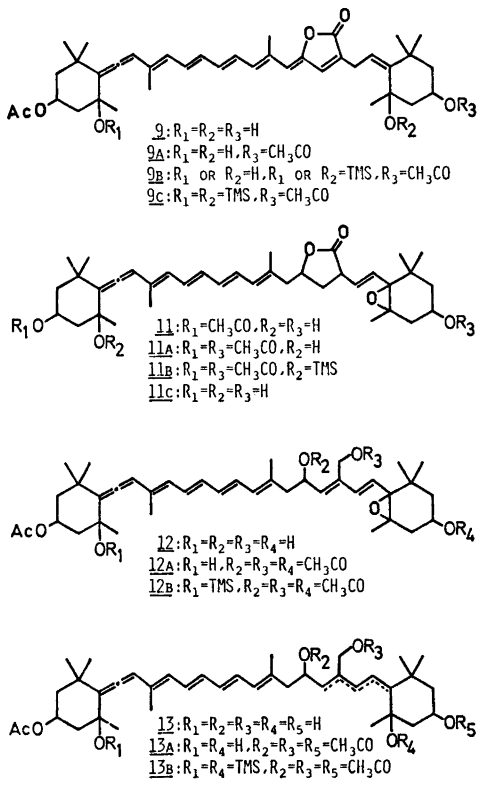
A fourth, acidic product *8* was obtained after acidification of the hypophase. It had λ_{max} 367 nm in methanol and could not be esterified with diazomethane. No conclusions as to structure are drawn for this product.

Dehydration with $POCl_3$. Reaction of peridinin (*1*) with $POCl_3$ under standard conditions[9] gave two monodehydrated (*m/e* 612=M) and two didehydrated (*m/e* 594=M) products. Further studies of this reaction are reported elsewhere.[10]

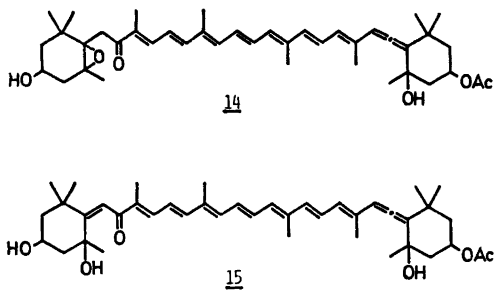
Reduction with $NaBH_4$. Reduction of peridinin (*1*) with $NaBH_4$ at room temperature resulted in only 50 % pigment recovery; unreacted peridinin 35 % and reduction products 15 %, Scheme 2. The major product (*9*) had λ_{max} 433, (452) nm in acetone. Acetylation gave an acetate (*9a*) which on silylation gave a mono-TMS ether (*9b*, *m/e* 746=M) and a di-TMS ether (*9c*, *m/e* 818=M) both with unchanged visible light absorption. Assuming that the acetylated product *9a* is a monoacetate, formation of the reduction product *9* (M=746−72−42=632) involved addition of two hydrogens with the formation of a new tertiary hydroxy group. Considering also the chromophoric change, the reduction has probably occurred as a 1,4-addition to the epoxidic system.

A UV-fluorescent product (*10*, *m/e* 634=M) had λ_{max} 331, 347 and 366.5 nm in ether, compatible with a pentaene chromophore. The IR spectrum showed absorptions for bonded OH (*ca.* 3300 cm^{-1}), allene (1930 cm^{-1}), carbonyl (1725 cm^{-1}, broad) and ester C−O stretch (1250 cm^{-1}). Acetylation gave a triacetate (*10a*, *m/e* 760=M) and silylation of the triacetate *10a* gave a mono-TMS ether (*10b*, *m/e* 832=M). Both derivatives had unchanged UV absorptions. Formation of a triacetate implies that two new acetylable hydroxy functions had been formed. Since total reduction of the lactone requires addition of six hydrogens, whereas only four were added, and partial reduction to a hydroxy ketone should give a coloured enol acetate, no plausible structure is proposed for *10*.

A third, isomeric product (*11*, *m/e* 634=M), also UV-fluorescent, had visible light absorption identical to product *10* above. Saponification gave a product (*11c*, *m/e* 592=M) with unchanged visible light absorption. Acetylation of *11* gave a monoacetate (*11a*, *m/e* 676=M), and silylation of *11* gave a di-TMS ether (*11b*, *m/e* 778=M) both with unchanged chromophores. Addition of four hydrogens to peridinin (*1*) to give a product with pentaene chromophore without formation of new reactive functional groups may be explained in terms of structure *11*. The formation of *11* may involve reduction of the butenolide double bond by 1,4-addition,[11] then opening of the lactone under the weakly alkaline conditions, followed



Scheme 2.



Scheme 3.

by reduction of the keto-enol function and subsequent ring closure.

Two products *12* and *13* had identical visible light absorptions, compatible with a pentaene chromophore, and identical adsorptive properties. They could only be separated after acetylation.

The acetate *12a* (*m/e* 762=M) gave a mono-TMS ether (*12b*, *m/e* 834=M) on silylation. Assuming that *12a* is a triacetate, it may be inferred that *12* is derived from peridinin by addition of six hydrogens with the formation of two new acetylable hydroxy functions and no new tertiary hydroxy group. This result may be explained by assuming complete reduction of the lactone system, Scheme 2.

The other acetate (*13a*, *m/e* 764=M) gave a di-TMS ether (*13b*, *m/e* 908=M) on silylation, consistent with the addition of eight hydrogens and formation of two new acetylable hydroxy functions and one new silylable, tertiary hydroxy group.

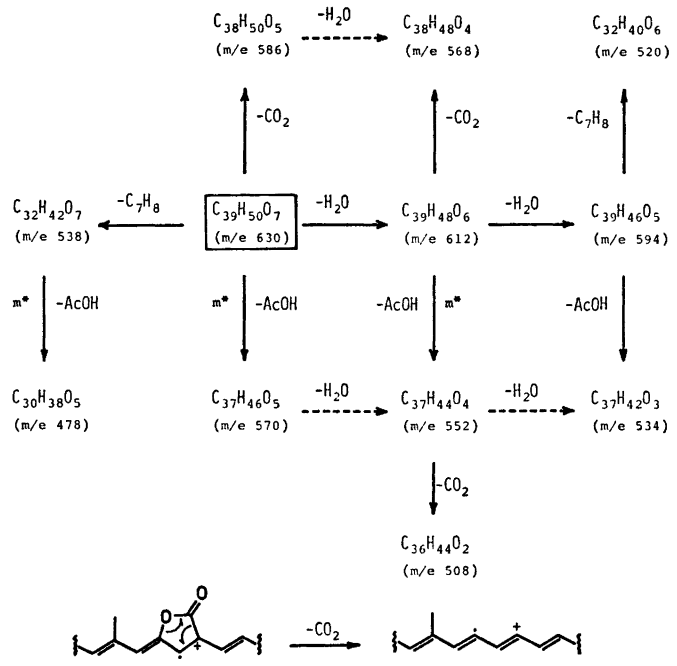
The derivatives *12a*, *12b*, *13a* and *13b* all displayed visible light absorption identical to that of the mixture of *12* and *13*.

Product *13* may be accounted for by assuming total reduction of the lactone moiety as well as reductive opening of the epoxide. The positions of the two double bonds, separated from the main chromophore, are uncertain.

Fragmentations of peridinin induced by electron impact. Important fragments observed in

Table 1. Electron impact induced fragmentation of peridinin, measured with high resolution.

m/e	Composition	Fragment	*m**	Rel.Int.
630.3553	$C_{39}H_{50}O_7$	M		7.9
612.3455	$C_{39}H_{48}O_6$	$M-H_2O$		49.5
594.3350	$C_{39}H_{46}O_5$	$M-2H_2O$		1.7
586.3659	$C_{38}H_{50}O_5$	$M-CO_2$		3.0
570.3346	$C_{37}H_{46}O_5$	M−AcOH	515.5	1.0
568.3550	$C_{38}H_{48}O_4$	$M-H_2O-CO_2$		4.0
552.3229	$C_{37}H_{44}O_4$	$M-H_2O$−AcOH	498	22.3
538.2936	$C_{32}H_{42}O_7$	$M-C_7H_8$		6.1
534.3134	$C_{37}H_{42}O_3$	$M-2H_2O$−AcOH		4.9
520.2856	$C_{32}H_{40}O_6$	$M-H_2O-C_7H_8$		2.7
508.3345	$C_{36}H_{44}O_2$	$M-H_2O$−AcOH$-CO_2$		2.0
478.2712	$C_{30}H_{38}O_5$	$M-C_7H_8$−AcOH	425	3.3
450.2416	$C_{28}H_{34}O_5$	A		1.1
397.2171	$C_{28}H_{29}O_2$	B−H_2O−AcOH		2.3
396.2288	$C_{25}H_{32}O_4$	C		0.5
358.1784	$C_{21}H_{26}O_5$	A−C_7H_8		1.2
257.1654	$C_{17}H_{23}O_3$	D		5.3
251.1287	$C_{14}H_{19}O_4$	E		1.2
234.1256	$C_{14}H_{18}O_3$	F		24.5
233.1203	$C_{14}H_{17}O_3$	E−H_2O		3.3
223.1480	$C_{17}H_{19}$	G−H_2O−AcOH		17.7
212.1572	$C_{16}H_{20}$	H−H_2O−AcOH		43.0
197.1325	$C_{15}H_{17}$	D−H_2O−AcOH		75.0
181.1218	$C_{11}H_{17}O_2$	I		100.0
163.1126	$C_{11}H_{15}O$	I−H_2O		28.0



Scheme 4.

the high resolution mass spectrum of peridinin (*1*) are compiled in Table 1.

In the high mass range losses of water, carbon dioxide, acetic acid and toluene as well as combinations of these are observed, Scheme 4.

Two consecutive losses of water are consistent with the two free hydroxy functions of peridinin. Loss of CO_2 has previously been observed for certain carotenoid carboxylic acids, *e.g.* azafrin,[12] and is readily accommodated with the lactone moiety of peridinin, Scheme 4.

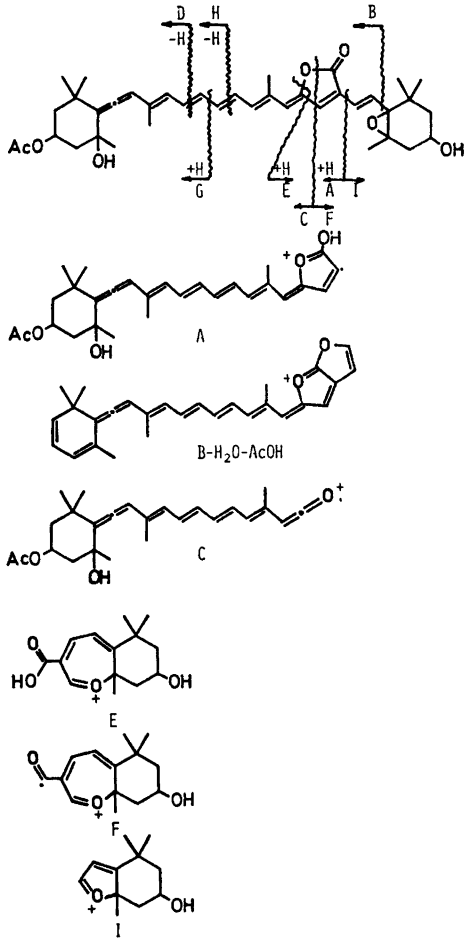
Losses of toluene (C_7H_8), but not of xylene, are observed. In view of the now accepted mechanism for these fragmentations,[13–16] losses of toluene are to be expected for structure *1* whereas loss of xylene is prohibited since no six consecutive carbons of the polyene chain carry the required two methyl groups.

Fragment A, $C_{25}H_{34}O_5$ (*m/e* 450) may be derived by cleavage of the 8′,9′-single bond of peridinin with transfer of the hydrogen at C-7′, resulting in the loss of the epoxy end group as an acetylenic species, Table 1, Scheme 5. Detection of this fragment, although not abundant, is important since it discloses the molecular boundaries for five of the seven oxygen functions of peridinin (*1*).[3] Another fragment, $C_{21}H_{26}O_5$ (*m/e* 358), obviously interconnected with A by an element of toluene (C_7H_8), may be derived by the same process.

Cleavage of the 6′,7′-single bond of peridinin should give rise to a fragment B. This fragment ion was not detected, but fragment $C_{28}H_{29}O_2$ (*m/e* 397) may be derived by this process after initial or subsequent losses of water and acetic acid, Scheme 5.

A fragment C, $C_{25}H_{32}O_4$ (*m/e* 396) may be derived by cleavage of the butenolide ring resulting in a substituted ketene as indicated in Scheme 5. This fragment, although of low abundance, demonstrates the location of the lactone ether oxygen relative to the allenic end group.

A fragment D, $C_{17}H_{23}O_3$ (*m/e* 257), of moderate abundance, is important since it is obviously interconnected with the fragment $C_{15}H_{17}$ (*m/e* 197) below by elements of water and acetic acid. Fragment D may be derived from the allenic end of the peridinin molecule by cleavage of the 11,12-double bond with hydrogen transfer away from the charge, Scheme 5.



Scheme 5.

Three abundant fragments of hydrocarbon composition $C_{17}H_{19}$ (*m/e* 223 = H − H_2O − AcOH), $C_{16}H_{20}$ (*m/e* 212 = G − H_2O − AcOH) and $C_{15}H_{17}$ (*m/e* 197 = D − H_2O − AcOH), may all be derived from the allenic end of the molecule by cleavages of the 13,14-double, 12,13-single and 11,12-double bonds, respectively, with hydrogen transfers as indicated in Scheme 5. No likely structures are, however, proposed for these fragments.

The *m/e* 197 ion is of great diagnostic importance since similar fragments are observed in the mass spectra of both fucoxanthin (*14*) [17] and isofucoxanthin (*15*),[18] Scheme 3. In the latter cases it has been suggested that these fragments are derived from the polyene chain.[17,18] However, to our knowledge this abundant fragment has only been observed for allenic carotenoids. In view of the assignment of fragment D above and the fact that several hydrogen transfers would be required for its formation from the polyene chain of peridinin (*1*) after the initial loss of CO_2, it is more likely to be derived from the allenic end of the molecule as indicated in Scheme 5.

Characteristic fragments of carotenoid 5,6-epoxides and 5,8-furanoxides are the furanoid and homopyryllium ions derived by rearrangements of the oxides and subsequent α-cleavages.[16,19] In the peridinin mass spectrum fragments E ($C_{14}H_{19}O_4$, *m/e* 251), F ($C_{14}H_{18}O_3$, *m/e* 234) and I ($C_{11}H_{17}O_2$, *m/e* 181) as well as the fragments $C_{14}H_{17}O_3$ (E − H_2O, *m/e* 233) and $C_{11}H_{15}O$ (I − H_2O, *m/e* 163) may be accommodated to these general fragmentations, Scheme 5.

Fragment E is of great diagnostic importance since the relative position of the butenolide to the epoxy end group is hereby revealed.[2] Fragment I is identical to that observed for most hydroxylated carotenoid 5,6-epoxides and 5,8-furanoxides.[19] Here its diagnostic importance is obvious since it reveals the epoxidic nature of peridinin.[2]

Several other fragments of the peridinin mass spectrum are of little diagnostic value, but all may be accommodated to structure *1* for peridinin.[20]

The over-all agreement of the twentyfive fragment ions discussed here with structure *1*, strongly support this proposed structure for peridinin.[2]

EXPERIMENTAL

Materials and methods were those commonly employed in the Trondheim laboratory.[21] R_F-values are quoted for Schleicher & Schüll No. 287 (kieselguhr filled) circular paper with mixtures of acetone in petroleum ether (APE) as developer.[22]

Properties of peridinin (*1*) are given in the preceding paper.[2] The high resolution mass spectrum of peridinin (*1*) was obtained with an AEI MS902 instrument with an on line PDP8 data processing unit.[20]

Acid treatment.[23] When dissolved in 1 % citric acid in methanol (3 ml) β-carotene diepoxide (*2*, 0.01 mg) gave a hypsochromic spectral shift of 40 nm after 10 h. Under the same conditions peridinin (*1* 0.1 mg) was quite inert

for the same period. With 15 % citric acid in methanol (5 ml) peridinin (*1*, 0.17 mg) exhibited a hypsochromic spectral shift of 20 nm after 5 h. Treatment of β-carotene diepoxide (*2*, 0.01 mg) in methanol (3 ml) with 0.1 % HCl in methanol (5 drops) resulted in total isomerization within 3 min.

To peridinin (*1*, 5 mg) in methanol (30 ml) was added 0.1 % aq. HCl in methanol (3 ml). After 1.5 h the hypsochromic spectral shift was 20 nm. The reaction mixture was worked up in the usual manner and the products separated by TLC on silica using 50 % APE as developer; pigment recovery was 75 %, including 25 % unreacted peridinin.

The two major products (*3a*, *b*), R_F 0.74 (28 %) and R_F 0.63 (14 %) with 20 % APE, had identical spectral properties: λ_{max} (acetone) 446 nm; *m/e* 630 (M), 612 (M−18), 686 (M−44), 570 (M−60), 552 (M−18−60), 538 (M−92), 234, 223, 212, 197 and 181.

The two minor products (*4* and *5*, 8 %) both had λ_{max} (acetone) 459, (474) nm and R_F 0.41 with 20 % APE. The mixture of *4* and *5* (0.8 mg) in dry pyridine (2 ml) was acetylated with acetic anhydride (0.2 ml) for 19 h and the acetates separated by TLC on silica using 40 % APE as developer. Pigment recovery was 93 %.

The major acetate (*4a*, 0.55 mg, 75 %) had R_F 0.76 with 20 % APE; λ_{max} (acetone) 459, (474) nm; *m/e* 704 (M), 686 (M−18), 672 (M−32), 654 (M−18−32), 644 (M−60), 626 (M−18−60), 612 (M−92), 594 (M−18−60−32), 223, 212, 197 and 149.

Silylation [6] of the acetate *4a* (0.37 mg) at room temperature for 67 h gave a mono-TMS ether [*4b*, R_F 0.67 with 10 % APE, λ_{max} (acetone) 459, (474) nm, *m/e* 776 (M)] and a di-TMS ether [*4c*, R_F 0.98 with 10 % APE, λ_{max} (acetone) 459, (474) nm, *m/e* 848 (M)].

The minor acetate (*5a*, 0.18 mg, 25 %) had R_F 0.77 with 20 % APE; λ_{max} (acetone) 459, (474) nm; *m/e* 672 (M), 654 (M−18), 612 (M-60), 594 (M−18−60), 223, 212, 197 and 149.

Silylation of the acetate *5a* (0.12 mg) at room temperature for 1 h gave a mono-TMS ether (*5b*, 0.11 mg) which had R_F 0.69 with 10 % APE, λ_{max} (acetone) 459, (474) nm, *m/e* 744 (M).

Base treatment.[23] To peridinin (*1*, 52 mg) in methanol (20 ml) was added K_2CO_3 (100 mg) in water (0.6 ml). After 16 h all peridinin had reacted and the reaction mixture was worked up by addition of water and extraction with ether giving 3.9 mg (7.7 %) neutral products. Acidification of the hypophase with 1 % aqueous H_2SO_4 gave 0.93 mg (1.8 %) of an acidic pigment.

The neutral pigments were separated by TLC on silica using acetone as developer.

The major product (*1c*, 3 mg, 5.8 %) had R_F 0.38 with 20 % APE λ_{max} (acetone) 464 nm, *m/e* 588 (M), 570 (M−18), 552 (M−2×18), 544 (M−44), 538 (M−50), 534 (M−3×18), 526 (M−18−44), 508 (M−80), 496 (M−92), 478 (M−18−92), 233, 230, 215, 209, 207, 197, 181 and 167.

Standard acetylation of *1c* (1.3 mg) gave two monoacetates, *1* [R_F 0.68 with 20 % APE, λ_{max} (acetone) 464 nm, *m/e* 630 (M)] which could not be separated from peridinin (*1*) on co-chromatography, and *1d* [R_F 0.58 with 20 % APE, λ_{max} (acetone) 464 nm, *m/e* 630 (M)], and a diacetate *1a* [R_F 0.88 with 20 % APE, λ_{max} (acetone) 464 nm, *m/e* 672 (M)] which could not be separated from peridinin acetate (*1a*) on co-chromatography.

Another neutral product (*6*, 0.83 mg, 1.6 %) had R_F 0.84 with 50 % APE, λ_{max} (acetone) 391 nm, *m/e* 620 (M), gave a diacetate [*6a*, R_F 0.69 with 20 % APE, λ_{max} (acetone) 391 nm, *m/e* 704 (M)] on acetylation and *6a* gave a di-TMS ether [*6b*, R_F 0.90 with 10 % APE, λ_{max} (acetone) 391 nm, *m/e* 848 (M)] on silylation.

A third neutral product (*7*, 0.09 mg, 0.2 %) had R_F 0.42 with 20 % APE, λ_{max} (acetone) 372 nm, *m/e* 600 (M). Acetylation of *7* gave a diacetate with R_F 0.88 with 20 % APE, λ_{max} (acetone) 372 nm and *m/e* 684 (M).

The acidic product *8* (0.93 mg, 1.8 %), purified by TLC on silica using 70 % methanol in acetone as developer, had λ_{max} (methanol) 367 nm and could not be esterified with diazomethane.

Dehydration with $POCl_3$.[24] Peridinin (*1*, 2 mg) in pyridine (2.5 ml) and $POCl_3$ (0.1 ml) were mixed at 0 °C and reacted at room temperature for 4 h. The products were extracted into ether after addition of water, and the extract washed with dilute aq. HCl and $NaHCO_3$ and dried. Evaporation of the solvent gave 1.7 mg (85 %) of oily products which were separated by TLC on silica using chloroform as developer. Four products with molecular ions at *m/e* 612, 612, 594 and 594, respectively, were obtained.

Reduction with $NaBH_4$.[23] Peridinin (*1*, 5.5 mg) in 96 % ethanol (10 ml) was reduced with excess $NaBH_4$ for 2 h at room temperature. The reaction mixture was worked up in the usual manner and the products (2.75 mg, 50 %) separated by TLC on silica using 80 % APE as developer. Unreacted peridinin (1.92 mg, 35 %) was recovered.

The major product (*9*, 0.45 mg, 8.1 %) had R_F 0.11 with 10 % APE, λ_{max} (acetone) 433, (452) nm, and gave a monoacetate, no intermediate, *9a* (R_F 0.28 with 10 % APE, λ_{max} (acetone) 433, (452) nm) on acetylation. Silylation of the acetate *9a* gave a mono-TMS ether *9b* [R_F 0.61 with 10 % APE, λ_{max} (acetone) 433, (452) nm, *m/e* 746 (M)] and a di-TMS ether *9c* [R_F 0.97 with 10 % APE, λ_{max} (acetone) 433, (452) nm, *m/e* 818 (M)].

Another product (*10*, 0.19 mg, 3 %) had R_F 0.15 with 10 % APE, λ_{max} (ether) 331, 347, 366.5 nm, ν_{max} (KBr) 1930 (C=C=C), 1725 (broad, C=O), 1250 (C−O) cm^{-1}, *m/e* 634

(M), 616 (M−18), 598 (M−2×18), 197, 181, 149. Acetylation of *10* gave a triacetate *10a* [R_F 0.55 with 10 % APE, λ_{max} (ether) 331, 347, 366.5 nm, *m/e* 760 (M)] and silylation of *10a* gave a mono-TMS ether *10b* [R_F 0.68 with 5 % APE, λ_{max} (ether) 331, 347, 366.5 nm, *m/e* 822 (M)].

A third product (*11*, 0.066 mg, 1.2 %) had R_F 0.29 with 10 % APE, λ_{max} (ether) 331, 347, 366.5 nm, *m/e* 634 (M), 616 (M−18), 574 (M−60), 556 (M−18−60), 541 (M−93), 523 (M−18−93), 491 (M−143), 251, 243, 223, 207, 200, 197, 185, 181, 163, 143, 125. Saponification of *11* gave a product *11c* [λ_{max} (ether) 331, 347, 366.5 nm, *m/e* 592 (M)]. Acetylation of *11* gave a monoacetate *11a* [R_F 0.44 with 10 % APE, λ_{max} (ether) 331, 347, 366.5 nm, *m/e* 676 (M)] and silylation of *11a* gave a di-TMS ether *11b* [λ_{max} (ether) 331, 347, 366.5 nm, *m/e* 778 (M)].

Two minor products (*12* and *13*) both had R_F 0.20 with 20 % APE, λ_{max} (acetone) 349, 368 nm and could only be separated as the acetates.

The minor acetate (*12a*, 0.05 mg, 0.9 %) had R_F 0.54 with 10 % APE, λ_{max} (acetone) 349, 368 nm, *m/e* 762 (M). Silylation of *12a* gave a mono-TMS ether *12b* [λ_{max} (acetone) 349, 368 nm, *m/e* 834 (M)].

The major acetate (*13a*, 0.074 mg, 1.35 %) had R_F 0.35 with 10 % APE, λ_{max} (acetone) 349, 368 nm, *m/e* 764 (M). Silylation of *13a* gave a di-TMS ether *13b* [λ_{max} (acetone) 349, 368 nm, *m/e* 908 (M)].

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