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# Algal Carotenoids 58.* Isomerization Studies on Peridinin

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Naturally occurring all-*trans*-(3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*R*)-peridinin (revised numbering) has been the subject of isomerization studies in benzene solution. Whereas geometrical *cis–trans* isomerization proceeded in light in the absence of iodine, iodine was required for noticeable isomerization of the allenic bond. This reaction was accelerated by intense light. The same quasi-equilibrium mixture was obtained upon iodine-catalyzed stereomutation of all-*trans*-(6′*R*)-peridinin and the allenic all-*trans*-(6′*S*)-isomer. There was no large difference in thermodynamic stability for the (6′*R*)- and (6′*S*)-isomers. The ratio 55:45 was obtained under photostationary conditions in the presence of iodine (quasi-equilibrium).

Detailed NMR studies including T-ROESY experiments resulted in unequivocal identification and complete $^1$H NMR assignments for all-*trans*-(6′*R*)-, 9′-*cis*-(6′*R*)-, 11-*cis*-(6′*R*)-, 13-*cis*-(6′*R*)- and all-*trans*-(6′*S*)-peridinin. Previously reported $^1$H NMR and $^{13}$C NMR assignments of all-*trans*-(6′*R*)-peridinin are partly corrected.

The identification of 9′-*cis*-(6′*S*)-, 11-*cis*-(6′*S*)- and 13-*cis*-(6′*S*)-peridinin rests on isomerization evidence, VIS absorption data and relative abundance, in comparison with data for the (6′*R*)-isomers.

Furanoxide constitutional isomers, obtained by acid-catalyzed isomerization (rearrangement) of peridinin have been identified and completely assigned by $^1$H NMR spectroscopy, partly including T-ROESY data. Included are three geometrical isomers of the (8*R*)-epimer, namely all-*trans*-(8*R*,6′*R*)-, 9′-*cis*-(8*R*,6′*R*) and 11-*cis*-(8*R*,6′*R*) and of the (8*S*)-epimer, all-*trans*-(8*S*,6′*R*). In addition the all-*trans*-(8*R*)-furanoxide of the allenic (6′*S*)-isomer has been studied. The results should facilitate identification of a claimed naturally occurring peridinin furanoxide.

Peridinin, the major carotenoid of dinoflagellates, is, together with fucoxanthin (**1**), the carotenoid produced in largest quantity in Nature.[1] Its constitution[1,2] and chirality (**2**)[3] were elucidated in the seventies, and confirmed by recent NMR data[4–6] and total synthesis.[7,8] The structural presentation in previous work[1–9] is reversed here in agreement with the IUPAC carotenoid nomenclature rules[10] (in order to give an unprimed 19,11-olide, because both end groups are β-ring derivatives) and to be consistent with the numbering of fucoxanthin (**1**), Scheme 1. Peridinin (**2**) consequently has the all-*trans*-(3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*R*)-configuration. The all-*trans* configuration corresponds to 9*Z*,11*Z*. Since the *cis–trans* nomenclature is simpler for in-chain substituted carotenoids it is employed here.

Preliminary studies on allenic and geometrical isomers of peridinin (**2**) were reported in 1984[11] and the results are rationalized below on the basis of available evidence. Recently Ito's group[12] isolated the allenic all-*trans*-(6′*S*) isomer (**3**) of peridinin after iodine-catalyzed photoisomerization of **2**. The 9′-*cis*-(6′*R*) (**2a**) and the 11-*cis*-(6′*R*) (**2b**) isomers were also isolated, the latter already prepared by total synthesis.[8] The structure of the all-*trans*-(6′*S*) isomer (**3**) was confirmed by total synthesis.[12] The isolation of the allenic (6′*S*) isomer (**3**) was unexpected in the light of the history of the corresponding (6′*S*)-isomer of fucoxanthin, where the isolation was claimed[13] and then disproved.[14–16]

In the present work we have extended our previous structural studies on peridinin[1–3,11] with (i) a detailed study of the stereoisomerization of natural peridinin (**2**) in the absence and presence of iodine, including the characterization of several geometrical isomers of both (6′*R*)-(**2**) and (6′*S*)-peridinin (**3**) and (ii) a stereochemical study of furanoxide constitutional isomers of (6′*R*)- (**2**) and (6′*S*)-peridinin (**3**), obtained by epoxide–furanoxide rearrangement of peridinin (**2**, **3**). A preliminary report has been presented.[17]

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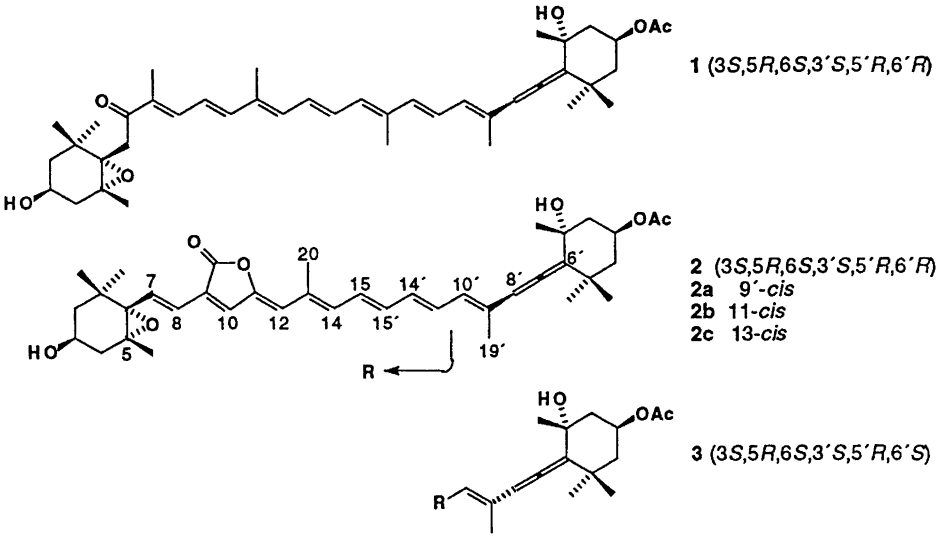



*Scheme 1.*

## Results and discussion

*Stereoisomerization studies*

All-*trans*-(6'*R*)-peridinin (**2**) was submitted to iodine-catalyzed stereoisomerization in benzene solution in direct sunlight. The photostationary mixture was subjected to preparative HPLC in the system used by Yamano *et al.*,[12] see Fig. 1 and Table 1. The individual isomers were characterized by VIS absorption data including $\lambda_{max}$, spectral fine-structure and *cis*-peak intensities[18,19] in addition to $^1$H NMR data, see below. The 9'-*cis*-(6'*R*) (**2a**) and 11-*cis*-(6'*S*) (**2b**) isomers could not always be separated in the HPLC system employed.

The second most abundant isomer, identified as the all-*trans*-(6'*S*) isomer **3**, was submitted to iodine-catalyzed stereoisomerization under the same conditions as for the all-*trans*-(6'*R*) isomer (**2**). The isomerization was monitored by HPLC until a photostationary condition was reached. The results, presented in Table 1, demonstrated that the same quasi-equilibrium mixture was reached, independent of the initial isomer.

Separate stereoisomerization experiments were carried out starting from the all-*trans*-(6'*R*) (**2**) and all-*trans*-(6'*S*) (**3**) isomers in benzene solution in direct sunlight in the absence of iodine for 2.5–3 h.

The results, presented in Table 2, revealed that *trans*–*cis* isomerization occurred readily, whereas isomerization of the allenic bond took place only to a very small extent.

The effect of light on the allenic isomerization was demonstrated by experiments with all-*trans*-(6'*R*)-peridinin (**2**) in benzene solution kept in darkness in the presence of iodine, Table 3. At high iodine concentrations a certain degree of isomerization of the allenic bond was noted.

From the data presented in Table 3, the relative proportions of (6'*R*)-isomers under iodine-catalyzed quasi-equilibrium conditions in darkness may be estimated as all-*trans* (**2**): 9'-*cis* (**2a**): 11-*cis* (**2b**): 13-*cis* (**2c**)≈

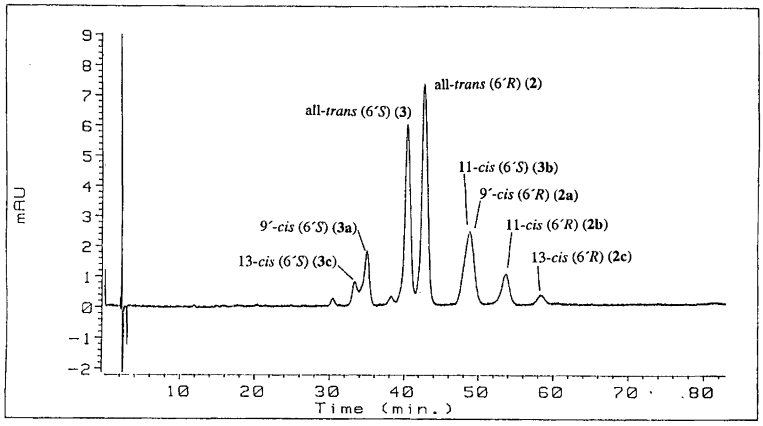



*Fig. 1.* Quasi-equilibrium mixture of peridinin isomers after iodine-catalyzed stereomutation with light, separated by HPLC (nitrile 5 μ Techsphere column, hexane–acetone–methanol 89:10:1,* flow 1.5 ml min$^{-1}$).

*Table 1.* Stereoisomerization of all-*trans*-(6′*R*)-peridinin (**2**) and of all-*trans*-(6′*S*)-peridinin (**3**) in benzene solution in direct sunlight in the presence of 1.8% (w/w) iodine relative to peridinin.

| Isomers obtained | | | | Initial isomer | | | |
|---|---|---|---|---|---|---|---|
| | VIS abs. data | | | all-*trans*-(6′*R*) (**2**) | | all-*trans*-(6′*S*) (**3**) | |
| Identification | | $\lambda_{max}$/nm | % III/II[9] | 2.5 h | | 1.5 h | |
| all-*trans*-(6′*R*) (**2**) | *a* | (427), 457, (479) | | 32 | | 35 | |
| | *b* | (425), 453, 482 | 61 | | | | |
| 9′-*cis*-(6′*R*) (**2a**) | *a,c* | 452, (476) | | (10)[d] | | (12)[e] | |
| | *b* | 325, (425), 449, 479 | 68 | | 55 | | 54 |
| 11-*cis*-(6′*R*) (**2b**) | *a* | 457, (480) | | 6 | | 5 | |
| | *b* | 325, (426), 452, 481 | 87 | | | | |
| 13′*cis*-(6′*R*) (**2c**) | *a* | 454, (477) | | 3 | | 2 | |
| | *b* | 422, 449, 477 | 44 | | | | |
| all-*trans*-(6′*S*) (**3**) | *a* | (427), 457, (480) | | 26 | | 28 | |
| | *b* | (426), 451, 481 | 69 | | | | |
| 9′-*cis*-(6′*S*) (**3a**) | *a* | 453, (477) | | 10 | | 8 | |
| | *b* | (427), 450, 477 | | | 46 | | 46 |
| 11-*cis*-(6′*S*) (**3b**) | *a,c* | 458, (480) | | (9)[d] | | (6)[e] | |
| | *b* | 453, 479 | | | | | |
| 13′-*cis*-(6′*S*) (**3c**) | *a* | 451 | | 4 | | 4 | |
| | *b* | 450, 477 | | | | | |

[a]In hexane-acetone-methanol 89:10:1. [b]In hexane. [c]Determined in separate experiments (Table 2). [d]Sum of **2a**+**3b** 19%, ratio estimated in separate experiment (Table 2). [e]Sum of **2a**+**3b** 18%.

*Table 2.* Stereoisomerization of all-*trans*-(6′*R*)-peridinin (**2**) and of all-*trans*-(6′*S*)-peridinin (**3**) in benzene solution in direct sunlight and absence of iodine.

| | Initial isomer | | | |
|---|---|---|---|---|
| | all-*trans*-(6′*R*) (**2**) | | all-*trans*-(6′*S*) (**3**) | |
| Isomers obtained | 3 h Components in % | | 2.5 h Components in % | |
| all-*trans*-(6′*R*) (**2**) | 64 | 98 | 8 | |
| 9′-*cis*-(6′*R*) (**2a**) | 20 | | | |
| 11′-*cis*-(6′*R*) (**2b**) | 13 | | | |
| 13-*cis*-(6′*R*) (**2c**) | 2 | | | |
| all-*trans*-(6′*S*) (**3**) | 2 | | 56 | 92 |
| 9′-*cis*-(6′*S*) (**3a**) | | | 17 | |
| 11-*cis*-(6′*S*) (**3b**) | | | 10 | |
| 13-*cis*-(6′*S*) (**3c**) | | | 8 | |

64:27:6:2. This ratio compares well with the results after photoisomerization (64:20:13:2) listed in Table 2. The relative abundance of the 9′-*cis*-(6′*R*) (**2a**) and 11-*cis*-(6′*S*) (**3b**) isomers was estimated from the results of the isomerization experiments in the absence of iodine. This results in a 55:45 ratio between the (6′*R*)- and (6′*S*)-isomers at quasi-equilibrium, reflecting the relative thermodynamic stability of (6′*R*)- and (6′*S*)-isomers of peridinin, cf. Table 1.

*Table 3.* Isomerization of all-*trans*-(6′*R*)-peridinin (**2**) in benzene solution at room temperature in darkness in the presence of iodine for 48 h.

| Isomer obtained | 1.8% (w/w) $I_2$ | 208% (w/w) $I_2$ |
|---|---|---|
| all-*trans*-(6′*R*) (**2**) | 69 | 55 |
| 9′-*cis*-(6′*R*) (**2a**) | 25 | 28 |
| 11-*cis*-(6′*R*) (**2b**) | 5 | 7 |
| 13-*cis*-(6′*R*) (**2c**) | 1 | 3 |
| all-*trans*-(6′*S*) (**3**) | 0 | 8 |

Following early attempts to identify peridinin isomers[11] 9′-*cis*-(6′*R*)-peridinin (**2a**), called neo V, was misidentified as all-*trans*-(6′*S*)-peridinin (**3**). A neo UI isomer assigned the (6′*R*) 11,13-di-*cis* structure is now considered identical with 11-*cis*-(6′*R*) (**2b**), whereas the neo UII isomer was correctly assigned the 13-*cis*-(6′*R*) (**2c**) structure. No allenic isomer was isolated.

The following conclusions can be reached from the stereoisomerization experiments. Whereas geometrical *cis–trans* isomerization of peridinin is effected with light in the absence of iodine, iodine is required for noticeable isomerization of the allenic bond, a reaction which is accelerated by strong light. The same quasi-equilibrium mixture is obtained upon iodine-catalyzed stereomutation of the all-*trans*-(6′*R*) (**2**) and all-*trans*-(6′*S*) (**3**) isomers. There is no large difference in thermodynamic stability for the allenic (6′*R*) and (6′*S*) isomers of peridinin (ratio ca. 55:45 at quasi-equilibrium).

Since this work was completed, four geometrical (6′*S*) isomers of fucoxanthin, cf. the (6′*R*) configuration of fucoxanthin (**1**) given in Scheme 1, have been isolated after isomerization under conditions similar to those described here for allenic isomerization of peridinin (**2**), and unequivocally identified.[20]

Consequently it may be expected that isomerization of the allenic bond of all other allenic carotenoids may be

achieved under appropriate isomerization conditions in the presence of iodine in strong light.

*NMR studies and structure elucidation of the isomers*

*All*-trans-*(6′R)-peridinin* (**2**) has already been subjected to detailed $^{1}H$ and $^{13}C$ NMR studies.[4,5] Since the derivation of the structures of the different isomers isolated relied on the correctness of the assignments for the *trans* compound, the $^{1}H$ and $^{13}C$ NMR assignments were reinvestigated for **2** by applying a number of well-proven NMR techniques such as $^{1}H,^{1}H$ 2D COSY, 1D TOCSY, and $^{1}H,^{13}C$ NMR heteronuclear one-bond and multiple-bond (long range) 2D COSY. Moreover, by measuring the longitudinal nuclear Overhauser effect (NOE) and, preferably, its transversal variant called rotating frame nuclear Overhauser effect (ROE),[21] additional information on interproton distances and hence on the assignments was accessible. It is known[15,21–23] that in the range of molecular masses typical for carotenoids, the ROE is much stronger than the longitudinal NOE. The spatial information accessible by this technique was particularly relevant for the deduction of the structure of the *cis*-isomers.

In regular carotenoids the deduction of the structure of geometrical isomers is greatly simplified by application of well-known isomerization shifts $\Delta = \delta_{cis} - \delta_{trans}$ (in ppm) deduced for a variety of *cis* configurations.[24] However, owing to the altered skeleton and the presence of the 19,11-olide as a strongly anisotropic group it was, *a priori*, unclear if these rules could be adapted for peridinin (**2**). Therefore, application of ROE spectroscopy (ROESY) was considered mandatory here.

As was shown previously,[23,25] a special variant of the ROESY experiment called T-ROESY[26] gives excellent results that are virtually free of undesired TOCSY artifacts often observed in ROE experiments. These artefacts are caused by coherent magnetization transfer via *J* coupling paths, disturbing the much weaker incoherent through-space effects of interest here.

The homonuclear 2D COSY of all-*trans* peridinin (**2**) was measured with a reduced spectral width of only 800 Hz with the transmitter placed at 6.5 ppm. As was demonstrated previously,[24,25,27] this results in highly resolved cross-peaks that simplify the identification of multiplet structures in crowded spectral regions. Moreover, it enables the detection of cross-peaks caused by very small long-range couplings, extending in the case of peridinin (**2**) up to eight bonds (H-19′/H-15; H-20/H-11′). These weak cross-peaks often provide valuable structural information.

A series of 1D TOCSY experiments is presented in Fig. 2. So far this technique has been used in the field of carotenoids mainly to disentangle the strongly overlapping signals of the aliphatic protons in end groups or attached carbohydrates.[15,22–25] This technique may equally well be applied to assign the protons of the olefinic chain in peridinin (**2**). All 1D TOCSY experiments depicted in Fig. 2 started with inversion of the magnetization of H-10′ by a selective 180° DANTE pulse sequence, followed by an MLEV-17 mixing period[28] the duration of which was increased from 7.5 ms in the lowest 1D TOCSY experiment up to 180 ms in the uppermost spectrum. A difference spectrum between on-resonance and off-resonance irradiation experiments resulted in a sub-spectrum containing only protons that are directly or indirectly (via relaying neighbours) coupled to H-10′. With increasing duration of the spin-locking or mixing period the magnetization of H-10′ is progressively relayed from proton to proton until H-12 and H-20 are reached, although only small couplings are involved in the latter transfer.

Thus, in the experiment with a 7.5 ms mixing time only the multiplets of the first neighbour H-11′ and, very weakly, of the second (H-14′) are seen in the difference spectrum in addition to the signal of H-10′ that was selectively excited. With increasing mixing time the signals of more protons appear according to the coupling constants involved. In this simple linear case the sequence of appearance can be directly translated into the 'distance' from the excited proton and hence the assignments are readily deduced.

It is apparent that even very small long-range couplings, also clearly detectable in the COSY spectrum, are sufficiently efficient to transfer magnetization provided the spin-locking time chosen was long enough. Based on experience with other carotenoids, it is predicted that a further increase of the spin-locking time to about 300 ms should suffice to detect even the signal of H-10 at 7.02 ppm that has approximately the same narrow line width as H-12 and hence only very small long-range couplings.

In the normal 400 MHz spectrum at the bottom of Fig. 2, it is seen that the chemical shifts of H-11′ and H-15 are identical. This caused some ambiguity in the assignment of C-11′ and C-15 which was only tentatively resolved by comparison with pyrrhoxanthin.[23]

The complete assignments of the $^{1}H$ and $^{13}C$ signals of all-*trans*-peridinin (**2**) are given in Scheme 2. Comparison with the previous assignments[4,5] demonstrates the required corrections.

Included in Scheme 2 are the results of a 2D T-ROESY experiment. Each arrow corresponds to ROE cross-peaks in the 2D spectrum of medium or strong intensity and hence reveals in a qualitative way the close spatial proximity of the corresponding protons. Thus, ROEs between the protons of the two geminal methyl groups and of these methyl groups with other neighbouring protons, or between the axially oriented methyl groups at C-1 or C-1′ and the axial H-3 or H-3′ contributed strongly to the reliability of their assignment. These data revealed the relative stereochemistry of the geminal methyl groups. Moreover, the ROE connectivities between olefinic protons and protons of the methyl groups attached to the olefinic chain clearly revealed the geometry of the different carbon–carbon double bonds. In this way the structures of the different geometrical isomers of

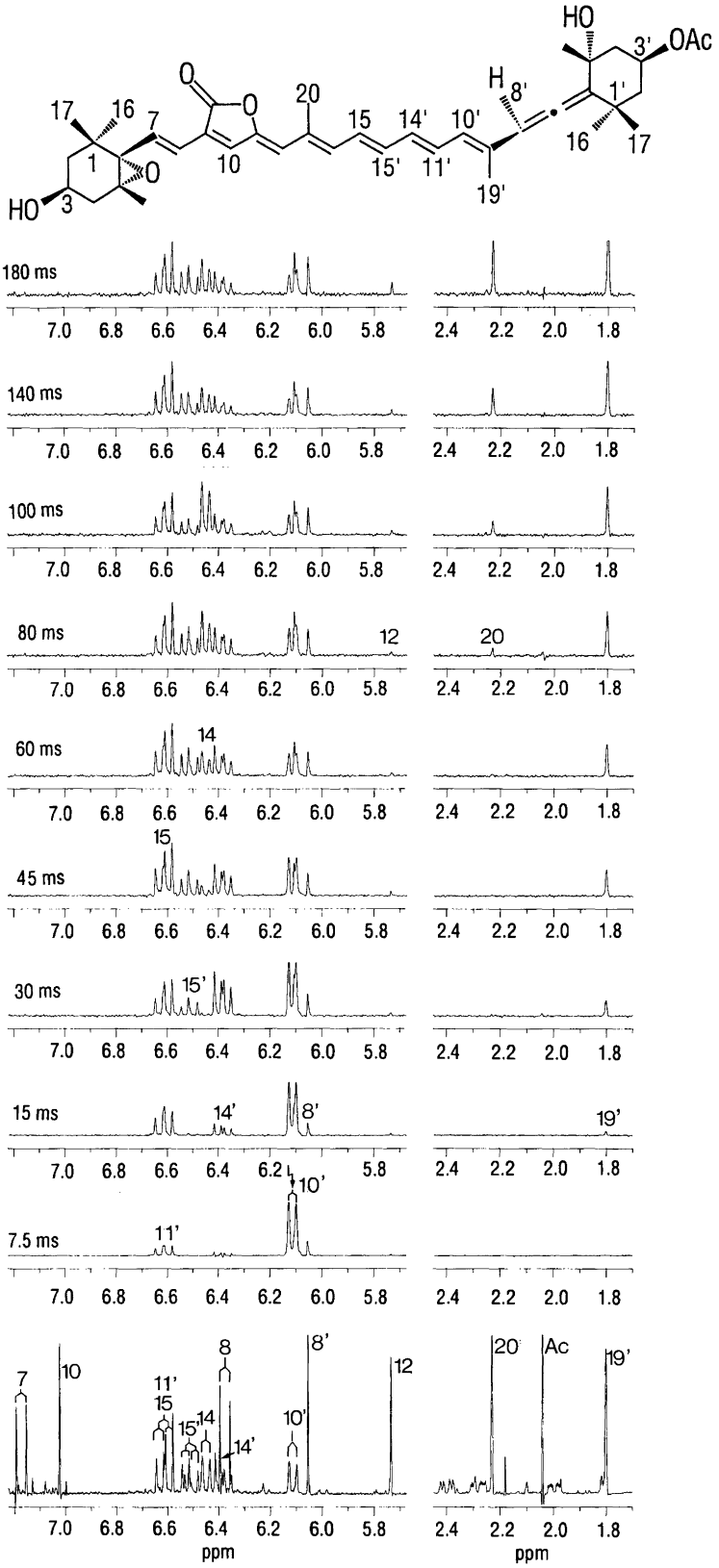
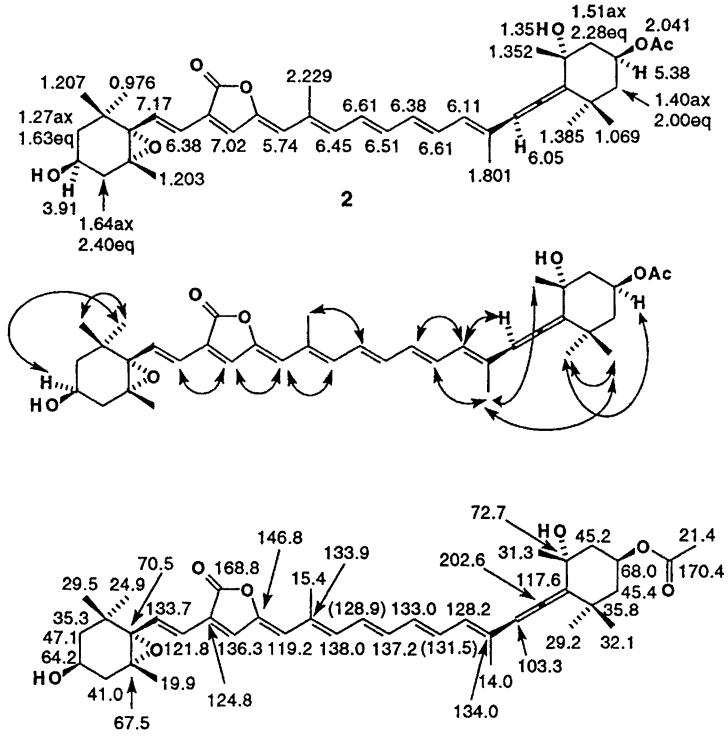


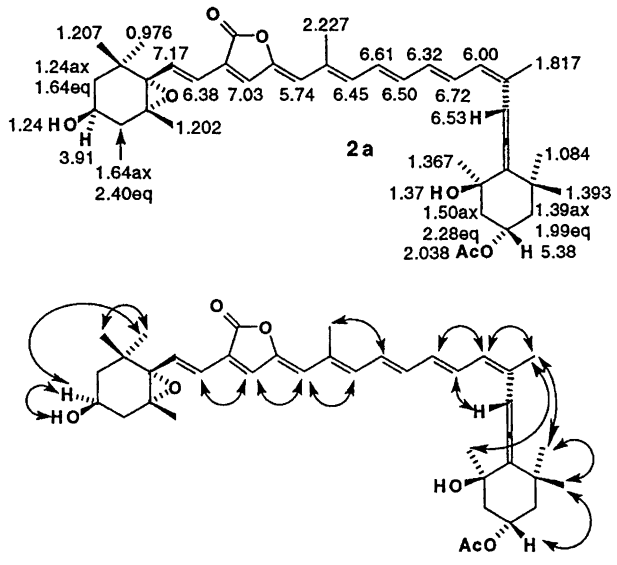

*Fig. 2.* Parts of the 400 MHz $^1$H NMR spectrum of all-*trans*-(6′*R*)-peridinin (**2**) (bottom; ca. 0.6 mg in 0.6 ml $CDCl_3$) and set of 1D TOCSY difference spectra obtained by selective inversion of the doublet of H-10′ at 6.11 ppm by a DANTE pulse train (580 times 2 μs with 70 μs delay between pulses at a power level 18H of the decoupler). Mixing was achieved by MLEV-17 with a last pulse of 60°. Mixing times between 15 and 180 ms were chosen. A z-filter with ten delays between 5 and 50 ms was used to improve the signal shapes.




*Scheme 2.*

(6′*R*)-peridinin (**2**) and (6′*S*)-peridinin (**3**) were unambiguously derived.

*9′*-cis-*(6′*R*)-Peridinin* (**2a**). HPLC-purified **2a** (ca. 100 μg) was available for $^1$H NMR studies. The assignments presented in Scheme 3 were based on a series of 1D TOCSY experiments starting with inversion of H-10′ at 6.00 ppm. Analogous results were obtained as discussed for the all-*trans* isomer **2**. The geometry of the double bonds was deduced from a 2D T-ROESY experiment, the results of which are depicted in Scheme 3. A relevant through-space contact was observed between the doublet of H-10′ and H-19′, whereas the remaining contacts along the olefinic chain were essentially as in all-*trans*-**2**.

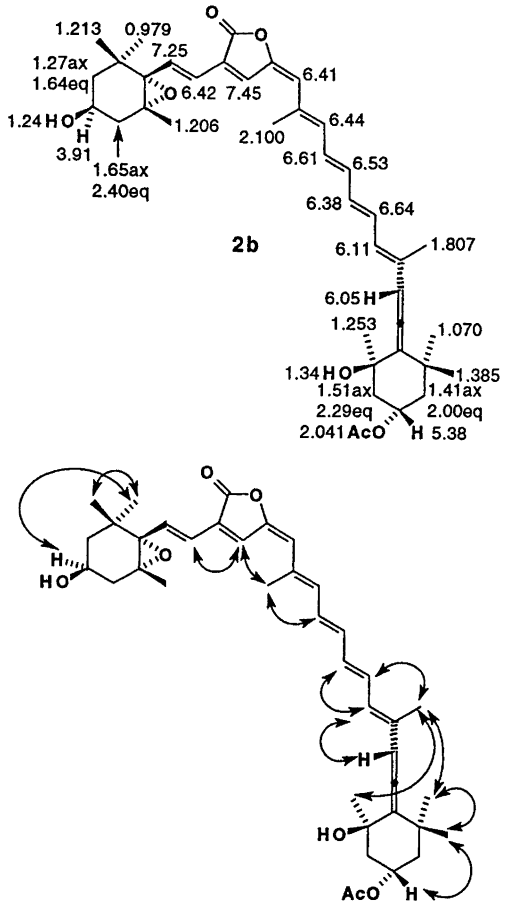



*Scheme 3.*

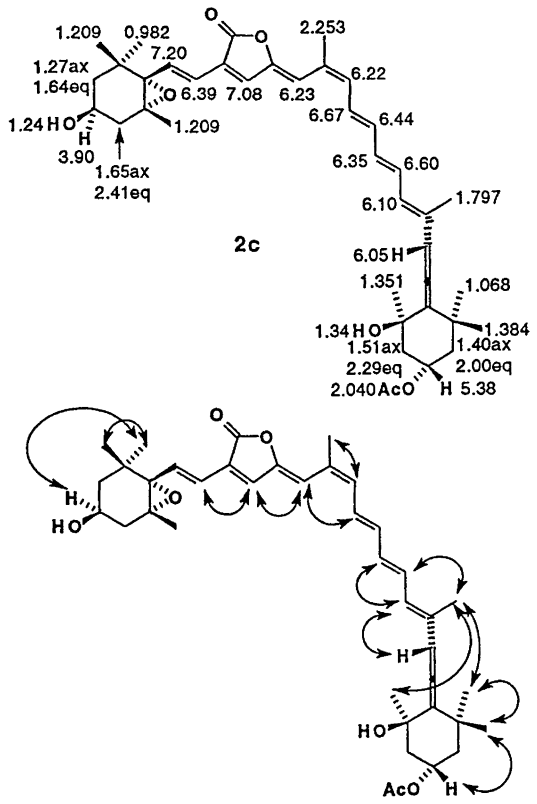
*11*-cis-*(6′*R*)-Peridinin* (**2b**). HPLC-purified **2b** (ca. 50 μg) was available for $^1$H NMR studies, including 2D COSY and 2D T-ROESY. The 11-*cis* configuration was derived mainly from a cross-peak between H-20 and H-10 in the T-ROESY experiment, see Scheme 4.

*13*-cis-*(6′*R*)-Peridinin* (**2c**). Only ca. 25 μg were isolated by HPLC and used for 2D COSY and 2D T-ROESY. Relevant ROEs were observed between H-20 and H-14 as well as between H-15 and H-12 in agreement with the structure, see Scheme 5.

*All*-trans-*(6′*S*)-Peridinin* (**3**). The complete $^1$H NMR assignments, based on $^1$H,$^1$H 2D COSY and 2D T-ROESY experiments, are given in Scheme 6. The isomerization shift of the allenic proton was 0.11 ppm relative to the all-*trans*-(6′*R*) diastereomer, as reported by Ito's group,[12] cf. the compilation in Table 4. The stereochemical assignment is further secured in the present work by ROESY data. Thus, in the (6′*R*) isomers ROE cross-peaks were observed between H-19′ and H-17′, H-18′, see Schemes 2–5, whereas in the (6′*S*) isomer through-space contacts were detected between H-19′ and H-16′, 5′-OH, as well as between H-8′ and H-18′, see Scheme 6.

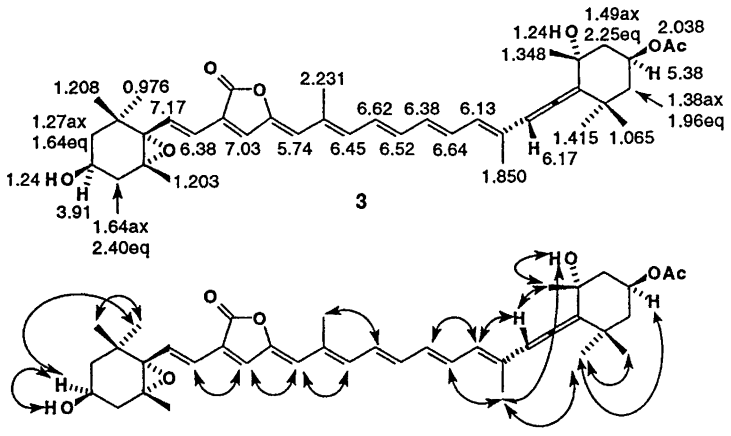



Scheme 4.

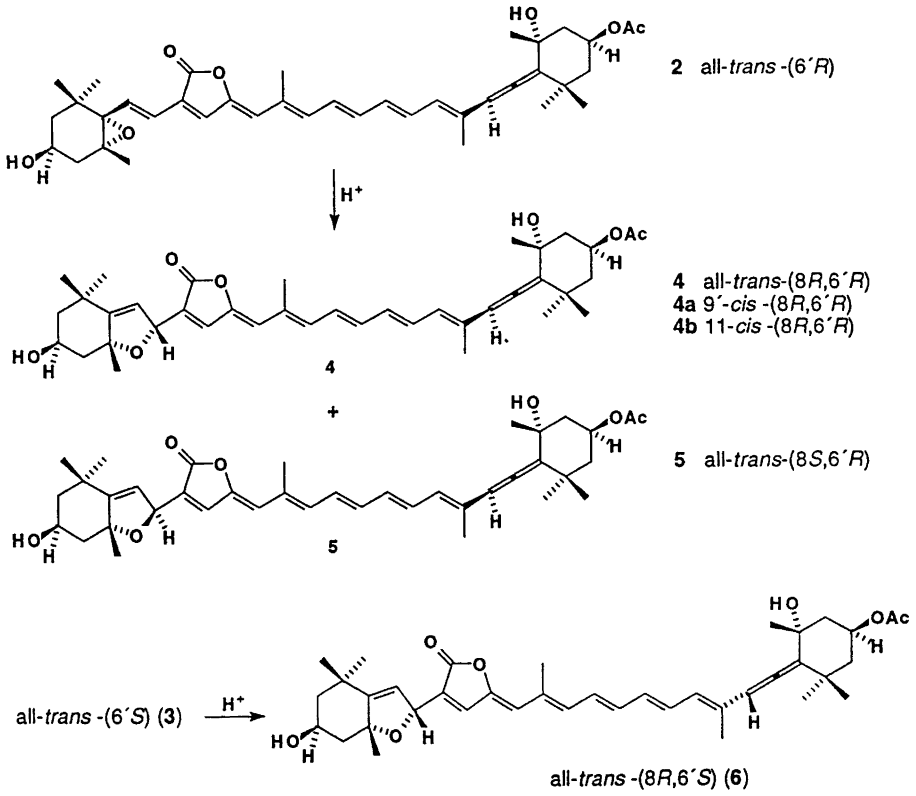



Scheme 5.

*9′*-cis-*(6′*S*)-Peridinin* (**3a**), *11*-cis-*(6′*S*)-peridinin* (**3b**) *and* *13*-cis-*(6′*S*)-peridinin* (**3c**) were only available for characterization by VIS spectroscopy (Table 1) and chromatographic behaviour. However, the VIS absorption data, including $\lambda_{max}$ in two solvent systems and spectral fine-structure % III/II were consistent with data for the corresponding (6′*R*) isomers **2a**, **2b** and **2c**, respectively (Table 1). The (6′*R*) and (6′*S*)-*cis* isomers isolated exhibited no significant *cis*-peaks, except the 13-*cis* isomer with $D_B/D_{II}$[19] = ca. 30 in the HPLC eluent, compatible with the general theory.[18] Moreover, the relative quantities of the *cis*-isomers in the (6′*R*)- and (6′*S*)-series in the iodine-catalyzed stereomutation mixture (Table 1) were, as predicted, in the same ratio namely all-*trans*: 9′-*cis*: 11-*cis*: 13-*cis* 100:43:19:10 in the (6′*R*)-series and 67:25:13:10 in the (6′*S*)-series. The isomerization experiment with all-*trans*-(6′*S*)-peridinin (**3**) in the absence of iodine (Table 2) lends further support to the identification of the geometrical isomers in the (6′*S*)-series.




Scheme 6.




*Scheme 7.*

*Furanoid isomers.* 5,6-Epoxide to 5,8-furanoxide isomerization, generally referred to as the furanoid rearrangement, is a well-known reaction in the carotenoid field. Consistent with the accepted mechanism[29,30] this reaction proceeds with retention of configuration at C-6 and provides two C-8 epimers due to a cationic intermediate, cf. Scheme 7.

Two presumed furanoid C-8 epimers of peridinin (**2**) had been isolated long ago after treatment with 0.1% HCl in methanol and characterized by VIS absorption data, mass spectroscopy and $R_F$-values.[2]

Recently the natural occurrence of peridinin furanoxide has been claimed in the dinoflagellate *Procentrum lima*.[31] Enzymatic formation of a carotenoid 5,8-furanoxide from the corresponding 5,6-epoxide is expected to provide one of the two possible C-8 epimers only. Detailed $^1$H NMR characterization of both C-8 epimers of peridinin furanoxide was consequently desirable for comparative purposes.

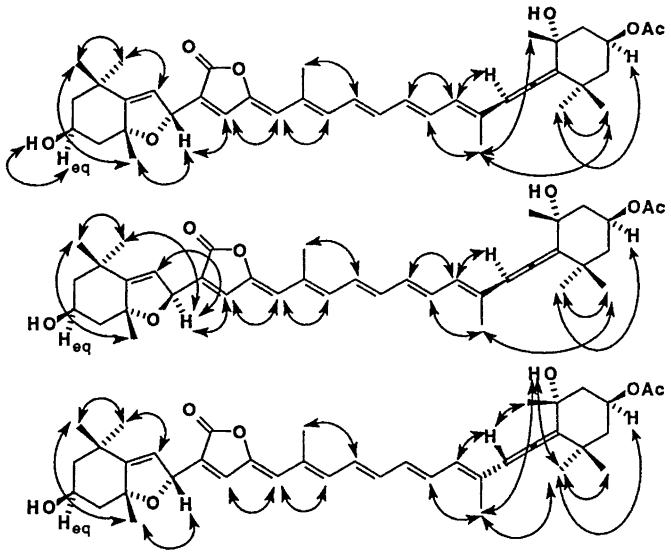
In the present work complete $^1$H NMR assignments have been made for the following furanoxides of peridinin obtained by acid-catalyzed rearrangement (see Scheme 7): three geometrical isomers of the (8*R*)-epimer, namely all-*trans*-(8*R*,6'*R*) (**4**) (2D T-ROESY with ca. 60 µg), 9'-*cis*-(8*R*,6'*R*) (**4a**) ($^1$H NMR only from 27 µg), 11-*cis*-(8*R*,6'*R*) (**4b**) ($^1$H NMR only from 12 µg) and of the (8*S*)-epimer, all-*trans*-(8*S*,6'*R*) (**5**) (2D T-ROESY). In addition, the all-*trans*-(8*R*,6'*S*) (**6**) furanoxide of the allenic (6'*S*)-isomer was studied. The results are presented in Table 5.

The T-ROESY data for the all-*trans*-(8*R*,6'*R*) (**4**) and all-*trans*-(8*S*,6'*R*) (**5**) C-8 epimers, as well as for the allenic isomer all-*trans*-(8*R*,6'*S*) (**6**) are summarized in Scheme 8.

The relative configuration in the (8*R*) (**4**, **6**) and (8*S*) (**5**) isomers was clearly evidenced by the T-ROESY data. Thus, only in the (8*R*) isomers was a relevant cross-peak observed between H-18 and H-8. The (8*R*) and (8*S*) iso-

*Table 4.* Relevant observed isomerization shifts $\Delta=\delta_{obs}-\delta$ all-*trans*-(6'*R*) (in ppm, > 0.03) for the olefinic protons in the $^1$H NMR spectra of three *cis* isomers and one allenic isomer of peridinin relative to the all-*trans*-(6'*R*)-isomer (**1**).

| Olefinic protons | 9'-*cis*-(6'*R*) **2a** | 11-*cis*-(6'*R*) **2b** | 13-*cis*-(6'*R*) **2c** | all-*trans*-(6'*S*) **3** |
|---|---|---|---|---|
| H-7 | | 0.08 | | |
| H-8 | | 0.04 | | |
| H-10 | | 0.43 | 0.06 | |
| H-12 | | 0.67 | 0.49 | |
| H-14 | | | −0.23 | |
| H-15 | | | 0.06 | |
| H-15' | | | −0.07 | |
| H-14' | −0.06 | | | |
| H-11' | 0.11 | | | |
| H-10' | −0.11 | | | |
| H-8' | 0.48 | | | 0.12 |




*Scheme 8.*

mers can also be distinguished by inspection of the size of the coupling constant $J_{7,8}$ which is $1.1 \pm 0.1$ Hz in (8*R*) and $2.1 \pm 0.1$ Hz in (8*S*) isomers.

Finally it should be mentioned that proton H-3 is equatorially oriented in the bicyclic end group of the furanoxides as evidenced by the observation that all couplings to its neighbours were approximately equal (ca. 3.5 Hz).

Similarly, the (6'*R*) and (6'*S*) diastereomers in the allenic end group can be readily distinguished in the same manner (see Scheme 8) as in the peridinin series.

*Table 5.* Chemical shifts (δ-values) of furanoxides of peridinin in deuteriochloroform.

| Proton | all-*trans* (8*R*,6'*R*) **4** | 9'-*cis* (8*R*,6'*R*) **4a** | 11-*cis* (8*R*,6'*R*) **4b** | all-*trans* (8*S*,6'*R*) **5** | all-*trans* (8*R*,6'*S*) **6** |
|---|---|---|---|---|---|
| H-2ax | 1.49 | 1.50 | 1.50 | 1.50 | 1.48 |
| H-2'ax | 1.40 | 1.39 | 1.41 | 1.40 | 1.38 |
| H-2eq | 1.76 | 1.76 | 1.77 | 1.79 | 1.76 |
| H-2'eq | 2.00 | 1.99 | 2.00 | 2.00 | 1.96 |
| H-3 | 4.26 | 4.25 | 4.27 | 4.27 | 4.25 |
| H-3' | 5.38 | 5.38 | 5.38 | 5.38 | 5.37 |
| H-4ax | 1.94 | 1.94 | 1.95 | 1.93 | 1.94 |
| H-4'ax | 1.51 | 1.50 | 1.51 | 1.51 | 1.49 |
| H-4eq | 2.20 | 2.20 | 2.22 | 2.19 | 2.20 |
| H-4'eq | 2.29 | 2.28 | 2.28 | 2.28 | 2.24 |
| H-7 | 5.54 | 5.53 | 5.58 | 5.51 | 5.53 |
| H-8 | 5.61 | 5.61 | 5.63 | 5.64 | 5.61 |
| H-8' | 6.05 | 6.53 | 6.05 | 6.05 | 6.16 |
| H-10 | 7.17 | 7.17 | 7.58 | 7.20 | 7.17 |
| H-10' | 6.11 | 5.99 | 6.10 | 6.11 | 6.12 |
| H-11' | 6.60 | 6.71 | 6.63 | 6.60 | 6.63 |
| H-12 | 5.70 | 5.70 | 6.39 | 5.72 | 5.71 |
| H-14 | 6.43 | 6.43 | 6.42 | 6.43 | 6.43 |
| H-14' | 6.38 | 6.33 | 6.37 | 6.37 | 6.37 |
| H-15 | 6.60 | 6.60 | 6.60 | 6.60 | 6.60 |
| H-15' | 6.50 | 6.49 | 6.52 | 6.50 | 6.51 |
| Me-16 | 1.161 | 1.162 | 1.165 | 1.197 | 1.161 |
| Me-17 | 1.343 | 1.343 | 1.348 | 1.316 | 1.345 |
| Me-16' | 1.384 | 1.393 | 1.384 | 1.384 | 1.413 |
| Me-17' | 1.068 | 1.084 | 1.070 | 1.069 | 1.064 |
| Me-18 | 1.666 | 1.665 | 1.679 | 1.683 | 1.665 |
| Me-18' | 1.351 | 1.367 | 1.351 | 1.351 | 1.345 |
| Me-19' | 1.798 | 1.815 | 1.804 | 1.797 | 1.846 |
| Me-20 | 2.213 | 2.211 | 2.087 | 2.214 | 2.214 |
| HO-C-3 | 1.30 | 1.30 | 1.31 | 1.31 | 1.30 |
| HO-C-5' | – | – | 1.38 | 1.34 | 1.24 |
| AcO | 2.040 | 2.038 | 2.040 | 2.039 | 2.037 |

In conclusion, the present chemical shift assignments of the various furanoxide isomers of (6′*R*)- and (6′*S*)-peridinin should allow the identification of any naturally occurring furanoxide isomer of peridinin, and serve to clarify the artefact issue.

## Experimental

*Materials and methods.* Peridinin (**2**) was isolated from dinoflagellates.[32] General precautions for work with carotenoids were taken.[33] Solvents were of *p.a.* quality. The time factor, critical when working with sterically labile geometrical isomers prior to and during spectroscopic examination, was paid particular attention. The instruments used have been specified previously.[16]

*HPLC.* Analytical HPLC was performed on a 5 μ Techsphere nitrile column with hexane–acetone–methanol 89:10:1 as the eluent,[12] flow = 1.5 ml $min^{-1}$. Semipreparative HPLC of peridinin isomers was carried out on a (i) 5 μ Techsphere semipreparative nitrile column (25 cm × 10 mm) with the same eluent, flow = 4.0 ml $min^{-1}$ [12] (system 1) or (ii) on a Spherisorb 55-W silica column (25 cm × 4 mm) with hexane–$CH_2Cl_2$–isopropyl alcohol–Hunig's base 62.4:25:2.5:0.1 as the eluent, flow = 0.8 ml $min^{-1}$ (system 2). The furanoid derivatives were separated in system 2.

*NMR experiments.* The stereochemical purity of the samples was checked by HPLC before and after spectra had been recorded.

All spectra were recorded in $CDCl_3$ (99.98% D) with $Me_4Si$ as an internal standard on a Bruker AM-400 spectrometer. The 2D $^1H,^1H$ and $^1H,^{13}C$ COSY, as well as the 1D TOCSY spectra were measured as recently described in detail.[15,22,25] The T-ROESY spectra were acquired using a ($180^\circ_x$–$180^\circ_x$) spin-lock[26] of 0.6 s duration.[23]

*Isomerization experiments.* Conditions for experiments including iodine concentrations are given in Tables 1–3. Peridinin concentrations were 40 μg $ml^{-1}$ benzene in all experiments. Glass of Quickfit quality was used. Irradiation experiments were carried out outdoors in early September sunlight at 63.5° latitude, temperature ca. 18°C.

Individual isomers were separated by HPLC (cf. Fig. 1), characterized by VIS data (Table 1) and $^1H$ NMR spectroscopy, see below and the Results section.

*Furanoid rearrangement.* All-*trans*-(3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*R*)-peridinin (**2**, 0.31 mg, $4.92 \times 10^{-4}$ mmol) was dissolved in dilute HCl in MeOH (0.03 M, 40 ml). The reaction was monitored by VIS spectroscopy. A 17 nm hypsochromic shift was observed for the main abs. max. after 30 min. Water was added and the product extracted with diethyl ether. The organic phase was washed with water until neutral and dried over anhydrous $Na_2SO_4$. Solvents were evaporated off and the residue dissolved in a minimal volume of benzene and subjected to preparative TLC.

*All*-trans-(8R,6′R)-*peridinin 5,8-furanoxide* (**4**). Available amount 0.12 mg, $1.91 \times 10^{-4}$ mmol, 39% yield. VIS absorption and mass spectral data were in accordance with reported data.[2] $^1H$ NMR assignments are given in Table 4.

*All*-trans-(8S,6′R)-*peridinin 5,8-furanoxide* (**5**). Available amount 0.07 mg, $1.11 \times 10^{-4}$ mmol, 23% yield. VIS absorption and mass spectral data were in accordance with previously reported data.[2] $^1H$ NMR assignments are given in Table 4.

Furanoid rearrangement of (6′*R*)- (**2**, **2a**, **2b**) and (6′*S*)- (**3**) peridinin occurred in $CDCl_3$ containing traces of DCl, and was often observed upon storage in $CDCl_3$ (quality for spectroscopic studies) for some days. Isomers were separated by HPLC (system 2) and characterized by VIS and $^1H$ NMR spectroscopy. In the HPLC eluent (system 2) $R_T$ and VIS data were as follows: 9′-*cis*-(8*R*,6′*R*) (**4a**), $R_T$ = 7.3 min, VIS $\lambda_{max}$ 440, (458) nm, % $D_B/D_{II}$[19] = 16; all-*trans*-(8*S*,6′*R*) (**5**), $R_T$ = 8.2 min, VIS $\lambda_{max}$ 442, (469) nm, % $D_B/D_{II}$ = 14; 11-*cis*-(8*R*,6′*R*) (**4b**), $R_F$ = 9.3 min, VIS $\lambda_{max}$ 441, (467) nm, % $D_B/D_{II}$ = 20; all-*trans*-(8*R*,6′*R*) (**4**), $R_F$ = 10.7 min, VIS $\lambda_{max}$ 442, (470) nm, % $D_B/D_{II}$ = 16. $^1H$ NMR data are given in Table 4.

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