Algal Carotenoids. 36*
NMR Studies of Prasinoxanthin – Stereochemical Analysis

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The 400 MHz ^{1}H NMR spectrum of prasinoxanthin (3′*R*,6′*R*)-3,6,3′-trihydroxy-7,8-dihydro-γ,ε-caroten-8-one *ex* Prasinophyceae was completely assigned (56 protons). Spin coupling patterns were determined by COSY-90 experiments. NOE difference spectroscopy experiments were further employed for stereochemical analysis.
The hydroxy groups of the γ-ring were shown to be *cis*-configurated (3*S*,6*R*) assuming 3*S*-chirality. The conformations in solution of the γ-ring and the adjacent hydrogen bonded keto moiety and of the ε-ring were established.
Improved ^{13}C NMR data are reported.

Prasinoxanthin (*1*), the major carotenoid of an algal group within the Prasinophyceae,[1,2] has previously been shown to be (3′*R*,6′*R*)-3,6,3′-trihydroxy-7,8-dihydro-γ,ε-caroten-8-one with 3,6-*trans* diol configurations (*1a*), *cf.* Scheme 1, including the relevant numbering of rings *A* and *B* and the 3,6-*cis* (3*S*,6*R*) structure (*1b*).

Results and discussion

In the present work further NMR studies are reported, aiming at complete assignment of the ^{1}H NMR signals, including relative stereochemistry of the γ-ring *A*.

Proton spin-spin coupling patterns were determined by two-dimensional homonuclear chemical shift-correlated spectroscopy (COSY),[4] Fig. 1. The cross peaks in the COSY matrix reveal the following proton-proton connectivities: H-3 is coupled to H-2_{ax}, H-2_{eq}, H-4_{ax} and H-4_{eq}, and the geminal relationships at C-2 and C-4 are demonstrated. The axial H-4 shows coupling to the methylene group at C-5, and the geminal coupling at C-7 is obvious. Furthermore, the connectivities between CH_3-9, H-10, H-11 and H-12 may be extracted, as well as for CH_3-13, H-14 and H-15. Starting from the ε-ring the couplings of H-3′ to H-$2'_{ax}$, H-$2'_{eq}$, H-4′ and CH_3-5′ are apparent. Moreover, the connectivity of CH_3-5′ to H-6′ is seen; then to H-7′, H-8′ and H-9′. Proton H-10′ is coupled to CH_3-9′ and to H-11′. In analogous manner all resonances up to H-15′ may be assigned.

The fragments assigned from the COSY experiments were connected by means of the NOE results.

Sixteen nuclear Overhauser effect (NOE) difference spectroscopy experiments[5] were carried out for assignment of protons closely located in space, Table 1.

The COSY-90 experiments, Fig. 1, allowed assignment of all 56 protons in prasinoxanthin (*1*). In the γ-end group *A* the weak *gem.* coupling between the protons of the exocyclic methylene group was established. A weak allylic coupling between these olefinic protons and the δ 2.6 methylene proton at C-4 was demonstrated. Ax-

*Part 35, see Ref. 1.
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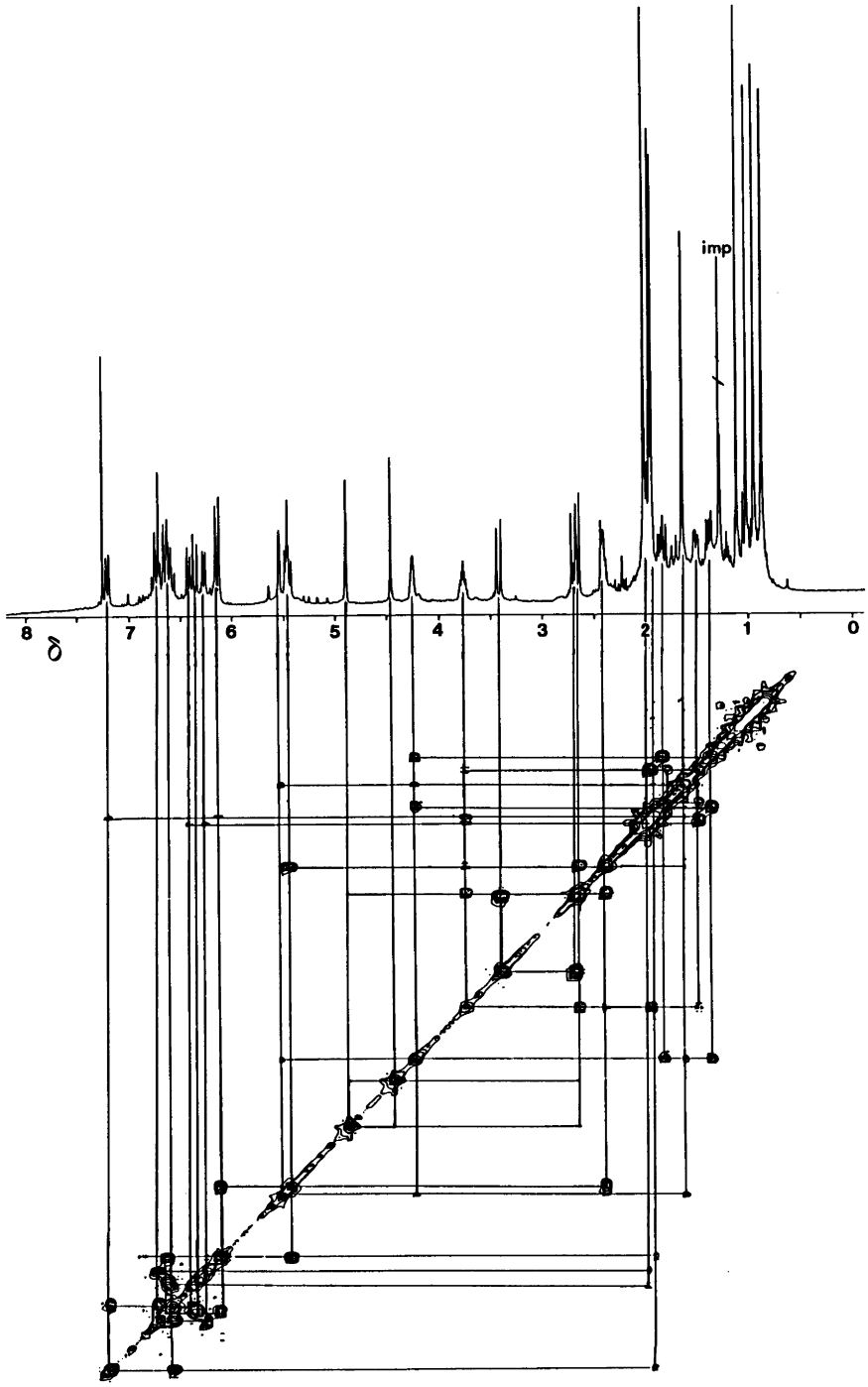


Fig. 1. 2D-^{1}H NMR spectrum of prasinoxanthin (*1*) in $CDCl_3$.

Table 1. The data matrix for NOE experiments with prasinoxanthin (*1*). The horizontal axis denotes which signals are irradiated and the vertical axis the signals affected.

	Lines irradiated (δ)																
	CH_3 0,85	CH_3 0.92	CH_3 1.00	CH_3 1.07	CH_3 1.62	CH_2 1.8	CH_3/CH_2 1,9	CH_3 1.95	CH_3 2.00	CH_2/H 2.4	CH_2 2.6	H 3.76	H 4.22	CH_2 4.46	CH_2 4.89	H 5.46	
H									+								6.7
H							+	+	+								6.6
H			+		+												6.15
H					+								+				5.55
H/OH	+						+				+						5.4
CH_2										+				+			4.89
CH_2								+							+		4.46
H	+				+	+											4.22
H		+								+							3.76
CH_2		+									+			+			3.4
CH_2				+						+							2.6
CH_2/H			+		+						+	+			+	+	2.4
CH_2				+													1.9
CH_2	+		+										+				1.8
CH_3										+							1.62
CH_2				+			+	+				+					1.5
CH_2			+			+							+				1.35
CH_3		+									+						1.07
CH_3	+																1.00
CH_3				+													0.92
CH_3			+														0.85

ial position of the δ 2.6 proton is required for the overlap between the π-orbitals of the exocyclic double bond and the σ-orbital of this proton.[6]

The NOE difference experiments, Table 1, presented as in a previous paper,[7] showed that H-3 (γ-ring *A*) was axial, since NOE was obtained upon irradiation of the δ 0.92 methyl signal, requiring a 1,3-diaxial relationship, see Scheme 1. Assignment of the δ 0.92 and δ 1.07 methyl groups at C-1 as axial and equatorial respectively, consequently followed. The relative position of the protons in the exocyclic methylene group could be determined since irradiation of the δ4.46 signal resulted in NOE for the δ 3.41 methylene proton at C-7, Table 1, Scheme 1. NOE for the latter methylene signal was also observed upon irradiation of the δ 0.92 methyl singlet. Moreover, irradiation of the methyl signal at δ 1.07 caused NOE for the δ 2.6 methylene proton at C-7, consistent with the above assignments at C-1. These results demonstrate that CH_2-7 must occupy a quasiequatorial position as to ring *A* and furthermore that this methylene group has one preferred orientation, ascribed to the intramolecular hydrogen bonding between the carbonyl group and the hydroxy group at C-6.[3] Since CH_2-7 is quasiequatorial, relative *cis* configuration between the hydroxy groups at C-3 and C-6 is established, Scheme 1. This result contradicts the previous tentative *trans* assignment[3] based on a biosynthetic hypothesis. However, other biosynthetic routes to prasinoxanthin (*1b*) may be envisaged.[8]

NOE experiments supporting the half-chair conformation of the ε-end group *B*, with quasiequatorial polyene chain and hydroxy group,[9] were also carried out, Table 1. Irradiation of the δ 0.85 methyl signal resulted in NOE for the δ 1.8 and δ 4.22 signals, consistent with the relative *cis*-position to ring *B* of the respective groups and with the 1,3-diaxial relationship between the δ 0.85 methyl group and the δ 4.22 methine.

Similar experiments confirmed a *cis*-relationship for the δ 1.00 methyl group and the δ 1.35 and δ 2.42 protons. Irradiation of the δ 1.00 and δ 0.85 methyl signals gave NOE for the δ 6.15 H-8′ and δ 5.4 H-7′ olefinic protons respectively. This demonstrates a preferred conformation of the ε-ring *B* relative to the polyene chain, minimizing the steric conflict between the methyl group at C-5′ and H-8′. Irradiation of the δ 1.00 methyl signal also caused weak NOE for the δ 1.8 methylene signal at C-2′. Models reveal that this result requires an equatorial position of the δ 1.00 methyl group, since irradiation at this frequency results in NOE for both protons at C-2. The conformation assigned to the ε-ring *B* is consistent with the 3′*R*,6′*R* (3′,6′-*trans*) configuration based on a simplified ^{1}H NMR analysis and CD data.[3]

For an unsubstituted ε-ring the *trans* relationship between the polyene chain and the least shielded *gem.* methyl group was first reported by total synthesis of a deuterated analogue,[9] and for a 3,6-*trans* 3-hydroxy-ε-end group established by ^{1}H NMR.[5,10] Individual assignment of the C-2′

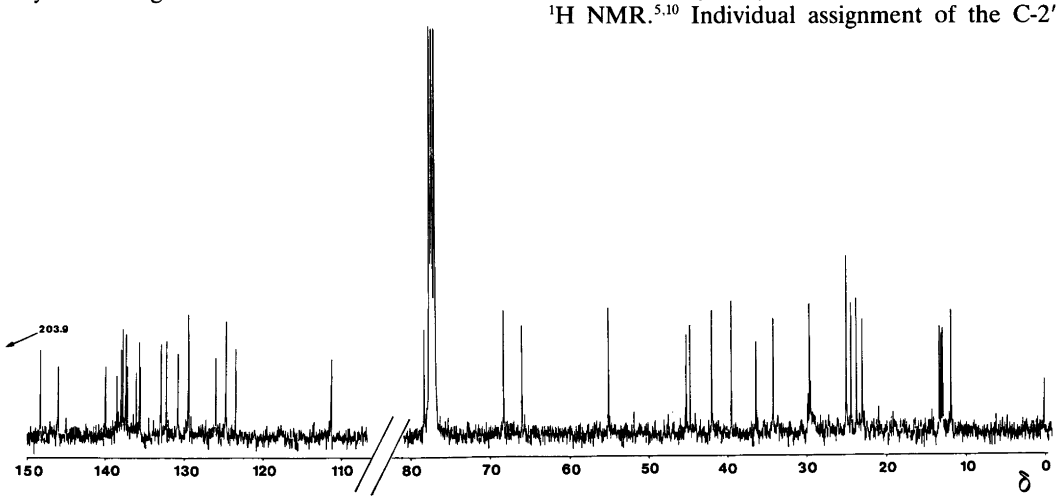


Fig. 2. ^{13}C NMR spectrum of prasinoxanthin (*1*) in $CDCl_3$.

methylene protons has previously only been reported for the 3,6-*cis* epimer.[5]

The conformation of ring *B* of known configuration[3] and the relative 3,6-*cis* glycol configuration (*1b*) are thus established, 3*S*,6*R*, assuming 3*S*-chirality. The previously suggested 3,6-*trans* structure[1] was incorrectly denoted 3*R*,6*R*.

The chirality at C-3 still rests on biogenetic analogy. Because of identical methylene substituents in both C-2 and C-4, the chirality does not lend itself to analysis by the Horeau procedure.[11,12]

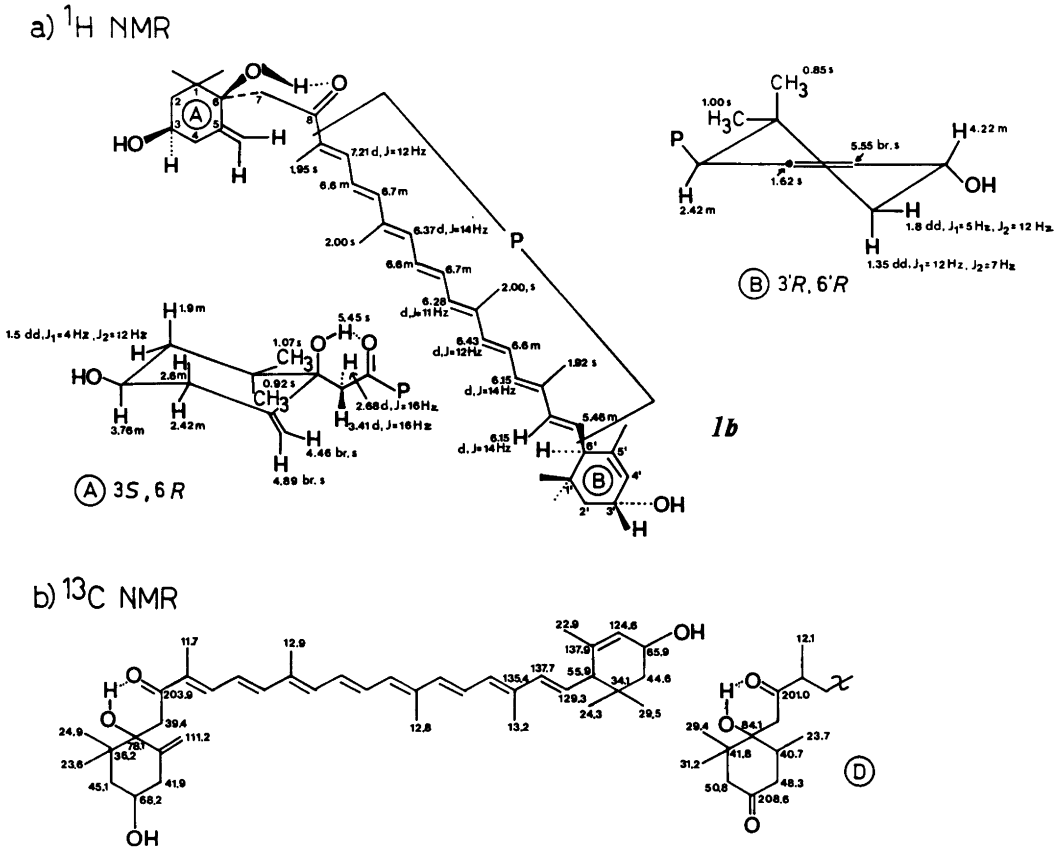
An improved broad band decoupled ^{13}C NMR spectrum of prasinoxanthin (*1*) was obtained, *cf.*,[3] for comparison with relevant model carotenoids. The small sample size available (4 mg) did not permit a two-dimensional proton carbon correlation for assignment of individual carbons.

The ^{13}C NMR spectrum contained individual signals for each of the 40 carbon atoms. Correlation with (3*R*,3′*R*,6′*R*)-lutein (with end group *B*)[13] and with isomytiloxanthin (with end group *D*)[14] identified the signals in the ε-ring *B*, *cf.*,[5] and led to the tentative assignments made for the other terminus in Scheme *1b*.

Experimental

Prasinoxanthin. Prasinoxanthin (*1*, 5 mg) was reisolated from the green algae clone BT-5[1] by the previously described procedure and was also characterized by R_f, visible absorption and mass spectra; see Ref. 3.

^1H NMR experiments. The 400 MHz ^1H NMR spectra were obtained in $CDCl_3$ at room temperature on a Bruker WM-400 instrument (Bruker Spectrospin) equipped with an Aspect 2000 com-



Scheme 1. NMR assignments of prasinoxanthin (*1*). The sequence of chemical shifts for the protons H-14-15-15′-14′ may be interchanged.

puter. All spectra were run with 32 K data points over 4000 Hz.

The nuclear Overhauser enhancement (NOE) measurements, see Table 1, were obtained by pre-irradiation of the different proton chemical shift frequencies for 3 s. After collecting 8 scans, preceded by 2–4 dummy scans, at one selected irradiation frequency, the free induction decay (FID) was stored, and the irradiation frequency was changed. For every fourth frequency a reference FID with irradiation at an off-resonance frequency was acquired. The entire cycle was repeated 10–100 times under computer control with addition of the new data to those already stored. The accumulated FID's were processed identically with the same line broadening and phase corrections.

The NOE experiments were not designed for quantitative measurements, since relaxation times were unknown, and degassing of the sample was not performed.

The 2D ^{1}H correlated spectrum, Fig. 1, was obtained with a (90°-t_1-90°-t_2) sequence (COSY-90, Bruker Automated Program). The spectral width in both frequency domains was 4000 Hz with a digital resolution of *ca* 8 Hz (1024 data points with zero filling in the *F*1 dimension). Sixty-four scans were stored for each of the 256 increments of T_1. After the 2D Fourier transformation with absolute value data and sine bell multiplication in both F1 and F2, the matrix was symmetrized.

^{13}C NMR experiments. The 100 MHz ^{13}C data were acquired on the same sample with noise decoupling of the attached protons, using a spectral width of 20,000 Hz and a digital resolution of *ca* 1 Hz/point. After acquisition of 90,000 scans Fourier transformation (FT) was made with an exponential multiplication giving an extra line broadening of 2 Hz.

The ^{13}C FT NMR spectrum is presented in Fig. 2, see partial assignments Scheme 1. δ11.72 (C-19), 12.76 (C-20′), 12.94 (C-20), 13.17 (C-19′), 22.87 (C-18′), 23.64 (C-16/17), 24.30 (C-16′/17′), 24.90 (C-16/17), 29.51 (C-16′/17′), 34.05 (C-1′), 36.22 (C-1), 39.39 (C-7), 41.88 (C-4), 44.63 (C-2′), 45.13 (C-2), 54.99 (C-6′), 65.90 (C-3′), 68.17 (C-3), 78.12 (C-6), 111.18 (C-18), 123.33, 124.58 (C-4′), 125.89, 129.30, 129.34, 130.63, 132.09, 132.77, 135.38 (C-9′), 135.51, 135.89, 137.06, 137.23, 137.64 (C-8′), 137.87 (C-5′), 138.37, 139.80, 145.78 (C-5/12), 148.01 (C-12/5), 203.9 (C-8).

A heteronuclear correlated experiment was carried out using Bruker software and computer control. An average proton carbon coupling of 140 Hz was chosen.

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