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PRASINOXANTHIN—A CHEMOSYSTEMATIC MARKER FOR ALGAE*

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Key Word Index—New coccoid marine alga; Prasinophyceae; Siphonales; chlorophylls *a,b* carotenoids; prasinoxanthin; chemosystematics.

Abstract—A new coccoid marine alga (clone Ω 48–23) contained chlorophylls *a* and *b* and carotenoids consisting of β,β-carotene (3% of total), β,ε-carotene (1%), zeaxanthin (2%), neoxanthin (21%), two minor unknowns (2 + 2%) and prasinoxanthin (69%). Prasinoxanthin is identical with xanthophyll K, previously considered characteristic of prasinophytes. From spectroscopic and chemical evidence prasinoxanthin is assigned the structure (3′*R*,6′*R*)-3,6,3′-trihydroxy-7,8-dihydro-γ,ε-caroten-8-one, with tentative 3*R*,6*R* chirality from biogenetic considerations, thus representing the first algal carotenoid with a γ-end group. The structural relationship between prasinoxanthin and siphonaxanthin (*ex* Prasinophyceae and Siphonales) is discussed in chemosystematic terms.

INTRODUCTION

The photosynthetic pigments are important chemosystematic indicators in algae [1–4]. This work shows how a newly isolated marine planktonic alga, initially assumed from its gross morphology to be a species of *Pelagococcus* and thus a member of Chrysophyceae [5], was found from its photosynthetic pigments to be related to the Prasinophyceae [6] and Siphonales (Chlorophyceae).

RESULTS AND DISCUSSION

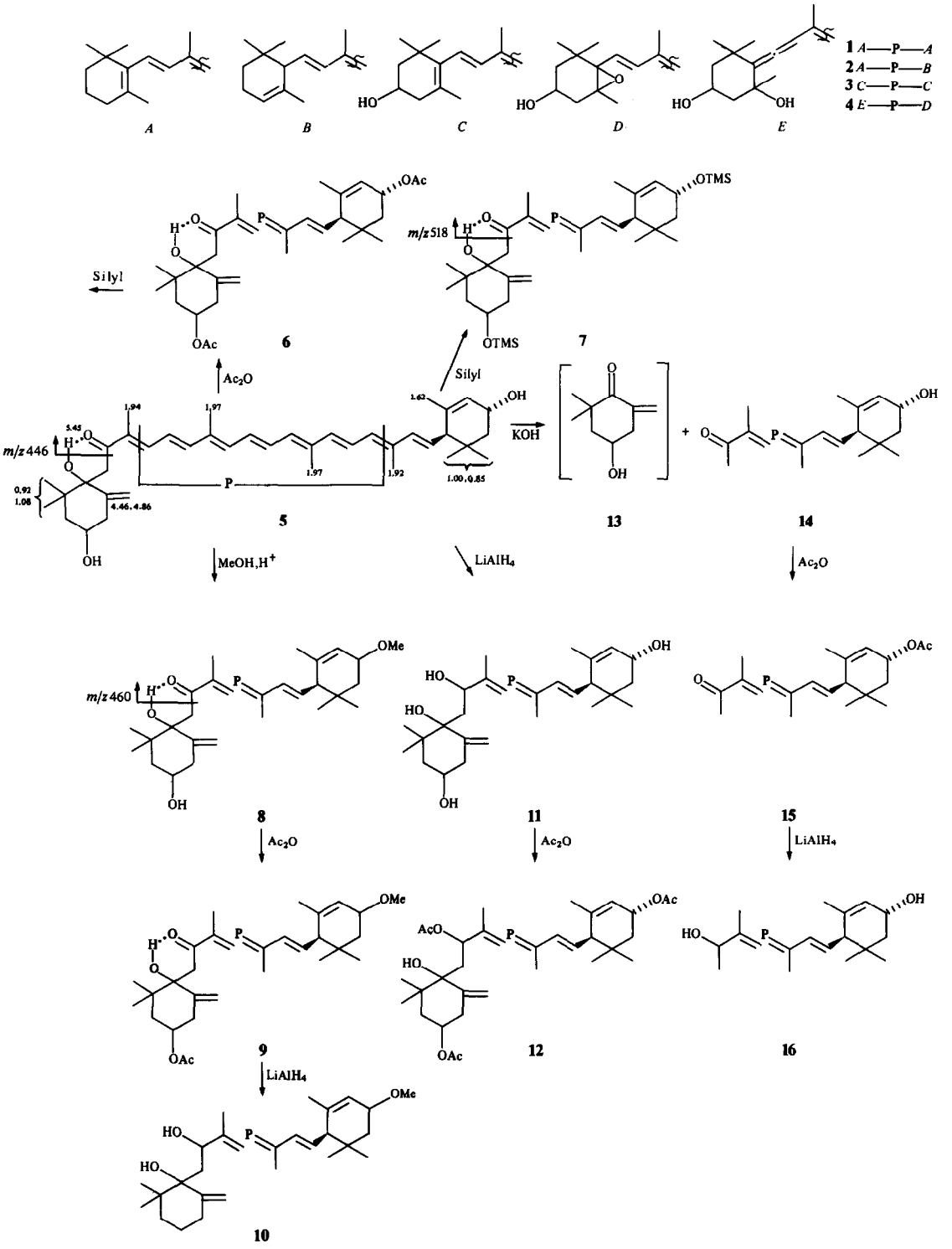
The identification of chlorophylls *a* and *b* revealed that clone Ω 48–23 belonged in the Chlorophyta [1, 3]. It had a high carotenoid content (0.39% of the dry wt) and these consisted of β,β-carotene (**1**, Scheme 1, 3% of total carotenoid) identified by R_f, visible and mass spectra, β, ε-carotene (**2**, 1%), tentatively identified by R_f and visible spectrum), zeaxanthin (**3**, 2%) identified by R_f, visible and mass spectra, neoxanthin (**4**, 21%) identified by R_f, visible and mass spectra and furanoid rearrangement, two minor unknowns (2 + 2%) and the major carotenoid, here called prasinoxanthin (**5**, 69%). Neither the allenic ketone fucoxanthin, nor the acetylenic diatoxanthin and diadinoxanthin, typical carotenoids of Chrysophyceae [7], were encountered.

The molecular ion of prasinoxanthin (**5**, *m/z* 600) was compatible with $C_{40}H_{56}O_4$. Of the four oxygen functions two were identified as secondary hydroxyl groups from the ^{1}H NMR spectrum of prasinoxanthin (**5**, Scheme 1), and by acetylation to the diacetate **6** and silylation of **5** to the di(trimethylsilyl) ether **7**. One of the hydroxyl groups was allylic, providing the monomethyl ether **8** upon treatment of prasinoxanthin (**5**) with acidified methanol. The second hydroxyl was non-allylic. Thus the monomethyl ether **8** gave the monoacetate **9** upon acetylation. The presence of a conjugated keto group followed from IR and ^{1}H NMR spectral data for prasinoxanthin (**5**) and the hypsochromic shift upon $LiAlH_4$-reduction to **11**, which provided a triacetate **12**. $LiAlH_4$-reduction of the monomethyl ether monoacetate **9** furnished the product **10** with the same chromophore as **11** and **12**. The fourth, inert oxygen function was ascribed to a tertiary hydrogen-bonded hydroxyl group with a concentration independent signal at δ5.45 in the ^{1}H NMR spectrum. The hydrogen was exchangeable with D_2O. This signal also disappeared upon addition of shift reagent and by reduction to the tetrol **11**. Its β-position to the carbonyl group was confirmed by retro aldol cleavage of prasinoxanthin (**5**) to the C_{31} methyl ketone **14**, the structure of which followed from visible and mass spectra, acetylation to the monoacetate **15** and $LiAlH_4$ reduction to the conjugated octaene **16**. Upon electron impact of prasinoxanthin (**5**) McLafferty rearrangement caused a prominent peak at *m/z* 446.3186 ($C_{31}H_{42}O_2$) corresponding to the loss of the conjugated ketone **13** ($C_9H_{14}O_2$). Corresponding cleavages were observed for the derivatives **6**, **7** and **8** as indicated in Scheme 1. ^{1}H NMR spectral assignments for prasinoxanthin (**5**; Scheme 1) are consistent with the presence of a 3,6-*trans* substituted ε-ring (δ 1.00*s*, 0.85*s*, 2.40*d* and 5.55*d*) [8]. S_N-1 type allylic methylation provided a mixture of the two epimeric C-3′ methyl ethers [methoxy signal at δ3.37*s* (70%) and δ3.39*s* (30%) in the ^{1}H NMR spectrum of **10**].

The substituted γ-end group was indicated by IR absorption for **5** at 900 cm^{-1} and ^{1}H NMR signals at δ4.46 and 4.88 for a terminal methylene. A considerable shift relative to the corresponding signals at δ4.54 and 4.69 in β,γ-carotene [9], may be explained by the proximity of the carbonyl function. Upon reduction of the keto group a broad singlet at δ 5.11 was observed for the tetrol **11**. Absorption at δ 112.0 in the ^{13}C NMR spectrum for the derivative **9** supports this assignment [10].

The magnetically non-equivalent protons of the meth-

*Part 30 in the series "Algal Carotenoids". For Part 29 see *Phytochemistry* **23**, 1711.



Scheme 1.

ylene group at C-7, adjacent to the chiral C-6 center, were seen in the ^{1}H NMR spectrum as an AB system with doublets at δ2.68 and δ3.42 (J = 16 Hz). The corresponding signals in fucoxanthin appear at δ2.59 and 3.63 (J = 18 Hz) [11]. Allocation of the secondary, non-allylic hydroxyl group to C-3 rather than C-2 is consistent with facile acetylation [12] and the ^{1}H NMR spectrum (δ 3.7 *m*) and analogy with most algal carotenoids [1, 2]. LIS experiments, with the secondary hydroxyl groups of prasinoxanthin (**5**) protected as the methyl ether (**8**, **9**) and

acetate (9), revealed downfield shifts of the C-1 methyl signals and an upfield shift of Me-19 compatible with complexation of the tertiary hydroxyl function.

Concerning the chirality of prasinoxanthin (5) its CD spectrum, closely similar to that of siphonaxanthin *ex Eutreptiella gymnastica* [13] with a positive Cotton effect, defines in comparison with (6*R*)-ε-apo-8′-carotenal [14] the 6′*R* chirality. Then 3′*R* chirality follows from the 3′,6′-*trans* relationship established by ^{1}H NMR spectroscopy. Biogenetic considerations, with hypothetic formation of the new, substituted γ-end group from a common 3-hydroxy-5, 6-epoxy precursor (Scheme 2) further indicate 3*R*, 6*R* chirality.

The major carotenoid **5** of clone Ω 48–23 had characteristics resembling those of the partly characterized xanthophyll K from Prasinophyceae [15], and these carotenoids were found identical by direct comparison. Xanthophyll K has been reported to give the less polar micronone of unestablished structure upon alkali treatment [16]. Micronone may now be assigned the methyl ketone structure **14** (C_{31}-skeleton, Scheme 1) and is considered an artefact obtained from the natural β-hydroxy ketone **5** under saponifying conditions. Microxanthin [17] is the corresponding diol **16**. Because studies by Ricketts [15–19] have revealed that xanthophyll K is abundant within the group Prasinophyceae, we have named this carotenoid prasinoxanthin (**5**). The carotenoid composition of clone Ω 48–23 resembles that of a non-chlorophycean-type pigment group of the Prasinophyceae [15], but does not appear to be identical with any, thus making it unlikely that clone Ω 48–23 is a non-motile stage of the *Mantoniella* (*ex Micromonas*) spp. or *Nephroselmis* sp. examined [15].

The Prasinophyceae (including Loxophyceae), comprising scaly, green flagellated cells is considered by some to be an algal class within the Chlorophyta [20, 21], while by others [22] its members are retained in the Chlorophyceae, mainly in the order Volvocales [22, 23]. In evolutionary terms they are considered the most primitive green algae [24], preceding for example, the siphonaceous green algae which have lost motility. Prasinophyte carotenoid synthesis appears more related to that of the Siphonales, with siphonaxanthin (**17**; Scheme 2) [25, 26] as the characteristic carotenoid, rather than to that of the Volvocales when judged by the carotenoids of *Chlamydamonas reinhardii* [27]. Xanthophyll K 1 [16] of prasinophytes has been identified as siphonein [26], a C-19 ester of siphonaxanthin (**17**).

A close biogenetic relationship between prasinoxanthin (**5**) and siphonaxanthin (**17**) may be formulated via the hypothetical precursor *A* (Scheme 2), consistent with the chirality for siphonaxanthin *ex Eutreptiella gymnastica* recently established [13].

In conclusion, the present study has led to the structural assignment for a new algal carotenoid with a novel substituted γ-end group (**5**). Its occurrence so far restricted to green algae classified as prasinophytes suggests a close systematic relationship for the new clone Ω 48–23 sp. from which prasinoxanthin (**5**) was obtained for this study. The structural relationship between prasinoxanthin (**5**) and siphonaxanthin (**17**) as well as the similar carotenoid pattern shown by the Prasinophyceae and siphonaceous algae suggest a phylogenetic relationship between them, as has been hypothesized on other grounds [24, 28].

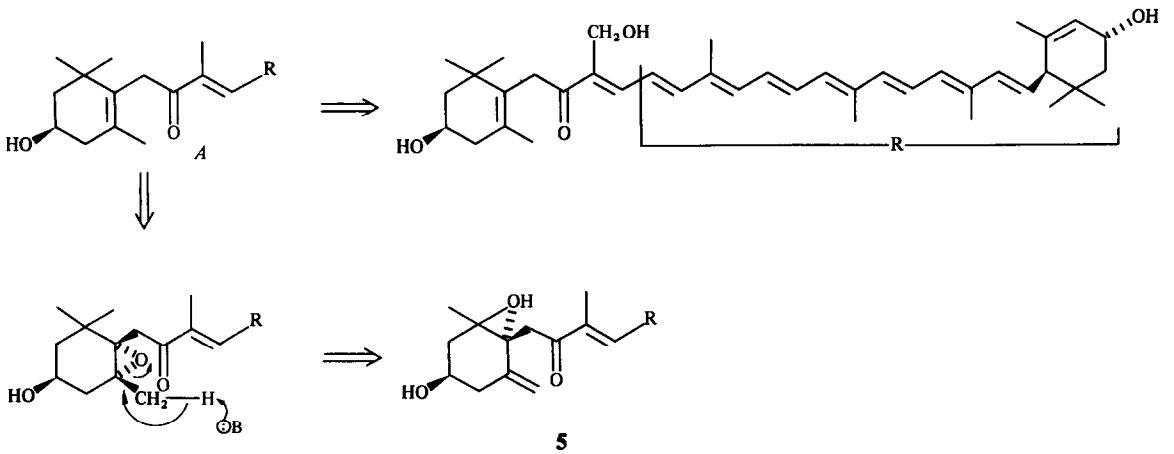
EXPERIMENTAL

Biological material. Clone Ω 48–23 was isolated by L. S. Murphy from a sample of slope water collected at 38° 19.5′N, 69° 34.5′W at 30 m depth in July 1978. It was rendered axenic by L. Provasoli and I. J. Pintner. In culture it grows as single non-motile cells of 2.5–3 μm diameter. For pigment analysis, 15 l. aerated carboy cultures in medium f/2 (without added silicon) were grown under a 16/8 hr light cycle provided by 'cool-white' fluorescent lamps yielding *ca* 150 μ einstein m^2 sec^{-1} at 20° [29]. These cultures were harvested by continuous centrifugation (Sharples Co.), frozen immediately and freeze-dried, all in subdued light.

Pigment isolation. General precautions for work with chlorophylls and carotenoids were taken [30]. Pigments were extracted with Me_2CO–MeOH (1:1) at room temp.

Chlorophylls. Prep. TLC (silica gel, 30% AH) of the algal extract gave a blue-green zone of chlorophyll *a* (R_f = 0.41; λ_{max} 663 nm in Me_2CO) and a green zone of chlorophyll *b* (R_f = 0.34; λ_{max} 641 nm in Me_2CO), inseparable from authentic standards.

Carotenoids. These comprised 0.39% of the dry wt when



5

Scheme 2.

spectrophotometrically determined. TLC was carried out on 1.0 mm silica gel (preparative), Merck DC Alufolien (analytical) or special plates [31] for separation of β,β-carotene (1) and β,ε-carotene (2). If not otherwise stated 30% Me_2CO in hexane (AH) was used as eluant. Visible spectra were recorded in Et_2O using $E^{1\%}_{1cm} = 2500$ at λ_{max}. Spectral fine-structure is expressed by the term % III/II [32]. ^1H NMR (100 MHz) and ^{13}C NMR spectra were recorded in $CDCl_3$. Mass spectra were recorded at 190–210° at 70 eV using a direct inlet system. Diagnostic peaks only are cited. Acetylation, silylation, furanoid rearrangement of epoxide, alkali treatment and $LiAlH_4$-reduction were carried out by general procedures [30]. Individual natural carotenoids and their derivatives are described below.

β,β-Carotene (1). $R_f = 0.98$ (30% AH), inseparable from synthetic 1; VIS λ_{max} nm: (424), 447, 475; MS *m/z* (rel. int.): 536 $[M]^+$ (20), 444 $[M-92]^+$ (2), 430 $[M-106]^+$ (1), 382 (100).

β,ε-Carotene (2). $R_f = 0.98$ (30% AH); VIS λ_{max} nm: (420), 442, 469, % III/II = 55.

Zeaxanthin (3). $R_f = 0.42$ (30% AH), inseparable from authentic 3; VIS λ_{max} nm: (424), 449, 474; MS *m/z* (rel. int.): 568 $[M]^+$ (80), 550 $[M-18]^+$ (10), 476 $[M-92]^+$ (10), 91 (100).

Neoxanthin (4). $R_f = 0.15$ (30% AH), inseparable from authentic 4; VIS λ_{max} nm: 414, 436, 463; MS *m/z* (rel. int.): 600 $[M]^+$ (50), 582 $[M-18]^+$ (10), 520 $[M-80]^+$ (5), 508 $[M-92]^+$ (3), 221 (50), 91 (100). Upon acid treatment a hypsochromic colour shift to VIS λ_{max} nm: 400, 422, 448 was observed.

Unknown 1. Isolated after alkaline hydrolysis of chlorophylls had $R_f = 0.60$ (30% AH); VIS λ_{max} nm: 405, 425, 449; MS *m/z* (rel. int.): 570 $[M]^+$ (10), 568 $[M-2]^+$ (2), 552 $[M-18]^+$ (3), 149 (100).

Unknown 2. $R_f = 0.43$ (30% AH); VIS λ_{max} nm: (430), 454, (484); MS *m/z* (rel. int.): 596 (?) 570, $[M]^+$ (70), 568 $[M-2]^+$ (10), 552 $[M-18]^+$ (10), 442 (3), 91 (100).

Prasinoxanthin (5). $R_f = 0.46$ (40% AH) inseparable upon cochromatography from xanthophyll K; VIS λ_{max} nm: 446, (466), (identical to xanthophyll K). IR ν_{max}^{KBr} cm^{-1}: 3400 *m* (broad, OH), 2960 *m*, 2930 *s* and 2860 *m* (CH), 1735 (imp.), 1680 *m*, 1640 *m* and 1605 *s* (chelated C=O), 1580 *w* and 1570 *w* (C=C), 1460 *s* (CH_2), 1380 *s* (Me), 1260 *s*, 1205 *m*, 1170 *s*, 1135 *w* (*tert.* OH), 1070 *m* (*sec.* OH), 1030 m (*sec.* allylic OH), 960 *s* (*trans* CHR=CHR), 900 *w* (=CH_2), 830 *w* (CHR=CR_2). ^1H NMR (100 MHz): δ 0.85 (3H, *s*, Me-17') [33], 1.00 (3H, *s*, Me-16') [8] 0.92 (3H, *s*) and 1.08 (3H, *s*, Me-16, 17), 1.62 (3H, *s*, Me-18'), 1.92 (3H, *s*, Me-19'), 1.94 (3H, *s*, Me-19), 1.99 (6H, *s*, Me-20, 20'), 2.41 (1H, *d*, *J* = 8 Hz, H-6'), 2.0–2.7 (*ca* 2H, *m*, CH_2-4), 2.68 (1H, *d*, *J* = 16 Hz, H_a-7), 3.41 (1H, *d*, *J* = 16 Hz, H_b-7), 3.7 (1H, *m*, H-3), 4.22 (1H, *m*, H-3'), 4.46 (1H, *s*) and 4.88 (1H, *s*, =CH_2), 5.46 (1H, *d*, *J* = 16 Hz, H-7') [34], 5.47 (1H, *s*, H-bonded OH at C-6), 5.55 (1H, *m*, H-4'), 6.1–7.0 (10H, *m*, conj. olefinic), 7.20 (*d*, *J* = 8 Hz, H-10). The δ 6.1–7.0 olefinic region was very similar to that of siphonaxanthin (17) *ex Eutreptiella gymnastica* [13]. The sharp δ 5.47 singlet disappeared upon shaking with D_2O. Irradiation at δ 3.42 gave a singlet at 2.68, and irradiation at 2.68 caused a collapse of the 3.42 doublet. MS *m/z* (rel. int.): 600 $[M]^+$ (1), 582 $[M-18]^+$ (1), 564 $[M-18-18]^+$ (1), 446.3186 (calc. 446.3185 for $C_{31}H_{42}O_2$, $[M-154]^+$ 100), 428 $[M-154-18]^+$ (30). The MS of xanthophyll K taken under similar conditions had *m/z* (rel. int.): 600 $[M]^+$ (8), 582 $[M-18]^+$ (8), 564 $[M-18-18]^+$ (18), 446 $[M-154]^+$ (100), 428 $[M-154-18]^+$ (8). CD (EPA) nm (Δε) 220 (+8), 230 (+2), 235 (+5), 245 (+4), 270 (+10).

Prasinoxanthin diacetate (6). Upon standard acetylation of 5, monitored by TLC, two intermediary monoacetates and a final diacetate (6) were formed in quantitative yield after 3 hr. Compound 6 had $R_f = 0.85$ (30% AH); VIS λ_{max} nm: 445; MS *m/z* (rel. int.): 684 $[M]^+$ (1), 666 $[M-18]^+$ (2), 606 $[M-18-60]^+$ (4), 488 $[M-196]^+$ (4), 428 $[M-196-60]^+$ (4), 149 (100). The diacetate 6 could not be silylated.

Prasinoxanthin di(trimethylsilyl) ether (7). Standard silylation of 5, monitored by TLC revealed two intermediary monoethers and the final diether 7, $R_f = 0.85$ (20% AH); VIS λ_{max} nm: 447; MS *m/z* (rel. int.): 744 $[M]^+$ (5), 518 $[M-226]^+$ (100).

Prasinoxanthin 3'-methyl ether (8). Allylic methylation with 0.03 N HCl in MeOH [35], monitored by TLC, caused quantitative transformation of 5 to 8. The methyl ether 8 had $R_f = 0.40$ (30% AH), VIS λ_{max} nm: 449; IR ν_{max}^{KBr} cm^{-1}: 3400 *m* (broad, OH), 2960 *m*, 2930 *s* and 2860 *m* (CH), 1730 (imp.), 1635 *s* and 1605 *s* (chelated C=O), 1570 *s* and 1520 *s* (C=C), 1440 *s* (CH_2), 1380 *s* and 1360 *s* (Me), 1262 *m*, 1195 *s*, 1090 *s* (OMe), 1070 *m*, 1040 *m* (*sec.* OH), 965 *s* (*trans* CHR=CHR), 900 *m* (=CH_2), 835 *w* (CHR=CR_2). ^1H NMR (100 MHz): δ 0.85 (3H, *s*, Me-16'), 0.92 and 0.98 (together 3H, *s*, Me-17'), 0.93 (3H, *s*) and 1.09 (3H, *s*, Me-16, 17), 1.63 (3H, *s*, Me-18'), 1.93 (3H, *s*, Me-19'), 1.95 (3H, *s*, Me-19), 1.99 (6H, *s*, Me-20, 20'), 2.14 (1H, *d*, *J* = 8 Hz, H-6' in 3',6'-*cis*), 2.43 (< 1H, *d*, *J* = 8 Hz, H-6' in 3', 6'-trans), 2.0–2.70 (2H, *m*, CH_2-4), 2.68 (1H, *d*, *J* = 16 Hz, H_a-7), 3.41 (1H, *d*, *J* = 16 Hz, H_b-7), 3.37 (2H, *s*, OMe in 3', 6'-*trans*) and 3.39 (1H, *s*, OMe in 3', 6'-*cis* [36], 3.8 (*ca* 2H, *m*, H-3, 3'), 4.47 (1H, *s*) and 4.90 (1H, *s*, =CH_2), 5.45 (1H, *s*, H-bonded OH at C-6), 5.55 (1H, *m*, H-4'), 6.1–7.0 (*ca* 10H, *m*, conj. olefinic), 7.20 (1H, *d*, *J* = 8 Hz, H-10). LIS experiment with stepwise addition of $Eu(fod)_3$ was carried out. Linear LIS were recorded at 0.1, 0.3, 0.6 and 1.0 mole $Eu(fod)_3$/mole 8. Largest LIS downfield shifts were observed for Me-16, 17, the terminal methylene, CH_2-7 and H-10. Upfield LIS was recorded for Me-19. MS *m/z* (rel. int.): 614 $[M]^+$ (2), 596 $[M-18]^+$ (4), 460 $[M-154]^+$ (100), 428 $[M-154-32]^+$ (8).

Prasinoxanthin 3'-methyl ether 3-acetate (9). Acetylation of 8 provided 9, $R_f = 0.50$ (20% AH); VIS λ_{max} nm: 449. ^1H NMR (100 MHz): δ 0.85 (3H, *s*, Me-16'), *ca* 0.97 (3H, *s*, Me-17'), 0.97 (3H, *s*, Me-16), 1.08 (3H, *s*, Me-17), 1.63 (3H, *s*, Me-18'), 1.94 (6H, *s*, Me-19, 19'), 2.00 (6H, *s*, Me-20, 20'), 2.02 (3H, *s*, Ac), 2.14 (< 1H, *d*, *J* = 8 Hz, H-6' in 3',6'-*cis*), 2.40 (< 1H, *d*, *J* = 8 Hz, H-6' in 3',6'-*trans*), 2.0–2.7 (*ca* 2H, *m*, CH_2-4), 2.68 (1H, *d*, *J* = 16 Hz, H_a-7), 3.41 (1H, *d*, *J* = 16 Hz, H_b-7), 3.36 (2H, *s*, OMe in 3',6'-*trans*), 3.38 (1H, *s*, OMe in 3',6'-*cis*), *ca* 3.8 (1H, *m*, H-3'), 4.51 (1H, *s*) and 4.92 (1H, *s*, =CH_2), 4.8 (1H, *m*, H-3), 5.43 (1H, *d*, *J* = 16 Hz, H-7'), 5.45 (1H, *s*, H-bonded OH at C-6), 5.55 (1H, *m*, H-4), 6.05–6.80 (*ca* 10H, *m*, conj. olefinic), 7.20 (1H, *d*, *J* = 8 Hz, H-10).

A LIS experiment with $Eu(fod)_3$ was carried out as for the 3'-methyl ether (8) described above. Linear LIS were recorded at 0.1, 0.3, 0.6 and 1.0 mole shift reagent/mole 9. Slightly smaller LIS downfield shifts were recorded for Me-16, 17, the terminal methylene CH_2-7 and H-10 than for the 3'-methyl ether (8). A similar upfield LIS was observed for Me-19. The LIS of the acetate methyl corresponded to those of Me-16, 17.

^{13}C NMR (25 MHz, 0.6 mg only) containing > 35 signals with gross assignments: δ 11.7, 12.7, 12.9 and 13.1 (four in-chain Me), δ 21.4, 22.9, 23.4, 24.2, 24.7, 29.4, 29.7, 33.8, 36.1, 37.5, 39.4, 40.5, 55.1, 55.6, 55.8 (together 15 signals, calc. 12 sp^3 C not bound to O), 70.4, 74.9 and 78.6 (C-3, 6, 3'), 112.0 (=CH_2) [10], 122.0, 123.3, 126.0, 129.3, 130.5, 131.5, 132.8, 135.4, 137.2, 138.5, 140.0, 141.7, 146.0, 147.1 (together 14 signals, calc. 19 other sp^2 C), 170.3 (C=O in Ac); conj. C=O not recorded. Signals associated with the lutein half of the molecule [37] were present: C-1' 33.8, C-2' 39.4, C-3' 74.6, C-4' 125.9, C-5' 137.2, C-6' 55.1, C-7' 129.3, C-8' 137.2, C-9' 135.4, C-10' 130.5, C-11' 123.3, C-12' 137.2, C-14' 132.8, C-15' 130.6, C-16', 17' 24.9, 29.4, C-18' 22.9, C-19' 13.1, C-20' 12.8. MS *m/z* (rel. int.): 638 $[M-18]^+$ (1), 460 $[M-196]^+$ (25), 93 (100), 91 (90).

8-*Dihydroprasinoxanthin 3'-methyl ether* (10). Reduction of 9 with $LiAlH_4$ in dry Et_2O provided 10, $R_f = 0.40$ (30% AH); VIS λ_{max} nm: 394, 417, 444, % III/II = 73; ^1H NMR (100 MHz): δ

(broad signals due to lipid impurity), 0.85 (3H, *s*, Me-16'), 0.93 (6H, *s*, Me-17' and Me-16/17), 1.08 (3H, *s*, Me-17/16), 1.63 (3H, *s*, Me-5'), *ca* 1.89 (*s*, Me-19), *ca* 1.94 (*s*, Me-19'), *ca* 2.0 (*s*, Me-20, 20'), 2.0–2.7 (*m*, CH-3', CH_2-4) 3.38 (3H, *s*, OMe), 3.9 (*m*, H-3, 3'), 4.3 (*m*, H-8), 5.11 (2H, *s*, $=CH_2$), 5.6 (1H, *m*, H-4), 6.1–7.0 (*m*, conj. olefinic).

8-Dihydroprasinoxanthin (**11**). Reduction of **5** with $LiAlH_4$ in dry Et_2O gave **11**; R_f = 0.35 (30% AH); VIS λ_{max} nm: 394, 417, 444, % III/II = 89; MS *m/z* (rel. int.): 602 $[M]^+$ (15), 584 $[M-18]^+$ (5), 446 (15), 91 (100).

8-Dihydroprasinoxanthin 3,8,3'-triacetate (**12**). Prepared by acetylation of **11**, R_f = 0.85 (30% AH); VIS λ_{max} nm: 394, 417, 444; MS *m/z* (rel. int.): 728 $[M]^+$ (2), 686 $[M-42]^+$ (6), 668 $[M-60]^+$ (20), 608 $[M-60-60]^+$ (8), 95 (100), 91 (60).

C_{31} methyl ketone (**14**). Treatment of **5** with 5% KOH in MeOH overnight gave unreacted **5** and product **14** (*ca* 70–80%), with R_f = 0.40 (30% AH); VIS λ_{max} nm: (415), 439, 463; MS *m/z* (rel. int.): 446 $[M]^+$ (70), 428 $[M-18]^+$ (70), 91 (100). ^{1}H NMR (100 MH_3): δ 0.86 (3H, *s*, Me-17), 1.00 (3H, *s*, Me-16), 1.26 (*s*, imp.) 1.63 (3H, *s*, CH_3-18), 1.94 (6H, *s*, Me-19, 19'), 2.00 (6H, *s*, Me-20, 20'), 2.37 (3H, *s*, Ac), 2.40 (1H, *d*, J = *ca* 8 Hz, H-6), 4.25 (1H, *m*, H-3), 5.55 (1H, *m*, H-4), 5.30–6.90 (*ca* 11H, *m*, olefinic), 7.15 (1H, *d*, J = 8 Hz, H-10').

C_{31} *methyl ketone acetate* (**15**). Acetylation of **14** provided **15** with R_f = 0.65 (30% AH); VIS λ_{max} nm: (415), 439, 463; MS *m/z* (rel. int.): 488 $[M]^+$ (100), 446 $[M-42]^+$ (2), 428 $[M-60]^+$ (25), 375.3 [m*, 488 → 428].

C_{31} *diol* (**16**). Reduction of **15** with $LiAlH_4$ in dry Et_2O provided **16**: R_f = 0.34 (30% AH); VIS λ_{max} nm: 393, 416, 444, % III/II = 78; MS *m/z* (rel. int.): 448 $[M]^+$ (40), 430 $[M-18]^+$ (7), 95 (100), 91 (40).

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