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CAROTENOIDS FROM EUCARYOTIC ULTRAPLANKTON CLONES (PRASINOPHYCEAE)*

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Key Word Index—Eucaryotic ultraplankton; Prasinophyceae; carotenoid pattern; prasinoxanthin; dihydroprasinoxanthin epoxide; uriolide; chlorophylls *a* and *b*.

Abstract—Three new eucaryotic ultraplankton clones contained chlorophylls *a* and *b* and carotenoids consisting of β,ε-carotene (1–6% of total), β,β-carotene (5–9%), (3*R*,3′*R*)-zeaxanthin (0–6%) (3*S*,3′*S*)-astaxanthin (0–17%), prasinoxanthin (50–65%, major) and neoxanthin (13–25%). One clone also produced two new minor carotenoids with saturated 7′,8′-bond shown to be 7′,8′-dihydroprasinoxanthin-4′,5′-epoxide (4′,5′-epoxy-3,6,3′-trihydroxy-7,8,4′,5′,7′,8′-hexahydro-γ,ε-caroten-8-one, 4%) and the lactone uriolide (5,6-epoxy-3,3′-dihydroxy-5,6,7′,8′-tetrahydro-β-ε-caroten-11′,19′-olide, 8%) by methods including extensive ^{1}H NMR spin decoupling and mass spectrometry. Chiralities are considered using CD and ^{1}H NMR. The pigment distribution pattern suggests a close relationship to certain members of the class Prasinophyceae.

INTRODUCTION

Recently we have elucidated the structure of prasinoxanthin (**1**, Scheme 1), the major carotenoid of a new coccoid marine ultraplankton clone Ω 48–23. The affinity of this green microalga with certain species in the class Prasinophyceae, and the use of prasinoxanthin (**1**) with its special structural features connected with ring A as a taxonomic marker were discussed [1].

We now report a pigment study involving three additional small coccoid non-flagellated marine algae that closely resemble clone Ω 48–23.

RESULTS AND DISCUSSION

Each clone contained chlorophylls *a* and *b*, demonstrating their affinity to the Chlorophyta [2]. In each case prasinoxanthin (**1**) was the major carotenoid (Table 1). In addition other β- and ε-derived carotenoids were encountered. These included β,ε-carotene (**2**) and β,β-carotene (**3**). (3*R*,3′*R*)-Zeaxanthin (**4**) and (3*S*,3′*S*)-astaxanthin (**5**) were isolated from clones BT-5 and 1326-1. The chiralities were determined by the carbamate [3] and camphanate [4] methods respectively. The allenic neoxanthin (**6**) was present in all clones.

Clone URI 266 G in addition produced two new, minor carotenoids, here shown to represent a dihydroprasinoxanthin epoxide **7** and the butenolide **8**.

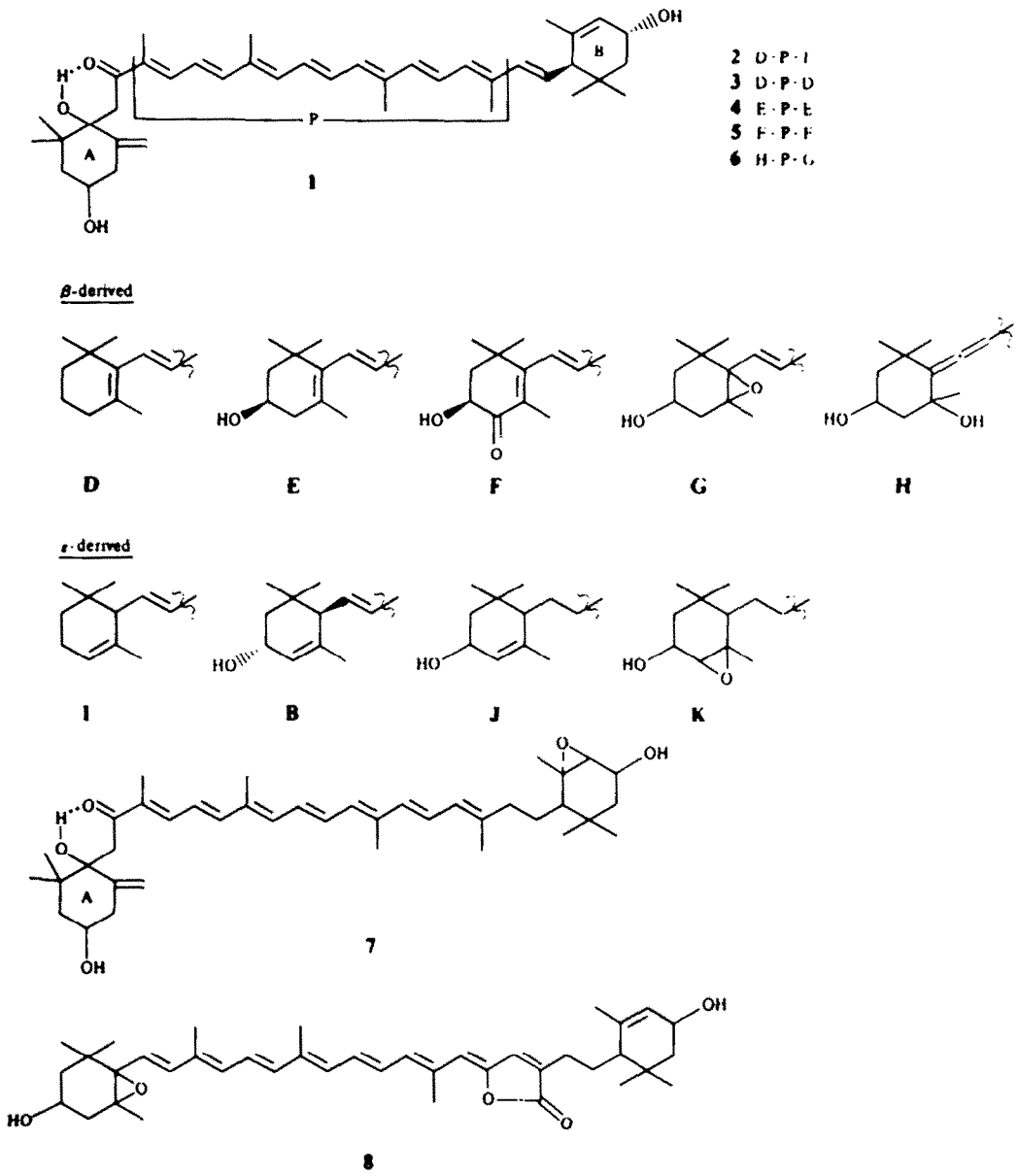
The naturally occurring prasinoxanthin derivatives **7** was more strongly adsorbed and had a shorter chromophore than prasinoxanthin (**1**). The molecular ion of natural **7** at *m/z* 618 was compatible with a molecular formula $C_{40}H_{58}O_5$. The ^{1}H NMR spectrum revealed the presence of the characteristic terminus *A*, (**1**, Schemes 1 and 2) of prasinoxanthin (**1**), confirmed by base catalysed retro aldol cleavage to the C_{31} methyl ketone **9** (Scheme 2); cf. previous results for **1** [1].

Natural **7** formed a diacetate **7a**, and from the ^{1}H NMR spectrum of **7** it was inferred that the fourth oxygen function, connected with the second terminus, was a secondary hydroxy group. ^{1}H NMR spectra of natural **7** and the C_{31}-methyl ketone **9** with a characteristic high-field methyl singlet at δ0.75 and a one-proton broad singlet at δ3.08, suggested that the final oxygen function represented a 4,5-epoxide of a formally epoxidized ε-ring. This was supported by literature data of a relevant 4,5-epoxy-ε-type model [5]. Allocation of the secondary hydroxy group in the unknown terminus to 3′-position appeared biogenetically plausible and was confirmed by spin decoupling.

Lithium aluminium hydride reduction of natural **7** and of the methyl ketone **9** caused hypsochromic shifts and formation of the products **10** and **11** respectively, both with aliphatic heptaene chromophores. Termination of the chromophore at C-9′ was consistent with the characteristic end-of-chain methyl signal (δ1.85) of Me-19′ for **7** and Me-19 for **9**. These data, together with the molecular formula $C_{40}H_{58}O_5$, compatible with the mass spectral data for natural **7**, reveal the presence of a saturated C-7′–C-8′ bond, consistent with ^{1}H NMR spectral data for **7** and the methyl ketone **9**. Natural **7** is thus 4′,5′,7′,8′-tetrahydroprasinoxanthin-4′,5′-epoxide.

One reaction deserves comment. Silylation of the acetylated C_{31} methyl ketone **9a** afforded a less polar product with visible light absorption compatible with an aliphatic octaene chromophore and considered to be the trimethylsilyl enol ether **12**. Upon electron impact mass spectrometry no molecular ion was observed for **12**, and the $[M-72]^+$ ion, rationalized in Scheme 2, represented the base peak. Weak acid treatment of the enol ether **12**

*Part 34 in the series "Algal Carotenoids". For Part 33 see *Biochem. Syst. Ecol.* **13**, 215.



Scheme 1.

caused reversion to the parent methyl ketone **9a**. Similar results were here observed upon silylation of the corresponding C_{31}-ketone obtained from prasinoxanthin (**1**).

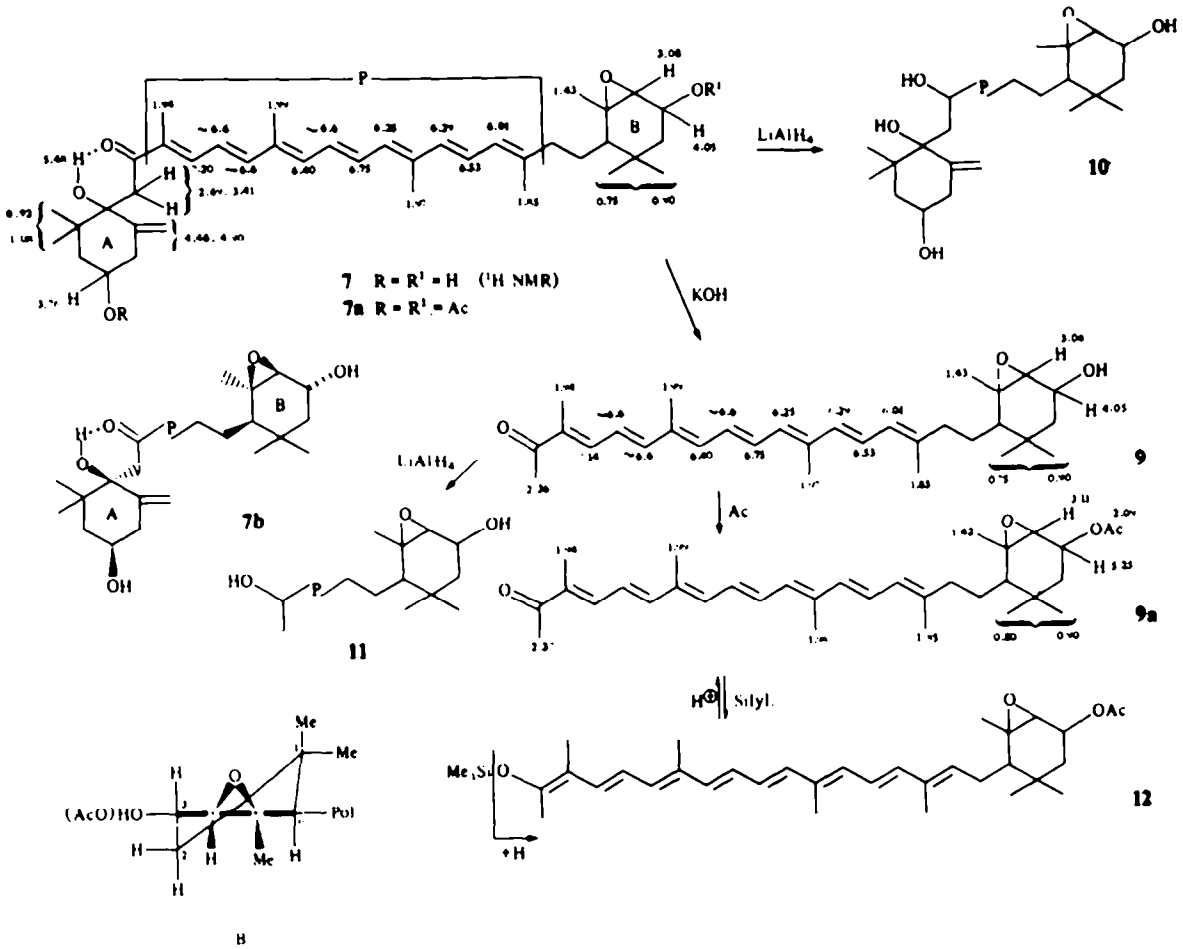
Concerning the configuration of its six chiral centres CD spectra of natural **7** and the C_{31} methyl ketone **9** were inconsistent and uninformative. The low coupling constant $J_{3',4'} = 1$ Hz for natural **7** suggested that H-3' and H-4' were *trans* and hence that the hydroxy and epoxy groups had the *trans*-configuration (Scheme 2). The coupling constants observed for H-3', 4' in **7** and H-3, 4 in **9** and **9a** and for H-2, 3 in the acetate **9a** are compatible with the conformation of ring B shown in Scheme 2. Common natural carotenoid 5,6-epoxides are generally *trans* to the C-3 hydroxy function [6], whereas 5,6-epoxides of caroten-2-ols have been considered to be *cis* [7].

Biogenetically the same chirality at C-3, 6, 3', 6' would be expected for prasinoxanthin (**1**) [1], recently shown to have the 3,6-*cis* diol configuration [8], and the natural epoxide **7**. The (3*S*,6*R*,3'*R*,4'*S*,5'*R*,6'*R*)-configuration for the epoxide **7b** may tentatively be postulated, taking the ^{1}H NMR data into account.

The second new carotenoid was assigned the butenolide structure **8**. Its molecular ion at m/z 614.3983 revealed the molecular formula $C_{40}H_{54}O_5$. Two of the oxygen functions, were, as judged by the ^{1}H NMR spectrum, secondary hydroxy groups, which was confirmed by formation of a diacetate (**8a**, Scheme 3). One of these hydroxy functions was allylic, as demonstrated by allylic methylation in acidified methanol with concomitant furanoid rearrangement to the methyl ether **13a**. The non-methylated **13**

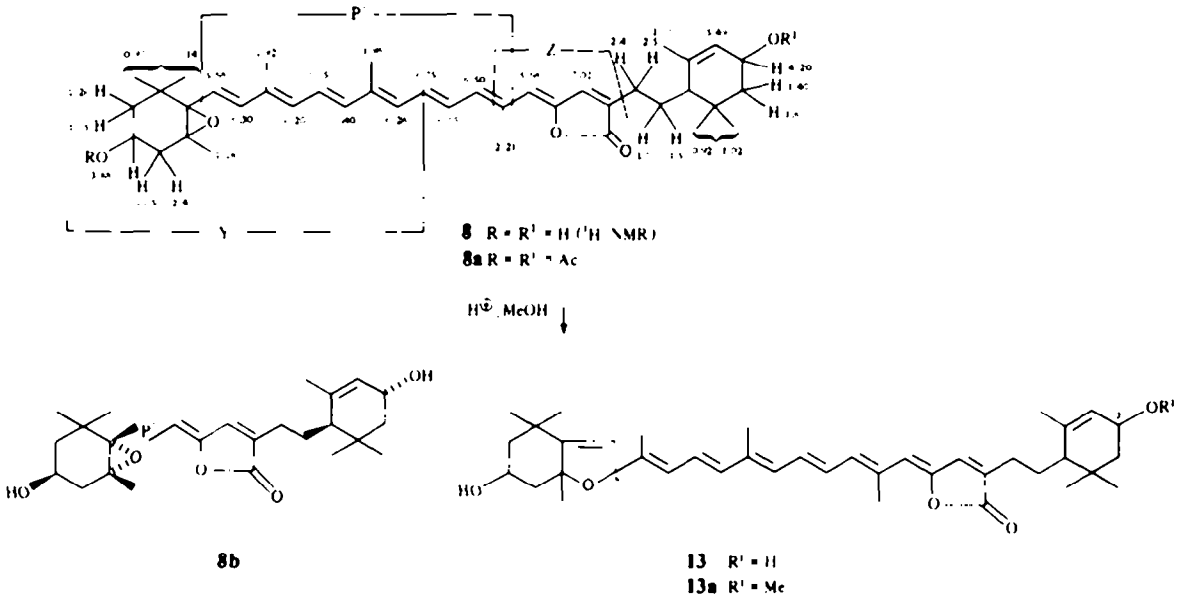
Table 1. Pigment composition of four coccoid marine microalgae

Pigment	% of total carotenoid			
	Ω 48–23 [1]	BT-5	1326.1	URI 266 G
β,ε-Carotene (2)	1	1	1	6
β,β-Carotene (3)	3	5	9	7
(3*R*,3'*R*)-Zeaxanthin (4)	2	6	3	–
(3*S*,3'*S*)-Astaxanthin (5)	—	17	6	- -
Prasinoxanthin (1)	69	58	65	50
Neoxanthin (6)	21	13	16	25
Unknown 1 + 2	2 + 2	—	—	—
Dihydroprasinoxanthin epoxide (7)	—	—	—	4
Uriolide (8)	—	—	—	8
Total carotenoid in mg/g dry wt	3.9	4.4	2.9	0.6
Chlorophyll *a*	+	+	+	+
Chlorophyll *b*	+	+	+	+



Scheme 2.

and methylated (13a) products exhibited hypsochromically shifted VIS spectra and diagnostically important fragment ions upon electron impact mass spectrometry for the hydroxylated furanoid end group; [M − 80], *m/z* 221 and 181 [9]. Product 13 was considered as a mixture of C-8 epimers, and product 13a as an unseparated mixture of four C-8 and C-3' epimers, according to the accepted reaction mechanism for their formation [10, 11]. Prasinoxanthin (1) is known to give a mixture of C-3' methyl ethers, non-separable in the same TLC system [1].



Scheme 3.

The two final oxygen functions in natural **8** were shown to be present in a butenolide moiety by ^{1}H NMR in comparison with peridinin [12]. ^{1}H NMR assignments are included in Scheme 3. A characteristic methyl signal at δ2.21 and two one-proton singlets at δ7.01 and 5.68 (δ2.23, 7.04 and 5.74 respectively for peridinin [12]) clearly defined the partial structure Z (Scheme 3) in natural **8**. Similar solvent shifts with pronounced spectral fine-structure in hexane were noted for both peridinin [12] and natural **8**, compatible with the conjugated butenolide moiety. Lithium aluminium hydride or sodium borohydride reduction of the butenolide **8** gave no defined coloured products.

Comparative ^{1}H NMR spectroscopy of violaxanthin (2 × Y, R = H, Scheme 3) [13] and of the butenolide **8** required allocation of the butenolide moiety Z to C-11'/C-19' rather than to the C-11/C-19 position, further confirmed by assignment of each individual olefinic proton by spin decoupling experiments.

Allocation of the allylic hydroxy group to C-3' and of the non-allylic one to C-3 followed from complete assignment of all ring protons using spin decoupling and was consistent with the mass spectrometric fragmentations of **8**, **8a**, **13** and **13a**.

Consideration of the molecular formula of natural **8**, requiring 14 double bond equivalences, and the conjugated heptaene–butenolide chromophore evident from the VIS spectrum, showed that the C-7'/C-8' bond had to be saturated as for the natural prasinoxanthin derivative **7** above. Eventually the predicted spin system for H-7'a, 7'b, H-8'a, 8'b was identified by decoupling experiments. Except for the allylic H-6' methine proton, expected in the crowded δ2.0 region all protons in the 400 MHz ^{1}H NMR spectrum of the butenolide **8** were assigned.

We propose the trivial name uriolide (**8**) for this new carotenoid butenolide isolated from clone URI266 G. Hitherto carotenoid lactones have been restricted to Dinophyceae [14]. Uriolide possesses five chiral centres. Its ^{1}H NMR spectrum in comparison with known carotenoid 3-hydroxy-5,6-epoxides [15] reveal the common *trans* relationship between the hydroxy and epoxide groups. The CD spectrum of **8** was obtained. However, no meaningful CD correlation could be made with related epoxidic carotenoids such as violaxanthin (2 × Y, R = H, Scheme 3) [16]. Biogenetically uriolide may be tentatively postulated to have the (3*S*,5*R*,6*S*,3'*R*,6'*R*)-configuration (**8b**).

The two new carotenoids **7** and **8** described here from clone URI 266 G offer complex structural features combined with the saturated C-7'/C-8' bond, which is commonly desaturated at an early biosynthetic stage.

In conclusion the pigment distribution patterns of the three coccoid ultraplankton clones studied here reveal a close chemosystematic relationship to that of clone Ω48-23 examined previously [1]. As compiled in Table 1 the carotenoid distribution pattern is characterized by a high proportion of carotenoids with peculiar γ-type end groups (27–35% of total end groups) and with ε-type end groups (30–35%), and the presence of carotenoids with epoxidic (7–15%) and allenic (7–13%) end groups.

The pigment distribution pattern of these coccoid, non-flagellated marine ultraplankters with prasinoxanthin (**1**) as the dominant carotenoid strongly suggests their affinity to some other species having a particular complement of pigments. These other algae are presently assigned to the Prasinophyceae [15].

EXPERIMENTAL

Biological material. Clone BT-5 was isolated from a surface sample taken at 32°10'N, 64°30'W on 20 April 1960 and rendered axenic by R.R.L.G. Clone 1326-1 originated from a surface sample at 25°25'N, 87°00'W, taken by L.E. Brand on 24 April 1980. Isolation into axenic culture was by L. Provasoli. Clone 266 G was derived from a 60 m sample taken at 18°57'N, 80°45'W on 5 March 1979 by P. W. Johnson, of the University of Rhode Island. The culture developed in one tube of medium 'f/20', and, though never formally cloned, has shown no evidence of algal contamination in electron microscope studies [P. W. Johnson,

pers. comm.], or in microscopic examination of mass cultures or stock cultures at the Bigelow Laboratory.

All cultures were grown and harvested as described before [1]. Clone 266 G is somewhat more sensitive to bright light than are the others (including Ω48-23, studied before [1]). Dilute cultures were therefore kept under about half the light supplied to the others. Cells of this clone attained a maximum diameter (*ca* 5 μm) a little larger than that of the other clones; all are otherwise very similar to Ω48-23 [1]. An electron microscope study of Ω48-23 by C. O'Kelly, G. Floyd, M. Keller and R. R. L. Guillard is to be published elsewhere; it shows the ultrastructure to be compatible with a position in the Prasinophyceae. All clones used are in the Culture Collection of Marine Phytoplankton at the Bigelow Laboratory. Clone 266G is coded as URI 266 G.

Pigment isolation. General methods were used [1].

Chlorophylls. The previous analytical procedure [1] was employed for the identification of chlorophylls *a* and *b*.

Carotenoids. Total carotenoid was spectrophotometrically determined and individual carotenoids determined after TLC, see Table 1. Chromatographic, spectrometric and chemical methods were as specified elsewhere [1]. VIS spectra were recorded in Me_2CO, using $E^{1\%}_{1cm} = 2500$ at λ_{max}. R_f-values refer to TLC (silica gel), eluent Me_2CO in hexane (AH) if not otherwise specified. Individual natural carotenoids and their derivatives are described below.

Prasinoxanthin (**1**). $R_f = 0.46$ (40% AH) as previously characterized [1]. Prasinoxanthin (**1**) was converted to the C_{31} methyl ketone micronone as previously described [1]. Standard silylation of the methyl ketone (0.1 mg) overnight gave the unreacted methyl ketone and two less polar products in a *ca* 1:1:1 ratio. The least polar product showed a 7 nm hypsochromic shift and was considered as the enolic ditrimethylsilyl ether, followed by the 3-mono trimethylsilyl ether of unchanged VIS spectrum.

β,ε-Carotene (**2**). $R_f = 0.98$ (30% AH), $R_f = 0.81$ (5% AH), special plates [17], inseparable from authentic **2**; VIS λ_{max} nm (420), 442, 469, % III/II [18] = 55; CD (EPA) inconclusive (impure).

β,β-Carotene (**3**). $R_f = 0.98$ (30% AH), $R_f = 0.62$ (5% AH) special plates [17], inseparable from synthetic **3**; VIS λ_{max} nm (424), 450, 475; MS *m/z* (rel. int.): 536 $[M]^+$ (20), 444 $[M-92]^+$ (12), 430 $[M-106]^+$ (1), 382 (100).

(3R,3'R)-Zeaxanthin (**4**). $R_f = 0.42$ (30% AH), inseparable from authentic **4**; 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). A 100% optical purity was demonstrated by the carbamate method [3].

(3S,3'S)-Astaxanthin (**5**). $R_f = 0.44$ (30% AH), inseparable from authentic **5**; VIS λ_{max} nm 473. A 100% optical purity was demonstrated by the camphanate method [4]. Upon acetylation **5** diacetate was produced: $R_f = 0.25$ (20% AH), VIS λ_{max} nm 473; MS *m/z* (rel. int.): 680 $[M]^+$ (10), 620 $[M-60]^+$ (8), 578 $[M-60-42]^+$ (5), 560 $[M-60-60]^+$ (10), 91 (100).

Neoxanthin (**6**). $R_f = 0.15$ (30% AH), inseparable from authentic **6**; VIS λ_{max} nm 414, 436, 463; MS *m/z* (rel. int.): 600 $[M]^+$, 582 $[M-18]^+$ (10), 520 $[M-80]^+$ (5), 508 $[M-92]^+$ (3), 221 (50), 91 (100).

Standard acid treatment of **6** caused a hypsochromic colour shift with the formation of neochrome; $R_f = 0.40$ (40% AH), inseparable from an authentic sample; VIS λ_{max} nm 397, 420, 447, % III/II = 78.

Dihydroprasinoxanthin epoxide (**7**). Natural **7**, available in total 0.7 mg; $R_f = 0.40$ (40% AH); VIS λ_{max} nm: 430, 448; ^{1}H NMR (400 MHz): δ0.75 (3H, *s*, Me-16'/17'), 0.90 (3H, *s*, Me-16'/17'), 0.92 (3H, *s*, Me-16/17), 1.08 (3H, *s*, Me-16/17), 1.43 (3H, *s*, Me-18'), 1.85 (3H, *s*, Me-19'), 1.94 (3H, *s*, Me-19), 1.97 (3H, *s*, Me-20'), 1.99 (3H, *s*, Me-20), 2.69 (1H, *d*, $J = 17$ Hz, H_a-7), 3.08 (1H, *br s*, H-4'), 3.41 (1H, *d*, $J = 17$ Hz, H_b-7), 3.76 (1H, *m*, H-3), 4.05 (1H, *m*, H-3'), 4.46 (1H, *s*) and 4.90 (1H, *s*), (=CH_2), 5.48 (1H, *s*, H-bonded OH at C-6), 6.01 (1H, *d*, $J = 11$ Hz, H-10'), 6.15 (1H, *d*, $J = 12$ Hz, H-14'), 6.29 (1H, *dd*, $J_1 = 15$ Hz, $J_2 = 11$ Hz, H-12'), 6.40 (1H, *d*, $J = 11$ Hz, H-14), 6.53 (1H, *m*, H-11'), *ca* 6.6 (3H, *m*, H-11, H-12, H-13), 6.75 (1H, *dd*, $J_1 = 15$ Hz, $J_2 = 12$ Hz, H-15'), 7.20 (1H, *d*, $J = 11$ Hz, H-10). MS *m/z* (rel. int.): 618 $[M]^+$ (0.2), 600 $[M-18]^+$ (1), 582 $[M-18-18]^+$ (0.5), 464.3298 (calc. 464.3291 for $C_{31}H_{44}O_3$) $[M-154]^+$ (100), 448 $[M-154-16]$ (1), 446 $[M-154-18]^+$ (1). Treatment of **7** with 0.03 N HCl in Et_2O caused no change in the VIS spectrum.

Dihydroprasinoxanthin epoxide diacetate (**7a**). Upon standard acetylation of **7** (0.1 mg), monitored by TLC, one intermediary acetate was observed and the final diacetate was formed in quantitative yield during 3 hr. The diacetate **7a** had $R_f = 0.36$ (30% AH); VIS λ_{max} nm: 430 (445); MS *m/z* (rel. int.): 702 $[M]^+$ (5), 684 $[M-18]^+$ (15), 506 $[M-196]^+$ (100).

C_{31} *methyl ketone* **9**. Treatment of **7** (0.3 mg) with 5% KOH in MeOH overnight provided **9** in quantitative yield. Product **9** had $R_f = 0.58$ (40% AH), 0.48 (30% AH); VIS λ_{max} nm: (400), 423, 442; ^{1}H NMR (400 MHz): δ0.75 (3H, *s*, Me-16/17), 0.90 (3H, *s*, Me-16/17), 1.43 (3H, *s*, Me-18), 1.85 (3H, *s*, Me-19), 1.94 (3H, *s*, Me-19'), 1.97 (3H, *s*, Me-20), 1.99 (3H, *s*, Me-20'), 2.36 (3H, *s*, Me-7'), 3.08 (1H, *br s*, H-4), 4.05 (1H, *m*, H-3), 6.01 (1H, *d*, $J = 11$ Hz, H-10), 6.25 (1H, *d*, $J = 12$ Hz, H-14), 6.29 (1H, *d*, $J = 15$ Hz), H-12), 6.40 (1H, *d*, $J = 11$ Hz, H-14'), 6.53 (1H, *dd*, $J_1 = 15$ Hz, $J_2 = 11$ Hz, H-11), *ca* 6.6 (3H, *m*, H-11', H-12', H-15'), 6.75 (1H, *dd*, $J_1 = 15$ Hz, $J_2 = 12$ Hz, H-15), 7.14 (1H, *d*, $J = 11$ Hz, H-10'). All spin couplings were established by appropriate decoupling experiments. MS *m/z* (rel. int.): 464 $[M]^+$ (100), 446 $[M-18]^+$ (5).

C_{31} *methyl ketone acetate* **9a**. Acetylation of **9** (0.2 mg) provided the acetate **9a**, $R_f = 0.58$ (30% AH); VIS λ_{max} nm: 423, (441); ^{1}H NMR (400 MHz): δ0.80 (3H, *s*, Me-16/17), 0.90 (3H, *s*, Me-16/17), 1.42 (3H, *s*, Me-18), 1.85 (3H, *s*, Me-19), 1.94 (3H, *s*, Me-19'), 1.98 (3H, *s*, Me-20), 1.99 (3H, *s*, Me-20'), 2.09 (3H, *s*, Ac), 2.37 (3H, *s*, Me-7'), 3.11 (1H, *br s*, H-4), 5.25 (1H, *m*, H-3). The δ6.0–7.2 olefinic region was similar to that of **9**. Irradiation at δ3.11 caused a collapse of the δ5.25 multiplet into a double doublet with $J_{2,3}$ ax, ax = 12 Hz and $J_{2,3}$ ax, eq = 6 Hz.

C_{40}-*tetrol* **10**. Reduction of natural **7** (0.05 mg) with $LiAlH_4$ in dry Et_2O gave **10**; $R_f = 0.25$ (40% AH); VIS λ_{max} nm 376, 397, 423, % III/II = 72.

C_{31} *diol* **11**. Reduction of the C_{31} methyl ketone **9a** (0.1 mg) with $LiAlH_4$ in dry Et_2O provided **11**, $R_f = 0.40$ (30% AH); VIS λ_{max} nm: 376, 398, 422, % III/II = 91; MS *m/z* (rel. int.): 466 $[M]^+$ (100), 450 $[M-16]^+$ (15), 448 $[M-18]^+$ (30).

C_{31} *methyl ketone acetate trimethylsilyl enol ether* (**12**). Standard silylation of **9a** (0.1 mg) overnight gave unreacted **9a** (50% of total) and the less polar product **12**, $R_f = 0.65$ (30% AH); VIS λ_{max} nm: (375) 392, 414, 441, % III/II = 92; MS *m/z* (rel. int.): 506 $[M-72]^+$ (100), 446 $[M-72-60]^+$ (3). Addition of acid (0.03 N HCl) caused a reversion of **12** to **9a**.

Uriolide (**8**). Natural **8**, available in total 1.5 mg; $R_f = 0.42$ (40% AH), more strongly adsorbed than prasinoxanthin (**1**). VIS λ_{max} nm (Me_2CO): 448, 472, % III/II = 11; (MeOH): 448, 470, % III/II = 2; (hexane): (427) 448, 478, % III/II = 38. ^{1}H NMR (400 MHz): δ0.92 (3H, *s*, Me-16'/17'), 0.97 (3H, *s*, Me-16/17), 1.02 (3H, *s*, Me-16', 17'), 1.14 (3H, *s*, Me-16/17), 1.18 (3H, *s*, Me-18), 1.26 (1H, *m*, H-2), 1.40 (1H, *dd*, $J_{gem} = 15$ Hz, $J_{vic} = 6$ Hz, H-2'), *ca* 1.5 (1H, *m*, H-7'), 1.65 (2H, *m*, H-2, H-4), *ca* 1.7 (1H, *m*, H-7'), 1.77 (3H, *s*, Me-18'), *ca* 1.8 (1H, *dd*, $J_{gem} = 15$ Hz, $J_{vic} = ca\ 7$ Hz, H-2'), 1.92 (3H, *s*, Me-19), 1.98 (3H, *s*, Me-20), 2.21 (3H, *s*, Me-20'), *ca* 2.4 (2H, *m*, H-4, H-8'), 2.50 (1H, *m*, H-8'), 3.88 (1H, *m*, H-3), 4.20 (1H, *m*, H-3'), 5.49 (1H, *br s*, H-4'), 5.68 (1H, *s*, H-12'), 5.88 (1H, *d*, $J = 16$ Hz, H-7), 6.20 (1H, *d*, $J = 12$ Hz, H-

10), 6.28 (1H, *d*, J = 11 Hz, H-14), 6.30 (1H, *d*, J = 16 Hz, H-8), 6.40 (1H, *d*, J = 15 Hz, H-12), 6.50 (1H, *d*, J = 11 Hz, H-14'), *ca* 6.65 (2H, *m*, H-11, H-15'), 6.75 (1H, *dd*, J_1 = 11 Hz, J_2 = 14 Hz, H-15), 7.01 (1H, *s*, H-10'). All spin couplings were established by appropriate decoupling experiments. MS *m/z* (rel. int.): 614.3983 (calc. 614.3971 for $C_{40}H_{54}O_5$) $[M]^+$ (30), 596 $[M-18]^+$ (50), 534 $[M-80]^+$ (25), 522 $[M-92]^+$ (35), 516 $[M-80-18]^+$ (25), 504 $[M-92-18]^+$ (15), 221 (100). CD (EPA) nm ($\Delta\varepsilon$) 215 (0), 225 (−5), 240 (0), 250 (+1), 260 (0), 267 (−2), 278 (0), 315 (−2.5), 340 (0).

Uriolide diacetate (**8a**). Standard acetylation of natural **8** (0.2 mg) provided the diacetate **8a**, R_f = 0.46 (30% AH), VIS λ_{max} nm 447, 474; MS *m/z* (rel. int.): 698 $[M]^+$ (15), 638 $[M-60]^+$ (100), 606 $[M-92]$ (15), 558 $[M-60-80]^+$ (60), 263 (70), 223 (40).

Furanoid rearranged uriolide (**13**). Standard treatment of natural **8** (0.3 mg) with 0.03 N HCl in MeOH, monitored by TLC, resulted in formation of **13** and **13a** in ratio 1:1. Compound **13** had R_f = 0.46 (40% AH); VIS λ_{max} nm: (405), 427 (455); MS *m/z* (rel. int.): 614 $[M]^+$ (30), 612 $[M-2]^+$ (20), 610 $[M-2-2]^+$ (15), 596 $[M-18]^+$ (100), 534 $[M-80]^+$ (20), 522 $[M-92]^+$ (30), 516 $[M-80-18]^+$ (60), 504 $[M-92-18]^+$ (20), 221 (50), 181 (50).

Furanoid rearranged uriolide 3'-methyl ether (**13a**). Compound **13a**, prepared above, R_f = 0.60 (40% AH); VIS λ_{max} nm: (405), 427, (455); MS *m/z* (rel. int.): 628 $[M]^+$ (50), 596 $[M-32]^+$ (20), 548 $[M-80]^+$ (20), 536 $[M-92]^+$ (25), 221 (100), 181 (100).

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