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# TEN MINOR CAROTENOIDS FROM PRASINOPHYCEAE (CHLOROPHYTA)*†

EINAR SKARSTAD EGELAND and SYNNØVE LIAAEN-JENSEN‡

Organic Chemistry Laboratories, The Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim, Norway

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**Key Word Index**—Prasinophyceae; Chlorophyta; carotenoids; anhydromicromonal; anhydromicromonol; anhydroprasinoxanthin; anhydrouriolide; deepoxyuriolide; dehydrouriolide; dihydrolutein; micromonal; micromonol; preprasinoxanthin.

**Abstract**—The characterization by chromatographic (TLC, HPLC) and spectroscopic (visible and mass spectrometric and, in part, $^1$H NMR, circular dichroism methods) and structural elucidation of 10 minor carotenoids isolated from prasinophytes (*Bathycoccus prasinos*, *Micromonas pusilla*, *Mantoniella squamata* and a clone IIA2) are reported. These $C_{40}$-carotenoids comprise the aldehydes micromonal and anhydromicromonal and the corresponding allylic *prim* carotenols micromonol and anhydromicromonol, and three natural butenolides derived from uriolide, namely anhydrouriolide, deepoxyuriolide and 3′-dehydrouriolide. In addition there are two prasinoxanthin related carotenoids, anhydroprasinoxanthin with a γ-end group and preprasinoxanthin, as well as a dihydrolutein. Several of the new carotenoids have short chromophores with saturated C-7,8 bond. A C-2,3 double bond is another frequent structural feature.

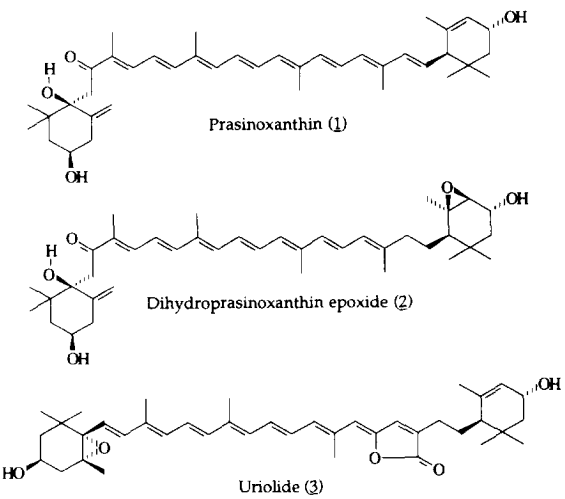
## INTRODUCTION

Structural and analytical studies on carotenoids from all algal classes have been published from our laboratory. In our previous studies on the carotenoids of Prasinophyceae [1–3], structures were assigned to prasinoxanthin (**1**) [1, 3], dihydroprasinoxanthin epoxide (**2**) [2] and uriolide (**3**) [2] with new structural features (Scheme 1).

In the present paper the structural evidence for 10 additional, minor carotenoids isolated from Prasinophyceae species is summarized. A preliminary report has been given [4]. The present paper forms the basis for subsequent chemosystematic considerations.

## RESULTS AND DISCUSSION

The new carotenoids were obtained from the prasinophytes listed in the Experimental section. The quantitative distribution pattern of individual carotenoids in the different species will be published separately. Individual carotenoids (7–650 μg) were available for structural studies on the micro scale.




Scheme 1.

### *Micromonal and derivatives*

The micromonal series represents novel algal carotenoids with an end-of-chain aldehyde or the corresponding *prim* alcohol in the 19′-position (Schemes 2 and 3). The parent carotenal, here named micromonal (3*R*, 3′*R*, 6′*R*)-3,3′-dihydroxy-7′,8′-dihydro-β,ε-caroten-19′-al, **4**, Scheme 2), was characterized by visible (VIS) absorption (Fig. 1), mass spectroscopy, CD, $^1$H NMR including COSY and ROESY, and $^{13}$C NMR–DEPT. Its polarity

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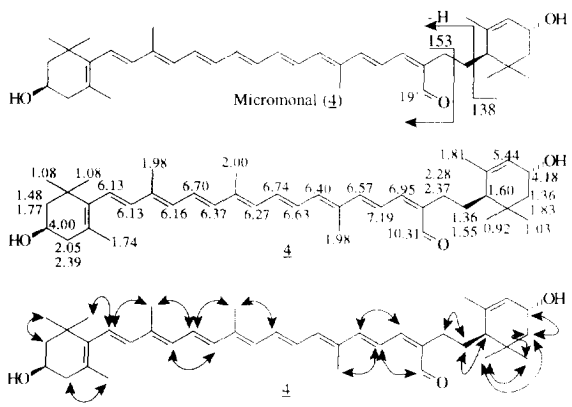
†Part 4 in the series "Carotenoids from Prasinophyceae". Parts 1–3, refs 1–3.

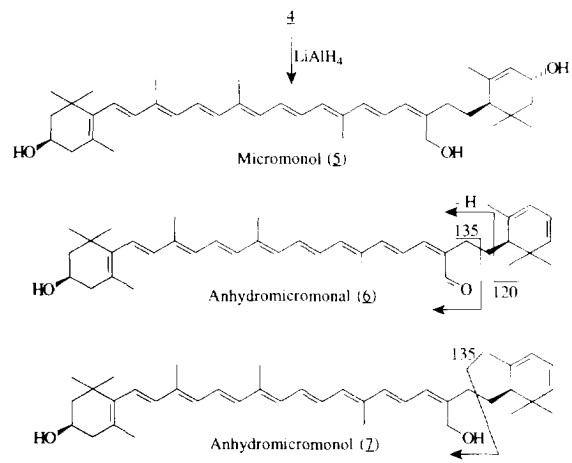
‡Author to whom correspondence should be addressed.

(TLC, HPLC) was compatible with a diol. The round-shaped VIS spectrum suggested a conjugated carbonyl function, demonstrated by $^1$H NMR and DEPT to be an aldehyde function ($^1$H NMR singlet $\delta$10.31, $^{13}$C NMR-DEPT CH=O $\delta$190.1), and $LiAlH_4$-reduction provided micromonol (**5**, Scheme 3) with a monocyclic nonaene chromophore. The mass spectrum of **4** showed characteristic fragment ions (M − 18, M − 18 − 18, M − 92 and M − 106) for a carotenoid diol [5]. The molecular ion was consistent with $C_{40}H_{56}O_3$ and fragment ions at *m/z* 434 (M − 153) and *m/z* 444 (M − 138) were compatible with a hydroxylated 7,8-dihydro-ε-end group (Scheme 2). The constitution was confirmed by $^1$H NMR correlations and ROESY experiments [6]. Chemical shift assignments and in-space NOE interactions are demonstrated in Scheme 2.




Scheme 2.

The *Z*-configuration of the Δ9′-bond of **4** was indicated by the VIS absorption of the $LiAlH_4$-reduced compound **5** ($\lambda_{max}$ and %III/II [7] values) and confirmed by the NOE effects (Scheme 2). A minor $^1$H NMR signal at $\delta$9.41 ppm (10% intensity of $\delta$10.31) was assigned to the corresponding *E*-isomer.




Scheme 3.

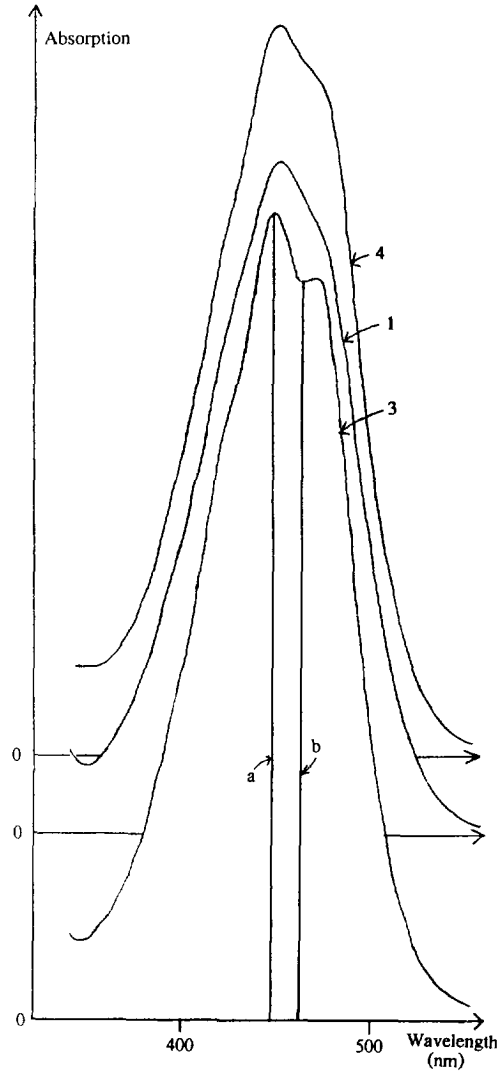
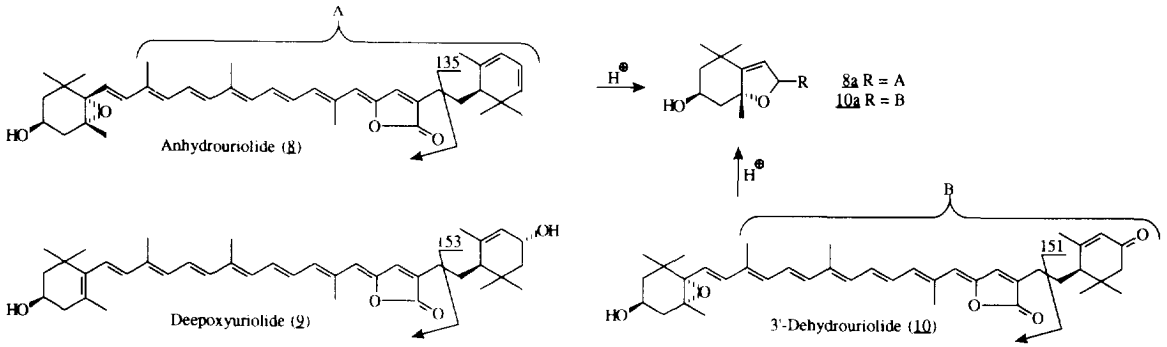



Fig. 1. Absorption spectra in acetone solution in visible light of prasinoxanthin (**1**), uriolide (**3**) and micromonal (**4**), and definition of $D_v = b/a$.

The Cotton effect of **4** resembled that of (3*S*,3′*S*)-β,β-carotene-3,3′-diol, compatible with a mono *cis* isomer of a 3*R* configurated carotenol. It is known that the Cotton effect is inverted for mono *cis* carotenoids with conservative CD spectra [8]. Finally, the chirality at C-3′ and C-6′ rests on biosynthetic analogy as a (3*R*, 3′*R*, 6′*R*)-lutein (**14**, Scheme 6) [9] derivative.

Micromonol [(3*R*, 3′*R*, 6′*R*)-7′,8′-dihydro-β,ε-caroten-3,3′,19′-triol, **5**, Scheme 3] was obtained by $LiAlH_4$ reduction of **4** and was also naturally occurring. The chemical correlation, VIS and mass spectral data were compatible with the structure assigned.

Anhydromicromonal [(3*R*,6′*S*)-3-hydroxy-3′,4′-didehydro-7′,8′-dihydro-β,ε-caroten-19′-al, **6**], less polar than **4**, exhibited the same characteristic VIS absorption as **4**. The mass spectrum showed a molecular ion compatible with $C_{40}H_{58}O_2$ and diagnostic fragment ions (M − 120 and M − 135) assigned to cleavage of the C-6′,7′ and the C-7′,8′-bond, *cf.* **4** and **7**, in the anhydro end group.




Scheme 4.

Anhydromicromonol [(3*R*,6'*S*)-3',4'-didehydro-7',8'-dihydro-$\beta,\varepsilon$-caroten-3,19'-diol, **7**] possessed the same chromophore as **5** according to the VIS absorption, but was less polar. The mass spectrum, including a fragment ion at *m/z* 433 (C-7',8'-cleavage), was compatible with the structure assigned.

*Natural uriolide derivatives*

Uriolide (**3**, Scheme 1) [2] represents, besides peridinin and pyrrhoxanthin derivatives [9], rare carotenoid butenolides. Three additional minor butenolides were encountered.

Anhydrouriolide [(3*S*,5*R*,6*S*,6'*S*)-5,6-epoxy-3-hydroxy-3',4'-didehydro-5,6,7',8'-tetrahydro-$\beta,\varepsilon$-caroten-11',19'-olide, **8**, Scheme 4] had the same chromophore as **3** [2], Fig. 1, but was less polar. Facile furanoid rearrangement provided the furanoxides **8a**. Both **8** and **8a** underwent C-7',8'-cleavage upon electron impact, and the hydroxylated epoxidic/furanoxidic end group showed characteristic fragmentation (M − 18, M − 80, *m/z* 221, *m/z* 181) [5]. The suggested chirality rests on biosynthetic analogy only.

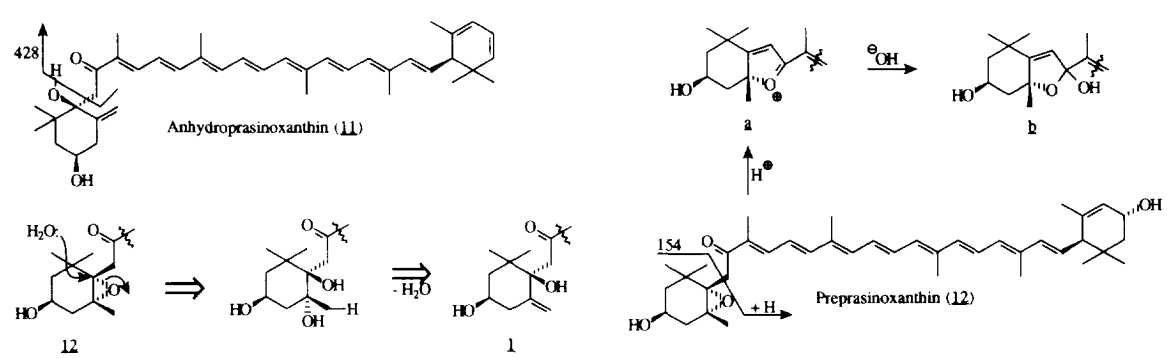
Deepoxyuriolide [(3*R*,3'*R*,6'*R*)-3,3-dihydroxy-7',8'-dihydro-$\beta,\varepsilon$-caroten-11',19'-olide, **9**, Scheme 4] exhibited the same characteristic VIS absorption as **3** [2]. Lacking furanoid rearrangement upon standard acid treatment, mass spectral data (M, M − 18, M − 18 − 18, M − 135 and no epoxide fragment ions) were compatible with the structure assigned. Tentative chirality is given.

Finally, 3'-dehydrouriolide [(3*S*,5*R*,6*S*,6'*R*)-5,6-epoxy-3-hydroxy-3'-oxo-5,6,7',8'-tetrahydro-$\beta,\varepsilon$-caroten-11',19'-olide, **10**, Scheme 4] had the predicted VIS absorption and mass spectrometric fragmentation in support of the butenolide (M − 44, $CO_2$), the hydroxylated epoxidic end group (M − 18, M − 80, *m/z* 221, *m/z* 181) and the second end group (M—151, corresponding to 7',8'-cleavage).

*Natural prasinoxanthin derivatives*

Prasinoxanthin (**1**) [1, 3] and **2** [2] exhibit a unique $\gamma$-type end group. A new minor carotenoid, less polar than **1**, exhibited the same characteristic VIS absorption (Fig. 1) and mass spectral data compatible with the anhydroprasinoxanthin [(3*S*,6*R*,6'*S*)-3,6-dihydroxy-2',3'-didehydro-7,8-dihydro-$\gamma,\varepsilon$-caroten-8-one, **11**, Scheme 5] structure. The molecular ion corresponding to $C_{40}H_{54}O_3$ and M − 18 ($H_2O$) and a strong *m/z* 428 ion (McLafferty rearrangement of the ketone) were compatible with the anhydro structure **11**. Chiralities are suggested by analogy with **1** [1, 3] and biosynthetic analogy (C-3).

Previously the unique end group of **1** was suspected to be formed biosynthetically from a hypothetical precursor [1] as cited in Scheme 5 [3, 10]. This carotenoid, here named preprasinoxanthin [(3*S*,5*R*,6*S*,3'*R*,6'*R*)-5,6-epoxy-3,3'-dihydroxy-5,6-dihydro-$\beta,\varepsilon$-caroten-8-one, **12**] has indeed now been isolated in reasonable amount. The VIS absorption was similar to that of **1**. The mass spectrometric data supported a $C_{40}H_{56}O_4$ diol structure and




Scheme 5.

the conjugated keto function and epoxy group were compatible with the $^1$H NMR data (Scheme 6). Assignments were made in comparison with data for fucoxanthin [11] and **1**. In the present work, all olefinic protons of **1** could be fully assigned by 2D $^1$H$^1$H NMR correlations, see Scheme 6 [3]. Confirmation for the rare 5,6-epoxy-8-keto end group in **12** was obtained by acid treatment, providing a blue product **a** which was converted into a yellow octaene **b** after the addition of base. This reaction sequence has been rationalized for fucoxanthin as oxonium ion and ketal formation, respectively [12].

*New lutein derivate*

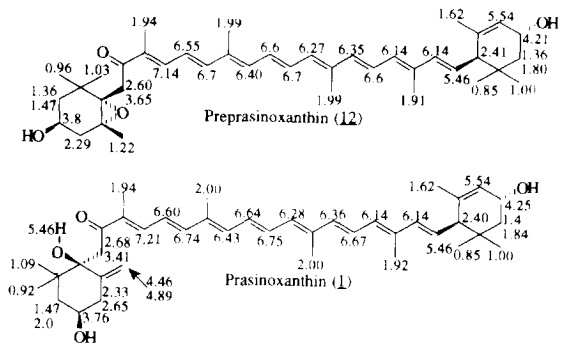
A minor carotenoid from the diol fraction exhibited VIS absorption spectrum ($\lambda_{max}$ and spectral fine structure) typical of a monocyclic nonaene chromophore and mass spectral data consistent with a $C_{40}H_{58}O_2$ diol. Due to co-occurrence with **4**, the same chirality is assumed at the three chiral centres. Hence, the structure (3*R*,3′*R*, 6′*R*)-7′,8′-dihydrolutein (**13**), Scheme 7, is tentatively assigned.

*Future identifications*

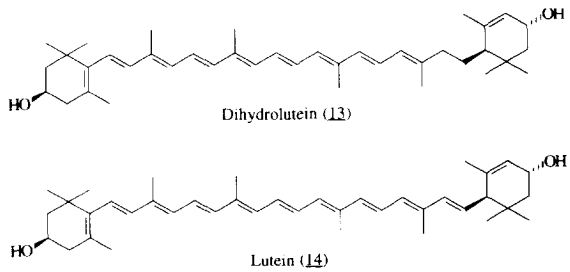
As an aid for future identification, the characteristic VIS absorption spectra of carotenoids with micromonal chromophore (**4**, **6**), uriolide chromophore (**3**, **8**, **10**) and prasinoxanthin chromophore (**1**, **11**) are depicted in Fig. 1. As for peridinin [13] the butenolides of uriolide (**3**, **8**, **10**) type exhibit improved spectral fine structure in hexane [2]. The prasinoxanthin and micromonal chromophores display very similar visible absorption in acetone. Also, in hexane no spectral fine structure is observed. However, a more pronounced long-wavelength shoulder is seen in the slightly more bathochromically shifted VIS spectrum of **1** relative to **4**.

The relative polarities of carotenoids **1**–**14** are described in Table 1.

The minimum identification criteria for a carotenoid include VIS absorption spectrum, co-chromatography in two different systems and mass spectrum [14]. The distribution pattern of carotenoids in Prasinophyceae is complex and identifications not including MS are not recommended, *cf.* recent HPLC studies [15–17].




Scheme 6.




Scheme 7.

## EXPERIMENTAL

*Biological sources.* The carotenoids characterized here were obtained from the following prasinophytes: *Bathycoccus prasinos* (isolated from the Gulf of Naples, 1986) (Department of Biology, Marine Botany, University of Oslo, Norway): **4**, **10** and **13**. *Micromonas pusilla* (isolated from the Gulf of Naples, 1987) (above Department): **4**, **5** and **8**. *Mantoniella squamata* (isolated from the Oslofjord) (above Department): **4**, **6**, **7**, **8**, **11** and **13**. Clone IIA2 (Bigelow Laboratory for Ocean Science, Maine, USA): **8**, **9**, **11**, **12** and **13**.

Details regarding the cultivation of the prasinophytes and the isolation of individual carotenoids will be published separately for *B. prasinos* [18], *M. pusilla* and *M. squamata* [19] and Clone IIA2 (E. S. Egeland *et al.*, to be published).

*General methods.* These were as generally employed in our laboratory [1, 20]. The individual carotenoids were isolated after extraction with $Me_2CO$–MeOH (7:3) and repeated chromatography by TLC (several systems). Relative polarities are given in Table 1.

Spectral fine structure is defined as %III/II [7] and $D_V$, defined in Fig. 1. Diagnostic peaks only are cited for the mass spectra.

*Prasinoxanthin* **1**: VIS $\lambda_{max}^{Me_2CO}$ nm: (426), 449, (473); $\lambda_{max}^{hexane}$ nm: (429), 452, (480).

*Micromonal* **4**. Available amount: 260 μg. VIS $\lambda_{max}^{Me_2CO}$ nm: (423), 449, (472), see Fig. 1; $\lambda_{max}^{diethyl\ ether}$ nm: (422), 447, (470); $\lambda_{max}^{hexane}$ nm: (425), 449, (474); $\lambda_{max}^{CHCl_3}$ nm: 462, (483). EIMS 70 eV, 210°C, *m/z* (rel. int.): 584 $[M]^+$ (74), 566 $[M - 18]^+$ (100), 548 $[M-18-18]^+$ (12), 538 $[M - 46]^+$ (10), 492 $[M - 92]^+$ (6), 478 $[M - 106]^+$ (6), 444 $[M - 138]^+$ (6), 431 $[M - 153]^+$ (8). CD $\lambda_{max}^{EtOH}$ nm $[\Delta\varepsilon]$: 220 [9.8], 232 [0], 247 [ − 7.0], 276 [ − 0.7], 339 [ − 2.5], 370 [ − 0.7], 399 [2.4]. $^1$H NMR (500 MHz, $CDCl_3$ including $^1$H$^1$H COSY); δ0.92 (3H, *s*, H-16′ or 17′), 1.03 (3H, *s*, H-16′ or 17′), 1.08 (6H, *s*, H-16 and H-17), 1.36 (1H, *m*, H-7′), 1.36 (1H, *dd*, *J* = 5.4, 13.9 Hz, H-2′ax), 1.48 (1H, *m*, H-2ax), 1.55 (1H, *m*, H-7′), 1.60 (1H, *m*, H-6′), 1.74 (3H, *s*, H-18), 1.77 (1H, *m*, H-2eq), 1.81 (3H, *s*, H-18′), 1.83 (1H, *dd*, *J* = 8.6, 14.8 Hz, H-2′eq), 1.98 (6H, *s*, H-19 and H-20′), 2.00 (3H, *s*, H-20), 2.05 (1H, *dd*, *J* = 9.5, 17.1 Hz, H-4ax), 2.28 (1H, *m*, H-8′), 2.37 (1H, *m*, H-8′), 2.39 (1H, *dd*, *J* = 5.0, 18.1 Hz, H-4eq), 4.00 (1H, *m*, H-3), 4.18 (1H, *m*, H-3′), 5.44 (1H, *s*, H-4), 6.13 (2H, *s*, H-7 and H-8), 6.16 (1H, *d*, *J* = 11.6 Hz, H-10), 6.27 (1H, *d*,

Table 1. Relative polarities (HPLC, TLC) for the new carotenoids, prasinoxanthin and uriolide

| Carotenoid | $t_R$ (HPLC)* | $R_f$ (TLC)† | $R_f$ (TLC) other systems |
|---|---|---|---|
| Anhydromicromonal (**6**) | 26.0 | 0.58 | 0.71‡ |
| Dihydrolutein (**12**) | 24.1 | 0.53 | 0.45§ |
| Anhydromicromonol (**7**) | | 0.46 | 0.30‡ |
| Anhydroprasinoxanthin (**11**) | | 0.45 | 0.20‡ |
| Anhydrouriolide furanoxide (**8a**) | 22.9 | 0.55 | — |
| Deepoxyuriolide (**9**) | 22.8 | 0.33 | 0.42‖ |
| Anhydrouriolide (**8**) | 21.9 | ≈ 0.44 | 0.70¶ |
| Dehydrouriolide (**10**) | | — | 0.32§ |
| Dehydrouriolide furanoxide (**10a**) | | — | 0.37§ |
| Micromonal (**4**) | 19.4 | 0.38 | 0.40‡ |
| Uriolide furanoxide (**3a**) | | 0.38 | |
| Prasinoxanthin (**1**) | 17.7 | 0.36 | ≈ 0.54¶ |
| Preprasinoxanthin (**12**) | 17.3 | 0.35 | 0.47¶ |
| Micromonol (**5**) | 17.0 | 0.32 | 0.59¶ |
| Uriolide (**3**) | 15.1 | 0.34 | ≈ 0.70¶ |

*Column: Spheri 5 RP-18, 5 μm, 220 mm × 4.6 mm (Brownlee Labs 0711-0017). Eluent (gradient): 0 min: 1 M $NH_4OAc$ (aq)–MeOH (1:4); 30 min; MeOH–$Me_2CO$ (7:3); 50 min: MeOH–$Me_2CO$–hexane (3:5:2).

†Silica Merck 5554; $Me_2CO$–hexane (1:1).

‡Silica Merck 5554; $Me_2CO$–EtOH–$CHCl_3$ (5:1:94).

§Laboratory-made plates, thickness 0.5 mm, silica Merck 11677–$Ca(CO_3)_2$ Merck 2066 (1:2); hexane–$Me_2CO$–MeOH (69:19:2).

‖Silica Merck 5554; EtOH–$CHCl_3$ (6:94).

¶Silica Merck 5554; $Me_2CO$–EtOH–$CHCl_3$ (25:1:74).

$J$ = 11.7 Hz, H-14), 6.37 (1H, *d*, $J$ = 15.2 Hz, H-12), 6.40 (1H, *d*, $J$ = 12.4 Hz, H-14'), 6.57 (1H, *d*, $J$ = 14.6 Hz, H-12'), 6.63 (1H, *dd*, $J$ = 11.7, 14.2 Hz, H-15'), 6.70 (1H, *dd*, $J$ = 11.5, 14.9 Hz, H-11), 6.74 (1H, *dd*, $J$ = 11.6, 14.2 Hz, H-15), 6.95 (1H, *d*, $J$ = 12.1 Hz, H-10'), 7.19 (1H, *dd*, $J$ = 12.3, 14.5 Hz, H-11'), 9.41 (1H, H-19' (10% 9'-*E*)), 10.31 (1H, *s*, H-19' (90% 9'-*Z*)). ROESY results are given in Scheme 2. $^{13}C$ NMR–DEPT (125 MHz, $CDCl_3$): $\delta$190.1 (CH=O). $LiAlH_4$ reduction provided **5**, inseparable from natural **5**. VIS $\lambda_{max}^{Me_2CO}$ nm: (404), *425*, 451; %III/II = 32; $D_V$ = 0.78. EIMS 70 eV, 210°C, *m/z* (rel. int.): 586 $[M]^+$, 568 $[M-18]^+$.

*Micromonol* **5**. Available amount: 60 μg. VIS $\lambda_{max}^{Me_2CO}$ nm: (406), *427*, 452; %III/II = 54, $D_V$ = 0.80, VIS $\lambda_{max}^{EtOH}$ nm: 310, (403), *424*, 450; %III/II = 49; $D_V$ = 0.77. EIMS 70 eV, 210°C, *m/z* (rel. int.): 586 $[M]^+$ (100), 568 $[M-18]^+$ (26), 550 $[M-18-18]^+$ (14), 532 $[M-18-18-18]^+$ (5), 494 $[M-92]^+$ (5), 480 $[M-106]^+$ (4), 446 $[M-140]^+$ (19).

*Anhydromicromonal* **6**. Available amount: 19 μg. VIS $\lambda_{max}^{Me_2CO}$ nm: 448, (470). EIMS 70 eV, 210°C, *m/z* (rel. int.): 566 $[M]^+$ (100), 548 $[M-18]^+$ (14), 474 $[M-92]^+$ (4), 460 $[M-106]^+$ (5), 446 $[M-120]^+$ (6), 431 $[M-135]^+$ (8).

*Anhydromicromonol* **7**. Available amount: 7 μg. VIS $\lambda_{max}^{Me_2CO}$ nm: (409), *427*, 453; %III/II = 65; $D_V$ = 0.85. EIMS 70 eV, 210°C, *m/z* (rel. int.): 568 $[M]^+$ (100), 550 $[M-18]^+$ (41), 532 $[M-18-18]^+$ (10), 476 $[M-92]^+$ (8), 433 $[M-135]^+$ (9).

*Anhydrouriolide* **8**. Available amount: 10 μg. VIS $\lambda_{max}^{Me_2CO}$ nm: *cf.* uriolide [2]. EIMS 70 eV, 210°C, *m/z* (rel. int.): 596 $[M]^+$ (92), 516 $[M-80]^+$ (28), 504 $[M-92]^+$ (9), 461 $[M-135]^+$ (17), 221 (67), 181 (53), 135 (100).

Furanoid rearrangement provided **8a**. Available amount: 8 μg. VIS $\lambda_{max}^{Me_2CO}$ nm: 423, 443; %III/II = 51; $D_V$ = 0.97. EIMS 70 eV, 210°C, *m/z* (rel. int.): 596 $[M]^+$ (100), 578 $[M-18]^+$ (14), 551 $[M-45]^+$ (15), 516 $[M-80]^+$ (26) ($m^*$ = 462), 504 $[M-92]^+$ (10) ($m^*$ = 426), 461 $[M-135]^+$ (17), 298 $[M]^{++}$ (7), 221 (35), 181 (23), 135 (62).

*Deepoxyuriolide* **9**. Available amount 23 μg. VIS $\lambda_{max}^{Me_2CO}$ nm: 450, (476); $\lambda_{max}^{hexane}$ nm: (430), *455*, 483; %III/II = 5; $D_V$ = 0.83; $\lambda_{max}^{CH_2Cl_2}$ nm: (430), 461, (485); $\lambda_{max}^{MeOH}$ nm: 450 (round), unchanged after addition of dilute hydrochloric acid. EIMS 70 eV, 210°C, *m/z* (rel. int.): 598 $[M]^+$ (9), 580 $[M-18]^+$ (100), 562 $[M-18-18]^+$ (11), 488 $[M-92-18]^+$ (4), 445 $[M-153]^+$ (4), 290 $[M-18]^+$ (6), 135, (40). Treatment with $LiAlH_4$ gave several unidentified uncoloured, fluorescent products and a coloured product, all of lower polarity (TLC).

*3'-Dehydrouriolide* (**10**). Available amount: 7 μg. VIS $\lambda_{max}^{Me_2CO}$ nm: (423), 448; $\lambda_{max}^{MeOH}$ nm: (430), 444. EIMS 70 eV, 210°C, *m/z* (rel. int.): 612 $[M]^+$ (35), 596 $[M-16]^+$ (5), 594 $[M-18]^+$ (8), 568 $[M-44]^+$ (5), 532 $[M-80]^+$ (9) ($m^*$ = 462), 520 $[M-92]^+$ (7), 479 $[M-133]^+$ (3), 461 $[M-151]^+$ (4), 221 (46), 181 (33), 135 (100).

*3'-Dehydrouriolide furanoxide* (**10a**). Available amount: 7 μg. VIS $\lambda_{max}^{Me_2CO}$ nm: 427, (450), unchanged after short treatment with $LiAlH_4$. EIMS 70 eV, 210°C, *m/z* (rel. int.): 612 $[M]^+$ (26), 532 $[M-80]^+$ (9), 520 $[M-92]^+$ (5), 503 $[M-109]^+$ (9), 429 $[M-183]^+$ (18), 221 (100), 181 (28), 135 (44).

*Anhydroprasinoxanthin* (**11**). Available amount: 8 μg. VIS $\lambda_{max}^{Me_2CO}$ nm: 450, EIMS 70 eV, 210°C, *m/z* (rel. int.): 582 $[M]^+$ (2), 564 $[M - 18]^+$ (3), 428 $[M - 154]^+$ (61), 400 $[M - 154 - 28]^+$ (5), 214 $[M - 154]^+$ (4), 69 (100).

*Preprasinoxanthin* (**12**). Available amount: 650 μg. VIS $\lambda_{max}^{Me_2CO}$ nm: *445*, 466; %III/II = 8; $D_V = 0.90$, VIS $\lambda_{max}^{hexane}$ nm: (423), *447*, 473; %III/II = 29; $D_V = 0.73$. EIMS 70 eV, 210°C, *m/z* (rel. int.): 600 $[M]^+$ (100), 584 $[M - 16]^+$ (48), 582 $[M - 18]^+$ (63), 566 $[M - 16 - 18]^+$ (40), 564 $[M - 18 - 18]^+$ (24), 446 $[M - 154]^+$ (29), 428 $[M - 154 - 18]^+$ (13), 221 (41). CD $\lambda_{nm}^{EtOH}$ $[\Delta\varepsilon]$: 255 [5.5], 270 [15.5], 300 [2], 360 [0], *cf.* parallel determination of (6′*R*)-β,ε-carotene $\lambda_{nm}^{hexane}$ $[\Delta\varepsilon]$ 260 [7], 305 [0.5], 315–335 [2], 360 [0.25]. $^1$H NMR (400 MHz, $CDCl_3$); δ0.85 (3H, *s*, H-16′), 0.96 (3H, *s*, H-17), 1.00 (3H, *s*, H-17′), 1.03 (3H, *s*, H-16), 1.22 (3H, *s*, H-18), ≈1.36 (2H, *m*, H-2ax, H-2′ax), 1.47 (1H, *dd*?, H-2eq), 1.62 (3H, *s*, H-18′), ≈1.80 (2H, *m*, H-4ax, H-2′eq), 1.92 (3H, *s*, H-19′), 1.94 (3H, *s*, H-19), 1.99 (6H, *s*, H-20, H-20′) 2.29 (1H, *m*, H-4eq), 2.41 (1H, *d*, *J* = ≈10, H-6′), 2.6 (1H, *d*, *J* = 18.6, H-7), 3.65 (1H, *d*, *J* = 18.1, H-7), ≈3.8 (1H, *m*, H-3), 4.21 (1H, *m*, H-3′), 5.46 (1H, *dd*, *J* = 10.2, 15,6, H-7′), 5.54 (1H, *s*, H-4′), 6.14 (2H, *d*, *J* = 15.1, H-8′, H-10′), 6.27 (1H, *d*, *J* = 11.2, H-14′), 6.36 (1H, *d*, *J* = 15.1, H-12′), 6.41 (1H, *d*, *J* = 11.7, H-14), ≈6.55 (1H, *m*, H-11), ≈6.6 (2H, *m*, H-15, H-11′), ≈6.7 (2H, *m*, H-12, H-15′), 7.15 (1H, *d*, *J* = 10.7, H-10). Treatment of **12** in acetone with dilute HCl resulted in a blue product (VIS $\lambda_{max}^{Me_2CO}$ nm: 690 (round, broad)), which upon subsequent addition of dilute NaOH was converted into a yellow product (VIS $\lambda_{max}^{Me_2CO}$ nm: (376), 397, *418*, 445; %III/II = 89; $D_V = 0.66$).

*7′,8′-Dihydrolutein* (**13**). Available amount: 25 μg. VIS $\lambda_{max}^{Me_2CO}$ nm: (408), *428*, 453; %III/II = 38; $D_V = 0.79$. EIMS 70 eV, 210°C, *m/z* (rel. int.): 570 $[M]^+$ (100), 552 $[M - 18]^+$ (35), 478 $[M - 92]^+$ (4) ($m^* = 401$).

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