Animal Carotenoids. 31.* Structure Elucidation of a Sponge Metabolite via Mesylate Elimination

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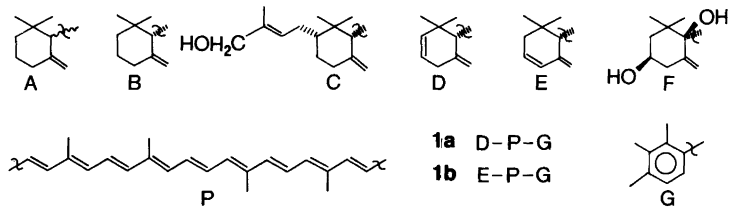
The structure of a sponge metabolite from *Microciona prolifera,* previously considered to be (6*S*)-2,3-didehydro- or 3,4-didehydro-γ,χ-carotene, has been further studied. Attempted total synthesis of the 3,4-didehydro derivative provided the hitherto unknown γ,χ-carotene, the synthesis of which is described. Hydrolysis of lutein methanesulfonate diester (dimesylate) gave elimination products possessing the 3,4-didehydro γ end-group. ^{1}H NMR data for this γ end-group were identical with those for the sponge carotenoid. The mesylate elimination reaction described may mimic the metabolic formation of the 3,4-didehydro-γ-carotenoid end-group.

In connection with other investigations on functionalized carotenoids we further report the preparation of zeaxanthin and lutein mesylates and their base-catalyzed elimination reactions. S_N2 type substitution reactions of zeaxanthin dimesylate with appropriate nucleophiles did not produce β,β-carotene, zeaxanthin diacetate or thiozeaxanthin.

Exocyclic methylene, common amongst terpenoids, was first encountered for carotenoids in the γ end-group **A**[2] (Scheme 1) of β,γ-carotene.[3] Subsequently, γ,γ-carotene,[4] γ,ψ-carotene, 3′,4′-didehydro-γ,ψ-carotene, 7′,8′-dihydro-γ,ψ-carotene and 7′,8′,11′,12′-tetrahydro-γ,ψ-carotene,[5] all possessing the unsubstituted γ end-groups **A** and **B** were reported. The C_{50}-carotenoid sarcinaxanthin has two C-2 isopentenyl-substituted γ-rings **C**.[6] A minor carotenoid from the marine sponge *Microciona prolifera* was believed to be either (6*S*)-2,3-didehydro-γ,χ-carotene (**1a**) or (6*S*)-3,4-didehydro-γ,χ-carotene (**1b**).[7] More recently, prasinoxanthin with assumed 3*S* configuration was assigned the hydroxylated γ-end group **F**.[8] All the γ-type carotenoids whose structures have been established until now have possessed the same absolute configuration at C-6.[6,8,9]

We now report further studies permitting a distinction between structures **1a,b** for the minor sponge carotenoid.



Scheme 1.

*For Part 30, see Ref. 1.

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Results and discussion

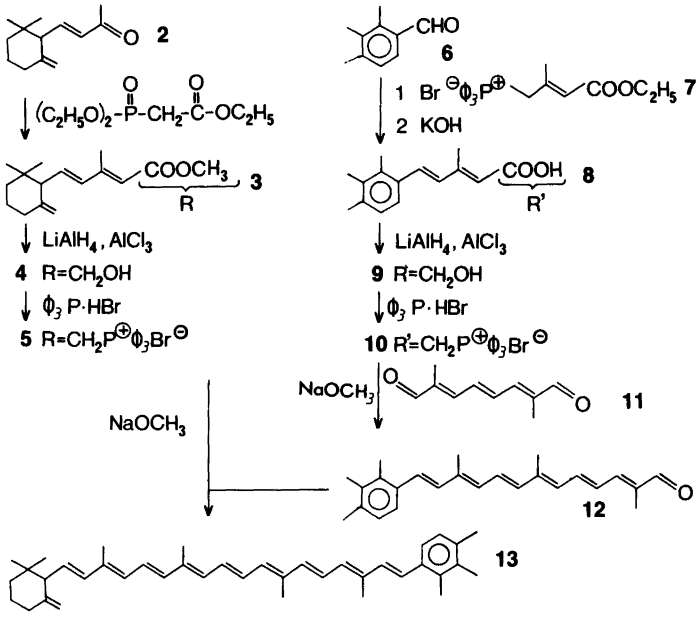
Total synthesis of 3,4-didehydro-γ,χ-carotene (**1b**) was attempted[10] using NBS-dehydrogenation of γ-ionone (**2**) as the key step (Scheme 2). However, the desired didehydro derivative was not obtained, and total synthesis of the previously unknown γ,χ-carotene was therefore resorted to, using standard methods. Optically inactive γ-ionone (**2**) was converted in a Horner reaction to the methyl ester **3**,[11] without noticeable isomerization of the γ to a β end-group. Reduction of the ester function with $LiAlH_4/AlCl_3$ by a method recommended for α,β-unsaturated esters[12] gave the known allylic alcohol **4** together with ca. 20 % (^{1}H NMR) of the β-analogue, subsequently converted to the crude phosphonium salt **5**.

The χ end-group of the molecule was built up from 2,3,4-trimethylbenzaldehyde (**6**), which was condensed in a Wittig reaction with the available 3-carbethoxy-2-methyl-prop-2-enyl triphenylphosphonium bromide (**7**)[13] to the methyl ester, saponified directly to the corresponding known[14] carboxylic acid **8** for easier purification. Reduction[12] to the allylic alcohol **9** and conversion to the phosphonium bromide **10**, followed by Wittig condensation with the available C_{10}-dialdehyde **11**,[15] afforded the previously undescribed χ-apo-12′-carotenal **12**. A final Wittig reaction between the phosphonium salt **5** and the aldehyde **12** provided, after chromatography, γ,χ-carotene **13** with the predicted spectral properties.

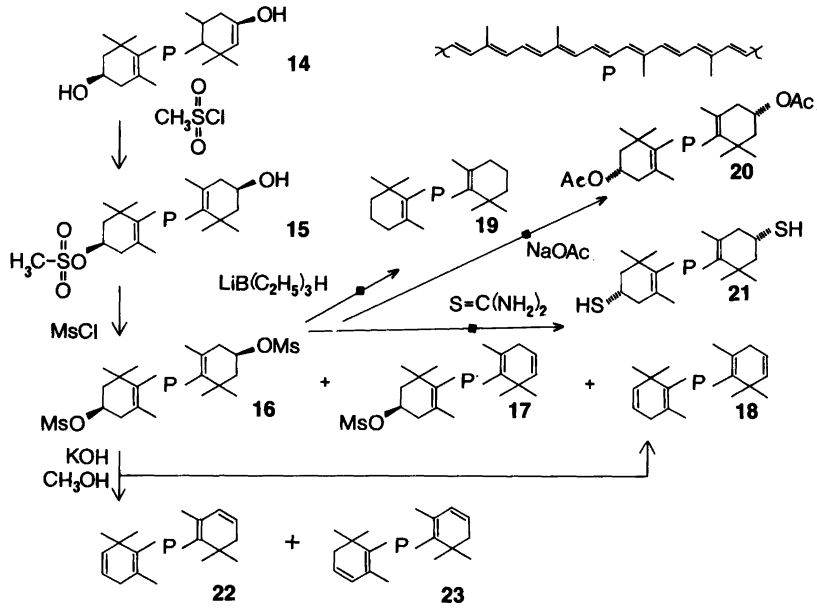
The ambiguity as to the structure (**1a** or **1b**) of the spronge carotenoid was subsequently resolved by a different approach. Alcohols react readily with methanesulfonyl chloride,[16] giving mesylates which are reactive intermediates for nucleophilic substitution and elimination reactions:

When (3*R*,3′,*R*)-zeaxanthin (**14**) (Scheme 3) was treated with mesyl chloride at room temperature according to Crossland,[16] at least four products were formed besides the monomesylate **15** and the desired dimesylate **16** (Scheme 3). Two of the minor products were identified as the elimination products **17** and **18**. By-product formation was minimized by carrying out the reaction with excess of mesyl chloride at 40 °C.

Zeaxanthin dimesylate (**16**) was first tested for its reactivity towards nucleophilic reagents. Attempted reduction with $LiB(C_2H_5)_3H$ ("superhydride")[17,18] to β,β-carotene (**19**) failed, in analogy with the behaviour of mesyl derivatives of hopanes.[19] The in-chain mesylate of renierapurpurin(χ,χ-carotene)-20-ol was also resistant to such



Scheme 2.

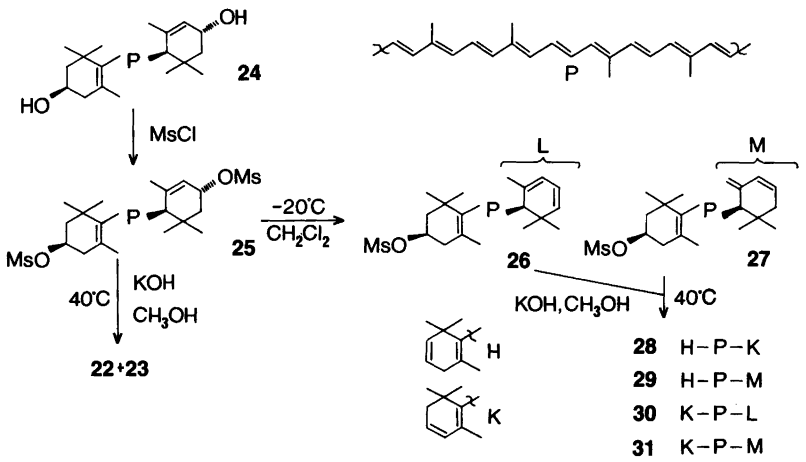


Scheme 3.

reduction. Attempted nucleophilic displacement in zeaxanthin dimesylate (**16**) by acetate ion[20] to give the 3*S*,3′*S* diacetate **20** and by thiourea[21,22] to give thiozeaxanthin (**21**) was also unsuccessful.

Elimination reactions, however, proceeded smoothly when the dimesylate **16** was treated with 10 % methanolic KOH, affording the carotenes **18**, **22** and **23**. Yamaguchi and co-workers[23] obtained mainly the conjugated carotene **23** upon hydrolysis of a corresponding sulfonamide derivative.

(3*R*,3′*R*,6′*R*)-Lutein (**24**) (Scheme 4), with one secondary, allylic hydroxy group, reacted faster with mesyl chloride than zeaxanthin (**14**). In contrast to the more stable zeaxanthin dimesylate (**16**), lutein dimesylate (**25**) (Scheme 4) underwent slow elimination under neutral conditions to give the two monomesylates **26** and **27**. Treatment of a mixture of the monomesylates **26** and **27** with methanolic KOH afforded a mixture of



Scheme 4.

the dehydrocarotenes **28–31** with four different end-groups. Similar elimination in the dimesylate **25** in methanolic KOH afforded the dehydrocarotenes **22** and **23**, as in the elimination reaction of zeaxanthin dimesylate (**16**).

Lutein dimesylate (**25**) was considerably more stable in solution than lutein (**24**) disulfate, recently studied in our laboratory.[24] The allylic sulfate underwent fast solvolysis by an S_N1 mechanism rather than elimination. As found for carotenoid sulfates,[24,25] no molecular ions ascribable to elimination prior to electron impact were seen in the mass spectra of carotenoid mesylates.

Detailed ^{1}H NMR data for zeaxanthin (**14**) diacetate[26] and zeaxanthin (**14**) disulfate,[27] as well as considerations based on models, reveal the equatorial position of the C-3,3′ substituents. Assuming that the same preference applies for zeaxanthin dimesylate (**16**), flipping of the cyclohexene half-chair to provide an axial leaving group is required in order to allow E2 antiperiplanar elimination of the mesyl group by protons at C(2) and C(4), resulting in the formation of the dehydrocarotenes with non-conjugated (**25**, **31**) and conjugated (**22**, **23**) end-groups.

The exocyclic methylene products reveal that neutral conditions allow lutein dimesylate (**25**) to eliminate the allylic mesyl group also by proton abstraction from CH_3-18′. Since E1 elimination via the resonance-stabilized allylic carbocation should also lead to the conjugated β-isomer, which was not observed in this case, a concerted elimination with the mesyl group in a quasi-axial position and the polyene chain in a quasi-equatorial position also seems to be favoured.

^{1}H NMR data for the elimination products **27**, **29** and **31** permitted the identification of the sponge carotenoid as 3,4-didehydro-γ,χ-carotene. The signals in question were those from the 3,4-didehydro γ end-group, particularly the characteristic signal for the exocyclic methylene, *gem*-dimethyl and the H-6 methine protons, as well as for the endocyclic olefinic protons.

A comparison with ^{1}H NMR data for γ,χ-carotene (**13**) shows that the 3,4 double bond significantly influences the chemical shift of the protons of one of the two methyl groups, and particularly that of the terminal methylene protons of the γ-ring.

Our ^{1}H NMR data for the 3,4-didehydro γ end-group are consistent with those of a product obtained (by an unknown mechanism) upon treatment of (3*R*,3′*R*,6′*R*)-lutein (**24**) with $LiAlH_4$/$AlCl_3$, and assigned the (3*R*,6*R*,*S*)-3′,4′-didehydro-β,γ-caroten-3-ol structure.[28] Since the location of the endocyclic double bond was not unequivocally established[28] and apparent inconsistencies in the interpretation of the VIS and the IR spectra existed, identification of the sponge carotene on the basis of this model alone was not justified.

6*R* Chirality of the new carotene (**1b**) has previously[7] been favoured on the basis of CD evidence. The negative Cotton effect observed for ε- and γ-type carotenoids with 6*R* configuration has been proposed to be associated with this particular chiral center.[29]

Structure **1b** is compatible with the metabolic formation of the γ,χ-carotene derivative by the sponge from a carotenoid precursor possessing a (6*R*)-3-hydroxy ε-ring, such as lutein (**24**), by an elimination reaction mimicking the elimination of the mesyl group demonstrated here.

The present work demonstrates the analytical application of mesylate elimination in carotenoid chemistry.

Allylic carotenols such as isocryptoxanthin, echscholtzxanthin and also astaxanthin are expected to react in a more regiospecific manner. Here, mesylate elimination may also be of preparative interest for the synthesis of conjugated elimination products.

Experimental

General Methods. The general method and instrumentation employed were as described previously.[11] The term % III/II[30] is used to express spectral fine-structure of VIS spectra. ^{1}H NMR spectra were recorded at 100 MHz. Only diagnostically useful ions are cited for the mass spectra.

(6-S)-2,3 or 3,4-Didehydro-γ,γ-carotene (**1a** or **1b**) *ex Microciona prolifera.*[7] ^{1}H NMR ($CDCl_3$) signals from the γ end group: δ 0.88s (3H) and 0.90s (3H) for *gem*-dimethyl, 2.63d (J = 9 Hz, 1H, H-6), 4.81s and 4.87s (1H+1H, $=CH_2$), 5.36–5.80m (H-7 and two endocyclic olefinic H); IR (KBr): 890 cm^{-1} ($=CH_2$).

γ-*Ionone* (**2**). Optically inactive **2**, obtained from Firmenich and Cie, was 96% pure by GC; UV

(hexane), ^{1}H NMR ($CDCl_3$) and MS data were in agreement with published spectra.[9,31]

NBS reaction of γ-ionone (**2**). Unsuccessful attempts to achieve 6,7-didehydrogenation are described elsewhere.[10]

Ethyl 5-(2,2-dimethyl-6-methylene)cyclohexyl-3-methyl-2,4-pentadienoate (**3**). **3** was prepared by the reaction of γ-ionone (**2**, 4.0 g) with ethyl diethylphosphonoacetate (5 g) in dry benzene (100 ml) at room temp. for 25 h, followed by extraction and isolation.[11] Yield 4.5 g (87 %). UV λ_{max} (CH_3OH): 265 nm; ^{1}H NMR ($CDCl_3$): δ 0.82s and 0.90s (6H, *gem*-dimethyl), 2.20s (3H, methyl), 2.5d (1H, J = 10 Hz, *tert* H), 4.45s and 4.65s (2H, $=CH_2$), 5.5–7.0 (3H, olefinic); MS (120 °C) m/z : 262 (M, 8 %), 217 (M-45, 18 %), 189 (M-73, 71 %), 125 (100 %), consistent with reported data for the methyl ester.[11]

5-(2,2-Dimethyl-6-methylene)cyclohexyl-3-methyl-2,4-pentadienol (**4**). **3** (4.0 g) was reduced[12] with $LiAlH_4$ (1.8 g) and $AlCl_3$ (2.1 g) in dry ether for 2 h. Yield after extractive isolation 3.1 g (88 %) of a mixture of 80 % **3** and 20 % β-isomer of **4** (by ^{1}H NMR); UV λ_{max} (CD_3OH): 235 nm; ^{1}H NMR ($CDCl_3$): δ 0.82s and 0.90s (*gem*-dimethyl γ-ring), 1.05s (*gem*-dimethyl β-ring), 1.8s (methyl β-ring), 4.25d (J = 6 Hz, CH_2OH), 4.45s and 4.65s ($=CH_2$), 5.5–7.0 (olefinic H); (MS 120 °C) m/z : 220 (M, 71 %), 202 (M-15, 5 %), 202 (M-18, 1 %), 105 (100 %), consistent with reported data.[11]

5-(2,2-Dimethyl-6-methylene)cyclohexyl-3-methyl-2,4-pentadienyl triphenylphosphonium bromide (**5**). **4** (3.0 g) and triphenylphosphonium bromide (5.9 g) in methanol–chloroform were allowed to react for 48 h.[11] Yield of crude **5** obtained from the chloroform phase remaining after extraction with water 5.1 g (69 %).

5-(2,3,4-Trimethylphenyl)-3-methyl-2,4-pentadienoic acid (**8**). Wittig condensation[32] between 2,3,4-trimethylbenzaldehyde (**6**, 10.0 g) and 3-carboethoxy-2-methyl-prop-2-enyl triphenylphosphonium bromide (**7**, 37.0 g) in methanol with sodium methoxide for 44 h afforded the **8** methyl ester, which was transferred to ether. Hydrolysis of the ester with KOH (10 %) in methanol for 17 h afforded **8**; yield 5.8 g (37 %); m.p. 214–216 °C, lit.[14] 214–215 °C; UV λ_{max} (ether): 240 (ε = 8900) and 310 (ε = 20300); ^{1}H NMR ($CDCl_3$): δ 1.7s (3H, in-chain CH_3), 2.1–2.3 (9H, aromatic CH_3), 4.7–6.5 (3H, olefinic), 6.5–7.5 (2H, aromatic), 11.2 broad s (1H, COOH); MS (100 °C) m/z : 230 (M, 67 %), 215 (M-15, 5 %), 185 (M-45, 100 %).

5-(2,3,4-Trimethylphenyl)-3-methyl-2,4-pentadienol (**9**). **8** (5.4 g) was allowed to react[12] with $LiAlH_4$ (3.2 g) and $AlCl_3$ (3.6 g) in dry ether; yield 3.7 g (73 %); UV λ_{max} (ether): 290 nm (ε = 7100); ^{1}H NMR ($CDCl_3$): δ 1.7s (in-chain CH_3), 2.1–2.3 (9H, aromatic CH_3), 3.5–4.3 (2H, CH_2OH), 4.5–6.5 (3H, olefinic), 6.5–7.5 (2H, aromatic); MS (100 °C) m/z : 216 (M, 33 %), 198 (M-18, 38 %), 185 (M-31, 100 %), 133 (31 %).

5-(2,3,4-Triphenylphenyl)-3-methyl-2,4-pentadienyl triphenyl phosphonium bromide (**10**). Reaction between **9** (2.7 g) and triphenylphosphonium bromide (5.5 g) afforded **10**; yield 5.2 g (77 %) of crude product; ^{1}H NMR ($CDCl_3$): δ 2.1–2.3 (9H, aromatic CH_3), 7.3–8.0 (15H, 3× phenyl on P).

χ-Apo-12'-carotenal (**12**). 2,7-Dimethyl-2,4,6-octatriene-1,8-dial[15] (**11**, 6.5 g) and **10** (5 g) in methanol containing Na-methylate (20 mmol), were allowed to react for 18 h. Ether extraction followed by chromatography on alumina afforded **10**; yield 0.66 g (21 %); VIS λ_{max} (acetone): 425 nm; ^{1}H NMR ($CDCl_3$): δ 1.9 (3H, CH_3-20'), 1.8–2.4 (12H, CH_3-15,17,18,19,20), 6.0–7.5 (olefinic and aromatic H), 9.4s (1H, aldehyde), MS (140 °C) m/z : 346 (M, 100 %), 331 (M-15, 1 %), 133 (40 %).

γ,χ-Carotene (**13**). The Wittig condensation was carried out with **5** (5.1 g) and **12** (0.6 g) in methanol, using excess sodium methoxide as base, for 26 h. The reaction mixture was extracted with ether, and the carotenoids were subjected to chromatography on alumina and finally TLC,[33] affording chromatographically homogeneous **13** (5.0 g) besides other products.

VIS λ_{max} (acetone): (432), 452 and 480 nm, % III/II = 2; IR ν_{max} (KBr): 960 (*trans* disubst. double bonds), 885 ($=CH_2$) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.83s and 0.90s (6H, *gem*-dimethyl γ-ring), 1.97s (9H, CH_3-19,20,20'), 2.06s (CH_3-19'), 2.21s (3H, aromatic CH_3) and 2.30s (6H,

aromatic CH_3), 2.5d (1H, J = 8 Hz, H-6), 4.60s and 4.78s (1H+1H, $=CH_2$ γ-ring), 6.0–6.9 (conj. olefinic H), 6.9–7.1 (2H, aromatic H); MS (190 °C) m/z: 532 (*M*, 58 %), 440 (*M*-92, 5 %), 426 (*M*-106, 10 %), 133 (100 %).

Preparation of zeaxanthin mesylates. (a) To (3*R*,3′*R*)-zeaxanthin[34] [**1**, 56.8 mg; $R_f = 0.34$ (SiO_2, 40 % acetone-hexane)] in CH_2Cl_2 (45 ml) containing triethyl amine (300 µl) was added mesyl chloride (160 µl) at 0 °C. The mixture was left at room temperature overnight, followed by extraction and chromatography. TLC revealed the formation of **15** (3.4 mg), **16** (0.5 mg), **17** (<0.5 mg) and **18** (1.1 mg), as well as unidentified minor products.
(b) **1** (11.4 mg) in CH_2Cl_2 (3 ml), triethyl amine (110 µl) and mesyl chloride (45 µl) were allowed to react for 5 h at 40 °C and then at room temperature overnight. Extraction and TLC gave mainly the dimesylate **16** (13.5 mg, 93 %).

Zeaxanthin monomesylate (**15**). Available quantity ca. 3 mg; R_f 0.38 (SiO_2, 40 % acetone–hexane); VIS as for **16**; MS (200 °C) m/z: 646 (*M*, 0 %), 532 (*M*-MsOH–H_2O, 90 %), 517 (532-CH_3, 6 %), 440 (532-92, 6 %), 105 (100 %).

Zeaxanthin dimesylate (**16**). Available quantity 3.4 mg; $R_f = 0.44$ (SiO_2, 40 % acetone–hexane), VIS λ_{max} (CH_2Cl_2): (433), 455 and 483 nm, % III/II = 14; ^{1}H NMR ($CDCl_3$): δ 1.12s (12H, *gem*-dimethyl), 1.62m (2H, H-2.2′), 1.74s (6H, CH_3-18,18′), 1.97s (12H, CH_3-19,20,19′,20′), 2.17m (2H, H-4,4′), 2.48 (2H, H-4,4′), 3.03s (6H, CH_3SO_3-), 4.94m (2H, H-3,3′), 6.1–6.6m (olefinic H); MS (200 °C) m/z: 724 (*M*, 0 %), 532 (*M*-2MsOH, 8 %), 440 (532-92, 3 %), 105 (100 %).

2′,3′-Didehydro-β,β-carotene-3-mesylate (**17**). Available quantity 1.1 mg; $R_f = 0.55$ (SiO_2, 40 % acetone–hexane); VIS λ_{max} (CH_2Cl_2): (435), 461 and (486) nm; (acetone): (432), 451 and (471) nm; ^{1}H NMR ($CDCl_3$): δ 1.25s (12H, *gem*-dimethyl), 1.74s (3H, CH_3-18), 1.78s (3H, CH_3-18′), 1.97s (12H, CH_3-9,13,9′,13′), 2.17m (1H, H-4), 2.48m (1H, H-4), 2.67m (2H, H-4′), 3.03s (3H, CH_3SO_3-), 4.94m (1H, H-3), 5.53m (2H, H-2′,3′), 6.1–6.7m (olefinic H).

2,3,2′,3′-Tetradehydro-β,β-carotene (**18**). Available quantity 0.46 mg; $R_f = 0.78$ (SiO_2, 40 % acetone–hexane); VIS λ_{max} (CH_2Cl_2): (440), 460 and (480) nm; ^{1}H NMR ($CDCl_3$): δ 1.25s (12H, *gem*-dimethyl), 1.78s (6H, CH_3-18,18′), 1.98s (12H, CH_3-19,20,19′,20′), 2.65 br.s. (4H, H-4,4′), 5.5–5.6m (4H, H-2,3,2′,3′), 6.1–6.7m (olefinic H), cf. Ref. 35; MS (200 °C) m/z: 532 (*M*, 16 %), 530 (*M*-2, 16 %), 517 (*M*-15, 1 %), 440 (*M*-92, 4 %), 157 (100 %).

Hydrolysis of zeaxanthin mesylates. Alkaline hydrolysis in 10 % methanolic KOH was carried out overnight. Both the monomesylate (**15**, 1.1 mg) and the dimesylate (**16**, 6.8 mg) gave the same products, viz. **18**, **22** and **23**, in ca. 70 % yield after TLC.

2,3,3′,4′-Tetradehydro-β,β-carotene (**22**) *and 3,4,3′,4′-tetradehydro-β,β-carotene* (**23**) were obtained in a mixture with 2,3,2′,3′-tetradehydro-β,β-carotene (**18**). Available quantity 3.3 mg; $R_f = 0.78$ (SiO_2, 40 % acetone–hexane); VIS λ_{max} of the mixture (hexane): 458 and (470) nm; ^{1}H NMR ($CDCl_3$): 2,3-didehydro-β-ring (see **18**), 3,4-didehydro-β-ring δ 1.04s (*gem*-dimethyl), 1.88s (CH_3-18), 2.08m (H-2), 5.3–5.4m (H-3), 5.8m (H-4), ratio of the two end groups ca. 1:3, polyene chain 1.98s (CH_3-19,20,19′,20′); MS (200 °C) m/z: 532 (*M*, 12 %), 440 (*M*-92, 4 %), 149 (100 %).

Attempted reaction of zeaxanthin dimesylate with acetate ions. **16** (1 mg) was stirred for 30 h at room temp. with conc. NaOAc in acetone. No zeaxanthin monoacetate or diacetate (**20**) were formed (TLC, MS evidence).

Attempted preparation of β,β-carotene (**19**). **16** (1 mg, vacuum-dried) in dry tetrahydrofuran was treated with $LiB(C_2H_5)_3H$ (Aldrich) at room temperature and the mixture was then maintained at 45 °C for 18 h. No β,β-carotene (**19**) was obtained (TLC evidence).

In an analogous experiment, the attempted reduction of the mesylate, prepared from synthetic renierapurpurin-20-ol,[36] failed.

Attempted preparation of thiozeaxanthin (**20**). **16** (3.4 mg) in abs. ethanol (2 ml) was treated with thiourea (6 mg); the solution was kept at room temperature for 3 days and then at 40 °C for 6 h, giving a polar product; $R_f = 0$ (SiO_2, 40 % acet-

one–hexane); VIS λ_{max} (methanol): (423), 442 and 464 nm; MS (200 °C) *m/z*: 647 (0.1 %), 633 (0.1 %), 618 (0.1 %), 532 (2 %), 440 (532-92, 0.4 %), 149 (100 %). Hydrolysis of this product with 10 % methanolic KOH did not yield carotenoids (VIS evidence).

Preparation of lutein mesylates. Natural (3*R*,3′*R*,6′*R*)-lutein (**24**, 56.8 mg) [*ex* alfalfa, National Chlorophyll and Chemical Co.; $R_f = 0.09$ (SiO_2, 20 % acetone–hexane)] in CH_2Cl_2 (5 ml) containing triethylamine (170 µl) was allowed to react with mesyl chloride (70 µl) at room temp. overnight. TLC revealed complete transformation to **25**. Storage of crude, conc. **25** at −20 °C for 2 months in CH_2Cl_2 led to transformation to **26**, **27** and decomposition products.

Lutein dimesylate (**25**). Available quantity 0.4 mg; $R_f = 0.16$ (SiO_2, 20 % acetone–hexane), same R_f as zeaxanthin dimesylate (**16**); VIS λ_{max} (CH_2Cl_2): 344, (435), 454 and (429) nm; ^{1}H NMR ($CDCl_3$): δ 0.89s and 0.93s (6H, CH_3-16′,17′), 1.11s (6H, CH_3-16,17), 1.62 (3H, CH_3-18′), 1.74s (3H, CH_3-16), 1.97s (12H, CH_3-19,20,19′,20′), 2.17m (1H, H-4), 2.48m (1H, H-4), 3.04s (6H, CH_3SO_3-), 4.98m (1H, H-3), 6.1–6.6m (olefinic H); MS (200 °C) *m/z*: 724 (*M*, 0 %), 628 (*M*-MsOH, 1 %), 532 (*M*-2 MsOH, 20 %), 440 (*M*-2 MsOH-92, 3 %), 105 (10 %).

2′,3′-Didehydro-β,ε-carotene-3-mesylate (**26**) *and 3′,4′-didehydro-β,γ-carotene-3-mesylate* (**27**). Available quantity 0.6 mg of ca. 1:1 mixture; $R_f = 0.32$ (SiO_2, 20 % acetone–hexane); VIS λ_{max} (methanol): 329 (420), 440 and 467, % III /II = 86; ^{1}H NMR ($CDCl_3$): β-ring of **17** and **18** as for **25** and **16**, 2,3-didehydro ε-ring δ 1.02s and 0.94s (*gem*-dimethyl), 1.73s (CH_3-18′), 2.21d (*J* = ca. 8 Hz, H-6), 5.32d (*J* = 8 Hz, H-2′), 5.57m (H-4), 5.60m (H-3), cf. Ref. 26, 3′,4′-didehydro γ-ring 0.88s and 0.89s (*gem*-dimethyl), 2.63d (*J* = 8 Hz, H-6), 4.82s and 4.88s (=CH_2), 5.5m (H-7), 5.66m (H-3,4), polyene chain 1.98s (CH_3-19,20,19′,20′), 6.1–6.6m (olefinic H), cf. Refs. 7 and 28; MS (200 °C) *m/z*: 628 (*M*, 3 %), 532 (*M*-MsOH, 3 %), 440 (*M*-MsOH-92, 4 %), 149 (100 %), 105 (100 %).

Hydrolysis of monomesylates **26** *and* **27**. The mesylates **26** and **27** (0.4 mg) were hydrolysed in 10 % methanolic KOH by treatment for 4 h at room temperature, then for 17 h at 35 °C and finally at 60 °C until TLC of an aliquot revealed complete hydrolysis. Yield 0.2 mg of a mixture of carotenes **28**, **29**, **30** and **31**; $R_f = 0.73$ (SiO_2, 20 % acetone–hexane); VIS λ_{max} (CH_3OH): (420), 442 and 461 nm; ^{1}H NMR ($CDCl_3$): 2,3-didehydro β-ring *H* of **28** and **29** as for **18**, 3,4-didehydro β-ring *K* of **30** and **31** as for **23**, 2′,3′-didehydro ε-ring *K* of **28** and **30** as for **26**, and 3′,4′-didehydro γ-ring *M* of **29** and **31** as for **27**.

In particular, the ^{1}H NMR evidence for end-group *M* was the observation of δ 0.88s and 0.89s (*gem*-dimethyl), 2.63d (H-6), and 4.82s and 4.88s (=CH_2).

Hydrolysis of lutein dimesylate (**25**). Hydrolysis of **25** (0.4 mg) was performed as for (**26** + **27**) above. The reaction was monitored by TLC, revealing formation of monomesylate as intermediate with $R_f = 0.32$ (SiO_2, 20 % acetone–hexane), as for **26** + **27**. Yield 0.1 mg of a mixture of **22** and **23**; $R_f = 0.73$ (SiO_2, 20 % acetone–hexane); spectral data as for **22** and **23** prepared from **16**. VIS λ_{max} (CH_3OH): (430), 455 and (470); ^{1}H NMR ($CDCl_3$): 2,3-didehydro β-ring as for **22**, and 3,4-didehydro β-ring as for **23**.

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