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Carotenoid Sulfates. 2.* Structural Elucidation of Bastaxanthin

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The structural elucidation of bastaxanthin, the major carotenoid of the marine sponge *Ianthella basta,* is reported.

Bastaxanthin, the first known naturally occurring carotenoid sulfate, has been characterized by spectroscopic data (electronic, IR, ^{1}H NMR, ^{13}C NMR, CD and mass spectra) and chemical evidence (20 sulfated and desulfated derivatives) including acidic and enzymatic hydrolysis to bastaxanthol, also encountered as a minor carotenoid in *I. basta*.

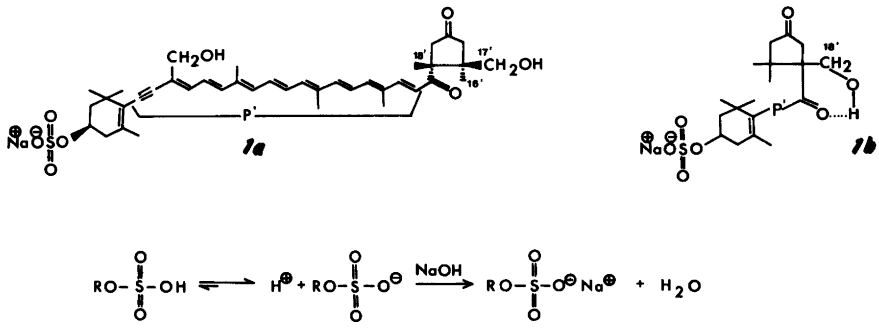
The evidence is consistent with the constitution 3,19,17′-trihydroxy-7,8-didehydro-β,κ-carotene-3′,6′-dione 3-sulfate. The absolute configuration of the three chiral centres is discussed in favour of (3*R*,1′*R*,5′*R*)-configuration.

Besides common phytoplankton type carotenoids the marine sponge *Ianthella basta* contains a group of strongly polar carotenoid sulfates, among which bastaxanthin *c* (*ca.* 40 % of the total carotenoids) is a major constituent.[1] Carotenoid sulfates are so far not encountered in other marine sponges [2] or other natural sources.

Details on the structural elucidation of bastaxanthin *c*, in this paper referred to as bastaxanthin, in favour of structure *1a* are now reported. In preliminary symposium contributions [3–5] the primary, non-allylic hydroxy function was allocated to C-18′ (*1b*), Scheme 1.

RESULTS AND DISCUSSION

Due to the large content of other extractives in the sponge and the high polarity of bastaxanthin and accompanying carotenoids the purification was particularly laborious. Reversed phase chromatography and ion exchange chromatography [6] were employed, but most efficient separation was obtained by pressurized silica columns followed by TLC on silica in combination with fractional precipitation.



Scheme 1.

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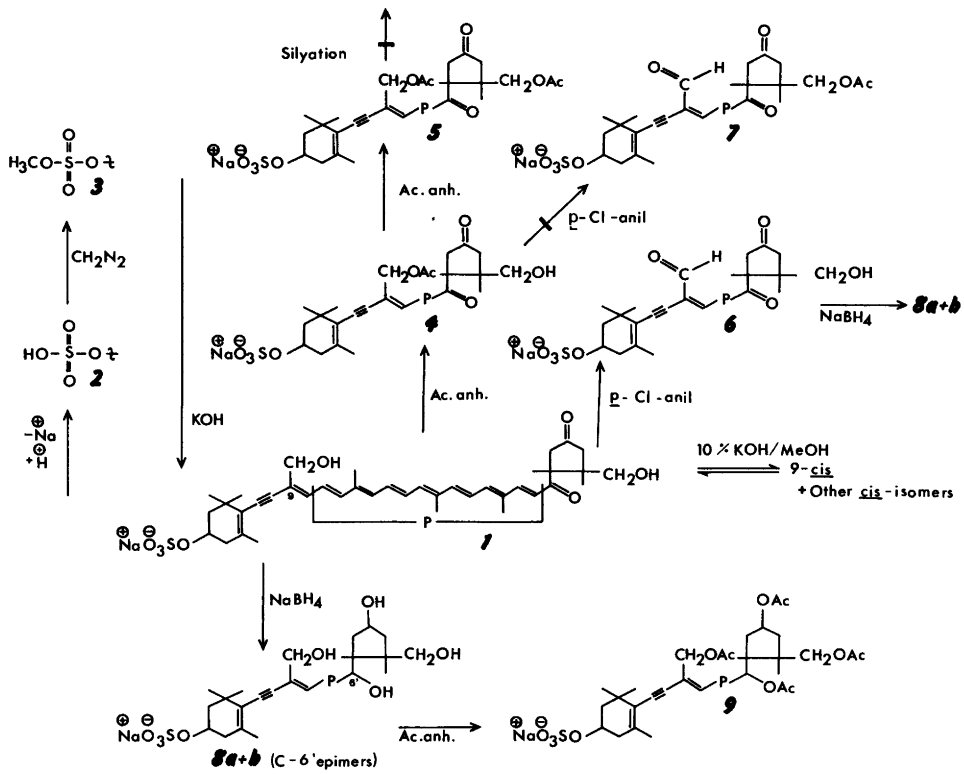
The high polarity and water solubility of bastaxanthin were striking. Previously high polarity of carotenoids has been associated with either carboxylic acids, phenols, enols or sugar derivatives. No such functions were present.

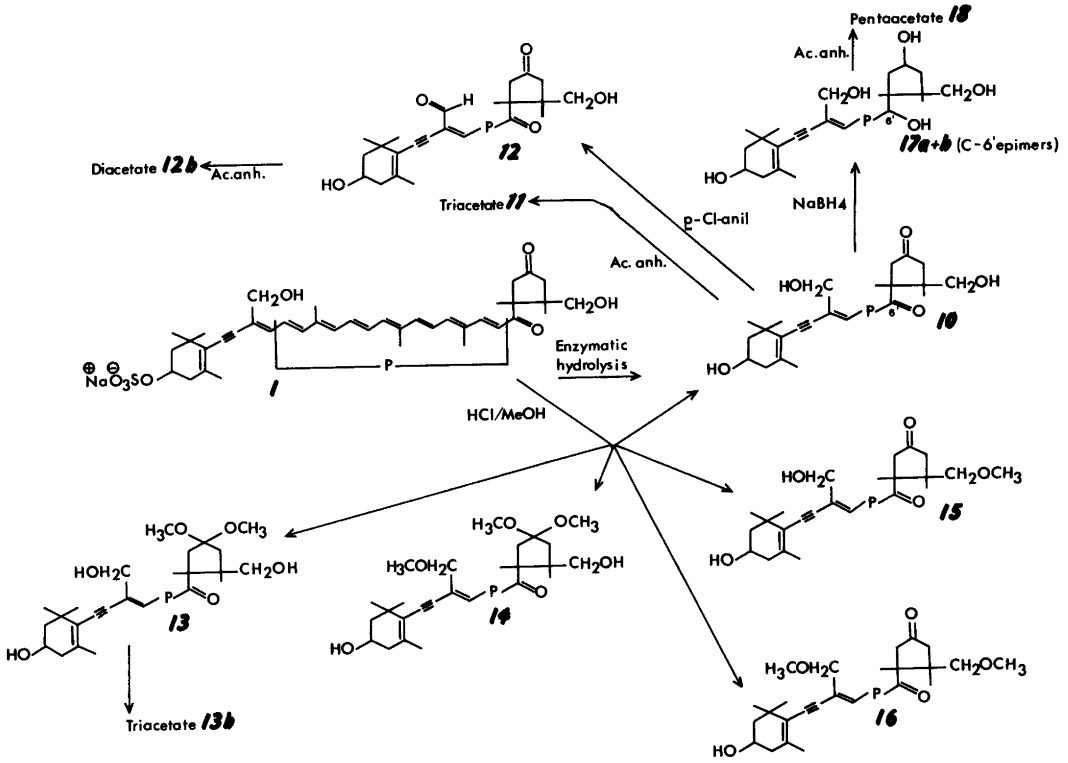
Monoesters of sulfuric acid, alkyl sulfuric acids, are approximately as acidic as sulfuric acid and readily form inorganic salts,[7] Scheme 1. Bastaxanthin was subsequently shown to be a sulfate ester of this type. Being ionized in neutral solution metal alkyl sulfates in general are strongly polar compounds and exhibit water solubility.

The recognition of bastaxanthin as a carotenoid sulfate was hampered by the initial failure of identifying the presumed molecular ion in the mass spectrum as that of a thermal elimination product. Partial syntheses of several model carotenoid sulfates [5,8] subsequently revealed that the thermal elimination of $NaHSO_4$ from their sodium salts prior to ionization is a general phenomenon, *cf.* Scheme 4.

The presence of a sulfate function in bastaxanthin (*1*) was indicated by strong IR absorption at 1240 cm^{-1}.[9] Micro sulfur analysis by X-ray fluorescence spectroscopy confirmed the presence of sulfur. The negative charge was confirmed by its electrophoretic behaviour in comparison with synthetic carotenoid mono- and disulfates. When passed through a suitable ion exchange column the free acid *2*, Scheme 2, was eluted, as confirmed by pH measurement. The acid *2* and the sodium salt *1* had the same R_F-value on adsorption chromatography and were stable in dilute solutions. Methylation with diazomethane gave in 12 % yield the methyl ester *3* of lower polarity. The methyl ester *3* upon electron impact showed a small (<1 %) molecular ion, but a strong M−30 fragment ion, consistent with the reported loss of methanal from the molecular ion of dimethyl sulfate.[10] Upon treatment with various commercial sulfatases bastaxanthin (*1*) was enzymatically desulfated to bastaxanthol (*10*, Scheme 3) of lower



Scheme 2. Sulfated derivatives of bastaxanthin (*1*).

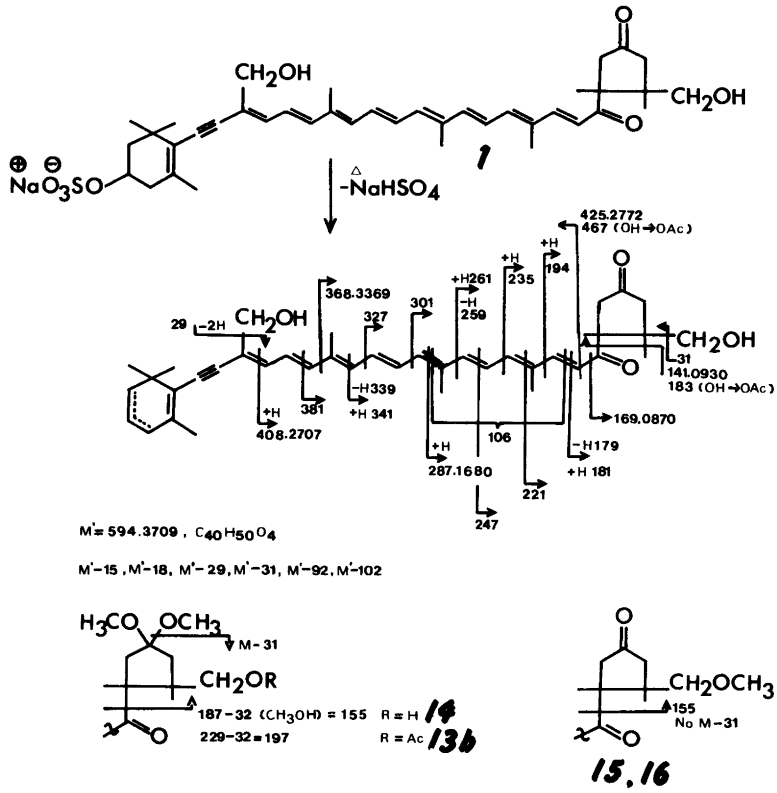


Scheme 3. Non-sulfated derivatives of bastaxanthin (*1*).

polarity, also encountered as a minor carotenoid constituent in *I. basta*. Acid hydrolysis gave the same product (*10*) in addition to secondary products (Scheme 3) to be discussed below. Finally the ^{1}H NMR and ^{13}C NMR spectra in comparison with those of synthetic model sulfates,[5,8] as well as the mass spectra of the therminal elimination products of bastaxanthin and its sulfated derivatives (Scheme 2) are compatible with a sulfate function.

Turning now to a consideration of the chromophore assigned to bastaxanthin (*1*, see Scheme 2) the presence of a disubstituted triple bond followed from IR absorption at 2170 cm^{-1} (KBr) and ^{13}C NMR signals at δ 91.7 and 98.1 (CD_3OD). The electronic spectra of native bastaxanthin (*1*), its reduction product *8* with $NaBH_4$ and allylic oxidation product *6* with *p*-chloranil is consistent with the monocyclic en-yn-octaenone chromophore assigned. Capsanthin [11] has the same chromophore as bastaxanthin (*1*) except for the triple bond, and crocoxanthin [12] has the same chromophore as the $NaBH_4$-reduced derivative *8*. The bathochromic shift (20 nm in methanol) observed upon allylic oxidation is compatible with the formation of a cross-conjugated aldehyde.[13,14] Preference for C-19 location for the allylic, primary hydroxy group follows from ^{1}H NMR, ^{13}C NMR and MS data discussed below (Schemes 5, 6 and 4, respectively). These spectra also support the common methyl substitution pattern of the chromophore. The reduced spectral fine-structure in the electronic spectrum of bastaxanthin (*1*) *versus* capsanthin and of *8* *versus* crocoxanthin is now ascribed to the influence of the hydroxy substituent at C-19, reducing the planarity of the chromophore, *cf.* similar effects for loroxanthin [15] (19-hydroxy-lutein) *versus* lutein.[16]

Isomerization to *Δ9-cis* is known to occur readily in related acetylenic carotenoids [17] and was effected by treatment with base. The presumed *Δ9-cis* isomer could only be isolated on analytical commercial silica plates, and was partly



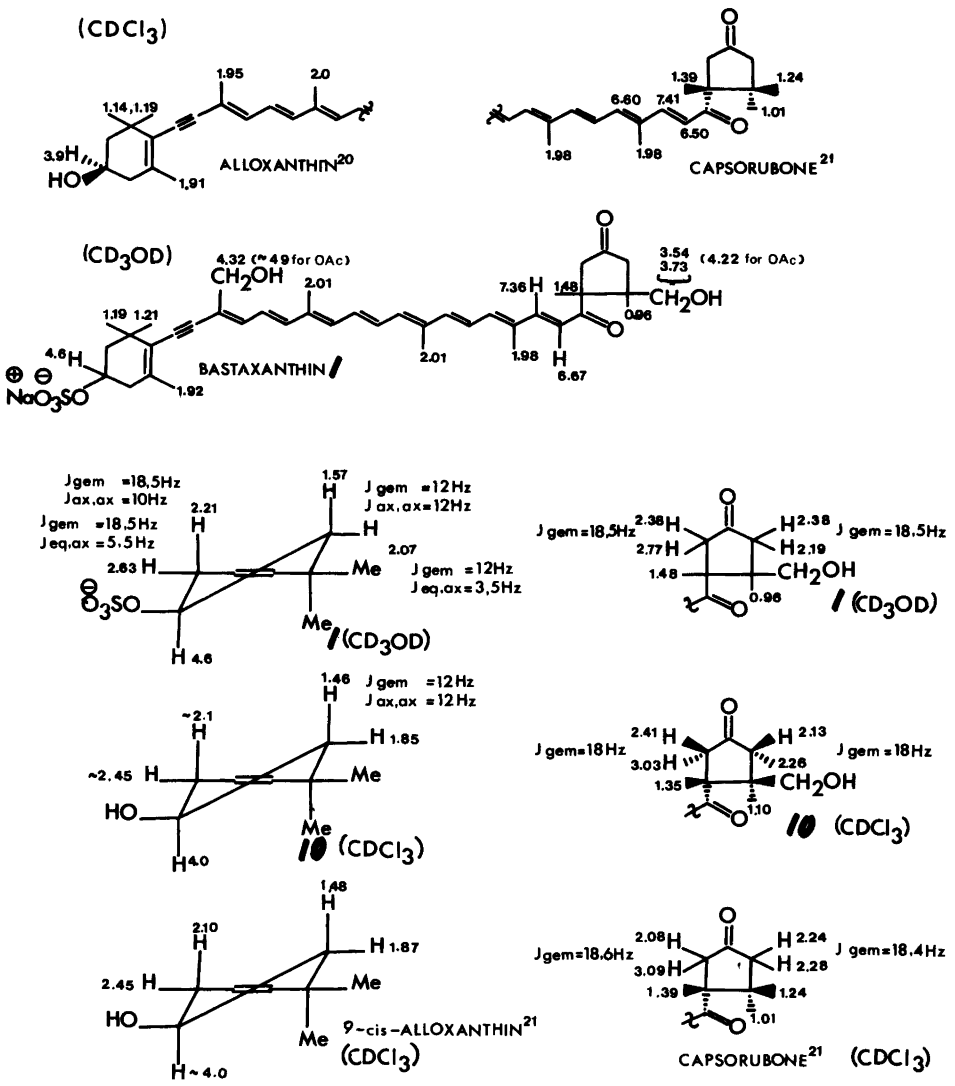
Scheme 4. Mass spectrometric fragmentations of bastaxanthin (*1*) and derivatives.

reversibly isomerized to the all-*trans* isomer in the presence of base. ^{1}H NMR (CD_3OD) of an isomerized mixture showed singlets at δ 4.32 (all-*trans*) and δ 4.18 (9-*cis*) for the $-CH_2OH$ protons at C-19, consistent with previous findings for related in-chain substituted allylic carotenols,[18] and concomitant, small downfield shifts of the CH_3-18 and CH_3-16 or -17 signals.

The mass spectrum of a therminal elimination product, $C_{40}H_{50}O_4$ by precise mass measurements (Scheme 4), obtained from bastaxanthin (*1*), showed characteristic losses of 92 (toluene) and 106 (xylene) mass units from the polyene chain.[19] The loss of 106 mass units defines the C-8′–C-13′ structural element in the non-acetylenic half of the molecule.[19] Other losses in the upper mass region are compatible with the loss of a CHO radical, previously noted for agelaxanthin with analogous C-19 hydroxy substitution,[14] CH_3 and CH_2OH radicals and H_2O. Observed fragment ions are rationalized by the in-chain cleavages indicated in Scheme 4, six of them were confirmed by precise mass measurements. Upon cleavage the charge is nearly exclusively retained on the ketonic part of the molecule. The *m/e* 141.0930 ion ($C_8H_{13}O_2$) assigned by cleavage of the C-5′–C-6′ bond occurs at *m/e* 183 for bastaxanthin diacetate (*5*, Scheme 2), consistent with the presence of one hydroxy group accessible for acetylation in this moiety.

Having now accounted for the presence of a sulfate function, a conjugated keto group, a primary allylic hydroxy group and a second *prim/sec* hydroxy group the final oxygen function in bastaxanthin *1* was, according to IR absorption at 1735 cm^{-1}, compatible with a five-ring ketone. Allocation of this keto group, the sulfate and the second hydroxy function rests on ^{1}H NMR (Scheme 5) and ^{13}C NMR (Scheme 6) data and chemical derivatizations (Schemes 2 and 3).

Assignments of the ^{1}H NMR spectrum (400 MHz) of bastaxanthin (*1*, in CD_3OD for solubil-



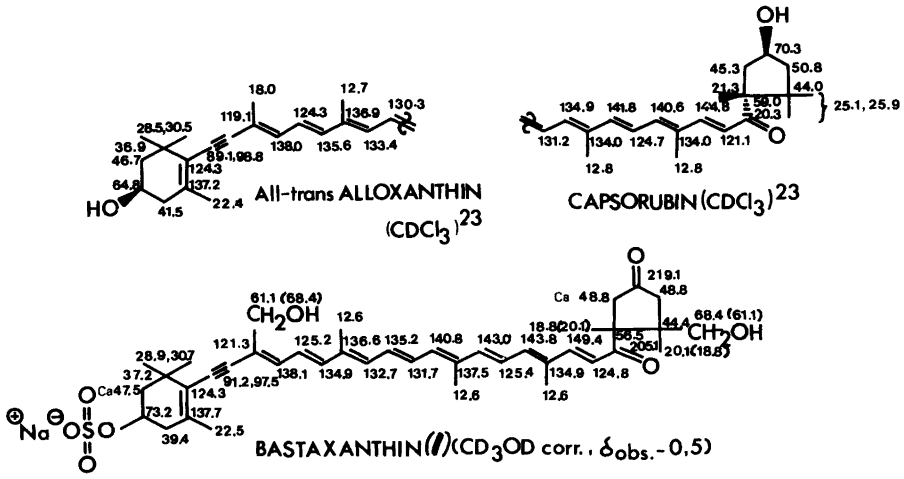
Scheme 5. ^{1}H NMR assignments for bastaxanthin (*1*) and bastaxanthol (*10*).

ity reasons) are aided by comparison with reported data ($CDCl_3$) for alloxanthin [20,1] and capsorubone,[21] Scheme 5.

The sulfate function is clearly assigned to C-3 in an alloxanthin end group by consideration of chemical shifts, coupling patterns and relevant decoupling experiments in comparison with data for alloxanthin [20,21] and related sulfated carotenols.[5,8] The ^{1}H NMR data ($CDCl_3$) for the desulfated bastaxanthol (*10*, obtained by enzymatic or acidic hydrolysis, Scheme 3) support this assignment.

C-3′ location for the five-ring ketone is consistent with the chemical shifts and coupling pattern (J=18 Hz) of four α-methylene protons in bastaxanthin (*1*, CD_3OD) and bastaxanthol (*10*, $CDCl_3$). Treatment with KOD in CD_3OD caused readily D-exchange of all four α-methylene protons, demonstrated by ^{1}H NMR and MS.

An AB system centered at δ 3.54 and 3.73 (2H, J=11.5 Hz), confirmed by spin tickling, is assigned to the diastereotopic methylene protons



Scheme 6. Tentative ^{13}C NMR assignments of bastaxanthin (*1*).

of a hydroxymethyl group attached to C-1′ or C-5′. Upon acetylation a singlet (2H) at δ 4.22 arises. Specific ASIS in pyridine of signals assigned to the two methyl groups and hydroxymethyl group of the cyclopentanone ring support carbonyl functions in their neighbourhood.[22] Preference for C-16′/C-17′ hydroxylation (*1*) *versus* C-18′ hydroxylation (*1b*, Scheme 1) rests on no retroaldol cleavage with formation of methanal upon alkali treatment as expected for a β-hydroxyketone, lacking McLafferty fragmentation with loss of methanal in the MS, no evidence of H-bonding for bastaxanthol (*10*) in ^1H NMR ($CDCl_3$) and the characteristic ^1H NMR chemical shift of CH_3-18′ in capsorubone,[21] bastaxanthin (*1*) and bastaxanthol (*10*). Plausible relative stereochemistry from chemical shift considerations is given for the cyclopentanone group of *10* (Scheme 5).

Tentative ^{13}C NMR assignments made in Scheme 6 by comparison with reported data for all-*trans* alloxanthin and capsorubin [23] and related sulfated carotenols [5] support structure *1* for bastaxanthin. The δ 219 signal is compatible with a five-ring ketone.

Chemical derivatizations of bastaxanthin (*1*) are given in Scheme 2 (sulfated derivatives) and Scheme 3 (non-sulfated derivatives). Scheme 2 summarizes the conversion of bastaxanthin (*1*) to its free acid *2* and the methyl ester *3*, relatively fast acetylation of bastaxanthin to the allylic monoacetate *4*, resistant towards allylic oxidation, and a diacetate *5* which could not be silylated. Allylic oxidation of bastaxanthin (*1*) gave the cross-conjugated aldehyde *6*, which upon acetylation afforded the monoacetate *7* and upon $NaBH_4$-reduction the tetrol *8* as two presumed C-6′ epimers *8a* and *8b*. The same products (*8a+b*) were obtained by complex metal hydride reduction of bastaxanthin (*1*) and were converted to the tetraacetate *9*.

Scheme 3 summarizes the non-sulfated derivatives obtained from bastaxanthin (*1*), including bastaxanthol (*10*) formed by enzymatic hydrolysis accompanied by pronounced 9-*cis* isomerization. Enzymatically produced bastaxanthol (*10*) was identical with natural *10*, characterized as a new, naturally occurring carotenoid. Bastaxanthol (*10*) provided a triacetate *11* upon acetylation, the cross-conjugated carotenal *12* upon allylic oxidation (*12* further gave the diacetate *12b* upon acetylation) and the pentol *17* (*a+b*, C-6′ epimers) upon $NaBH_4$-reduction. Unexpected for a carotenoid, bastaxanthin (*1*) did not decompose with strong acid and provided upon treatment with 0.1 N HCl in methanol for 30 min (70 % pigment recovery) in addition to unreacted bastaxanthin (*1*, 20 % of total) five less polar products of unchanged chromophore. The functional group modifications were determined by MS and acetylation. The most polar product bastaxanthol (*10*) was preceded by the triol

dimethyl ketal *13* and its allylic methyl ether *14*. Dimethyl ketal formation was demonstrated by MS, see also characteristic fragmentation in Scheme 4 for *13b* and *14*. Unusual, facile formation of a non-cyclic ketal may be explained by release of ring strain in the substituted cyclopentanone end group. It is also noteworthy that bastaxanthol dimethyl ketal (*13*) was isolated as a minor carotenoid from *I. basta* (Batch 4+5) and probably represents an artefact of bastaxanthol (*10*) and produced during manipulations with methanol. Low yield of a dimethyl ketal was also obtained in parallel experiments involving treatment of capsanthinone [26] with HCl–methanol. Two less polar, minor products had polarity and MS properties (Scheme 4) compatible with the monomethyl ether *15* and the diether *16*, respectively. Nucleophilic attack of methanol on a protonated *prim.* hydroxy function (C-17′) by S_N2 mechanism is considered more likely than intramolecular attack of the *prim.* hydroxy group on the 5-ring ketone to a cyclic hemiketal followed by methyl ketal formation, since the intermediary cation would not be planar.

Regarding the chirality of bastaxanthin (*1*), natural *1*, bastaxanthin diaceatate (*5*) and bastaxanthol (*10*) exhibited similar CD spectra with a positive peak at 285 nm (with measured $\Delta\varepsilon$= +10.2, +12 and +2.5, respectively). Alloxanthin (half-structure, Scheme 5) has a weak, negative Cotton effect.[25] Capsorubin [26] (half-structure, Scheme 6) has a positive peak at 300 nm (methanol, $\Delta\varepsilon$=*ca.* +10) and also capsorubone (half-structure, Scheme 5) has a positive peak at 304 nm (dioxane, $\Delta\varepsilon$=+6.3; Dr. H. Mayer, personal communication), consistent with previously reported ORD data.[11] The positive Cotton effect seems therefore largely to be governed by the chirality at C-5,5′, and the same chirality at C-5′ for bastaxanthin (*1a*) and capsorubone is assumed. The additivity hypothesis has previously been used successfully for carotenoids with κ end groups.[11] On biogenetic grounds [27] bastaxanthin is expected to exhibit the same chirality at C-3 as alloxanthin. Guided by CD and ^{1}H NMR the tentative stereochemical assignment *1a*, Scheme 1, for bastaxanthin (3*R*,1′*R*,5′*R*) is considered.

EXPERIMENTAL

Methods. If not specified, these were as commonly employed for carotenoid work in our laboratory.

Electronic spectra were recorded on a Coleman Hitachi 124 or Beckmann-DB spectrophotometer, using $E^{1\%}_{1\,cm}$=2500 at λ_{max} for calculation of concentrations, % III/II as a measure of spectral fine-structure and D_B/D_{II} as a measure of *cis*-peak intensity;[28] IR spectra on a Perkin Elmer 257 or 580 B instrument in KBr disc; ^{1}H NMR spectra on a Jeol JNM-FX 100 (100 MHz) FT instrument or a Bruker WM (400 MHz) spectrometer; ^{13}C NMR spectra on the above Jeol instrument (25.1 MHz); MS on an AEI MS902 instrument with direct inlet and CD spectra on a Roussel-Jouan Dicrograph. MS peak intensities are quoted for selected spectra. Diagnostically useful ions only (often without intensities) are cited for less purified derivatives. X-Ray fluorescence spectroscopy was carried out by cand.real. S. Melsom, Central Institute for Industrial Research, Blindern, Oslo, on a Philips 1410 X-ray spectrometer using the Kα-radiance of S as a measure of concentration. R_F-values for sulfated carotenoids are not well reproducible and only meaningful in relation to a reference sulfate.

Biological source. The marine sponge *Ianthella basta* (Porifera, class Desmospongiae, subclass Ceractinomorpha, order Verongida, family Ianthellideae),[1] RRIMP Museum specimen FN 1784/01/000, was collected by Roche Research Institute of Marine Pharmacology, Dee Why, Australia, on the Great Barrier Reef off the coast of Queensland.

In total 5 batches, each up to 6 kg sponge, were examined. No marked difference in the content of polar carotenoids [1] were noted for lyophilized or frozen sponge material.

Extraction was effected with MeOH or acetone–MeOH at room temperature, for Batches 4 and 5 followed by a partition into epiphasic (non-polar) and hypophasic (polar) carotenoids in ether–H_2O 2:1. Hypophasic carotenoids were transferred to EtOAc from H_2O.

Chromatography. Suitable systems for separation of the sulfated carotenoids were: Sephadex LH20 (MeOH), ion exchange chromatography,[30] kieselgel G60 Merck Labor Ferligsaüle (Art. No. 10401) (column packed wet in acetone, EtOAc or 5–10 % MeOH/EtOAc, for preliminary purification), pressurized (0.3–2 atm.), flow *ca.* 15 ml/min) kieselgel G60 (40–63 μm) columns (20 % MeOH in EtOAc, for further purification), cellulose columns (Linters No. 124 or

Avicel, eluant MeOH-acetone), preparative TLC (SiO_2, MeOH-EtOAc) and analytical TLC (Merck No. 5553 DC-Alufolien Kieselgel 60, 0.2 mm). Unsuitable adsorbents were acetylated polyamide and $CaCO_3$ columns.

Several different combinations were used for the various batches depending on quantities and the presence of non-carotenoid contaminants.

Precipitation of contaminants from crude extracts and chromatography fractions were effected at −20 °C from (i) acetone and (ii) MeOH/EtOAc. The colourless precipitates were removed by centrifugation and the process repeated up to 8 times.

Saponification. Particularly oily fractions were submitted to standard saponification (5 % KOH in EtOH−ether overnight) after it had been demonstrated that such alkali treatment caused no other modification of bastaxanthin than *cis*-isomerization.

Yield. The yield was greatly reduced by repeated chromatography and precipitations. Chromatographically purified bastaxanthin available for further studies were *ca.* 3.5+20+10+13+22 mg from the five batches.

Bastaxanthin (*1*)

Bastaxanthin, as salt (1). 1 was isolated as an unspecified salt by extraction and chromatography, as the Na-salt by standard saponification with NaOH in methanol−ether of the diacetate *5* below or as the Ba-salt by precipitation with Ba-acetate in aqueous methanol.

Crystallization of tiny samples was effected from acetone−hexane or EtOH−ether; m.p. (corr., evacuated tube) *ca.* 190 °C.

Sulfur analysis by X-ray fluorescence spectroscopy gave 44 μg S in 0.51 mg *1* (calc. 22.5 μg S).

VIS λ_{max} (MeOH) 360, 474 nm, % D_B/D_{II} <16; (acetone) 474 nm.

IR ν_{max} (KBr) 3430 (vs, OH), 3040 (w, CH=), 2975, 2930 and 2880 (s, CH), 2170 (w, C≡C), 1735 (s, 5-ring C=O), 1660 (s, conj. C=O), 1550 and 1520 (vs, C=C), 1490 (w), 1460 (vw, CH_2), 1420 (vw), 1400 (w), 1240 (vs, S=O), 1160 (vw), 1120 (vw), 1065 (vs, S−O?), 1050 (vs, C−O in CH_2OH), 1010 (w, allylic CH_2OH), 1000 (w). 965 (vs, *trans* CH=CH), 910 (vw), 835 (m, R_2C=CHR) and 790 (w) cm^{-1}.

^1H *NMR, cf.* assignments Scheme 5, δ (CD_3OD, fresh solution, 400 MHz) 0.96 s (3H, CH_3−16′), 1.19 s (3H) and 1.21 s (3H, CH_3−16, 17), 1.48 s (3H, CH_3−18′), 1.57 t (J=12 Hz, 1H, H−2_{ax}), 1.92 s (3H, CH_3−18), 1.98 s (3H, CH_3−19′), 2.01 s (6H, CH_3−20,20′), 2.07 dd (J_{gem}=12 Hz, $J_{ax,eq}$=3.5 Hz, 1H, H−2_{eq}), 2.19 d (J_{gem}=18.5 Hz, 1H, H−2′), 2.21 dd (J_{gem}=18.5 Hz, $J_{ax,ax}$=10 Hz, 1H, H−4_{ax}), 2.38 d (J_{gem}=18.5 Hz; 2H, H-2′, H-4′), 2.63 dd (J_{gem}=18.5 Hz, $J_{eq,ax}$=5.5 Hz, 1H, H-4_{eq}), 2.77 d (J_{gem}=18.5 Hz, 1H, H-4′), 3.54 d (J=11.5 Hz, 1H, H_a-17′), 3.73 (J=11.5 Hz, 1H, H_b-17′), 4.18 s (trace, H-19 in Δ9-*cis*), 4.32 s (2H, H-19), 4.6 m (1H, H-3), 6.67 d (J=14.5 Hz, 1H, H-7′), 7.36 d (J=14.5 Hz, 1H, H-8′), 6.3−6.8 m (10H, olefinic). Homonuclear spin decoupling was effected. The first figure cites frequency of irradiation in ppm and the second figure observed change at ppm: 7.36−6.67 (d→s), 6.68−7.36 (d→s), 4.6−1.57 (t→d), 2.07−2.21 (dd→d) and 2.63 (dd→d), 2.63−2.21, 2.6−4.61.

Spin tickling confirmed the relationship between the δ 3.54 and δ 3.73 doublets. Storage in CD_3OD or treatment with KOD/CD_3OD caused disappearance of the δ 2.19, 2.38 and 2.77 signals.

δ (D-pyridine) 1.13 s (3H, CH_3−16′), 1.22 s (3H) and 1.30 s (3H), CH_3−16,17, 1.63 s (3H, CH_3−18′), 1.90 s (6H, CH_3−18,19′), 2.01 s (6H, CH_3−20,20′), 2.34−3.28 m (*ca.* 6H, CH_2), 3.78 d (J=12 Hz, H_a−17′), 4.00 d (J=12 Hz, H_b−17′), 4.80 s (2H, H-19), 5.2 m (1H, H-3), 6.4−7 m (olefinic H), 7.68 d (J=15 Hz, H-8′).

^{13}C *NMR, cf.* tentative assignments, Scheme 6, δ (CD_3OD, 10 mg *1*): 13.15 (C-20,19′,20′), 19.35 (C-18′ or C-16′), 20.58 (C-16′ or C-18′), 22.97 (C-18), 29.41 and 31.28 (C-16,17), 37.72 (C-1), 39.94 (C-4), 44.91 (C-1′), *ca.* 48 (C-2), *ca.* 49.3 (C-2′ and C-4′), 56.96 (C-5′), 61.59 (C-19 or C-17′), 68.96 (C-17′ or C-19), 73.75 (C-3), 91.71 (C-7), 98.09 (C-8), 121.84 (C-9), 124.83 (C-6), 125.35 (C-7′), 125.76 (C-11), 125.94 (C-11′), 132.20 (C-14′), 133.25 (C-14), 135.47 (C-12 and C-9′), 135.77 (C-15), 137.11 (C-13), 138.05 (C-13′), 138.22 (C-5), 138.57 (C-10), 141.27 (C-14′), 143.49 (C-12′), 144.31 (C-10′), 149.92 (C-8′), 205.62 (C-6), 219.60 (C-3′).

MS (210 °C, 70 eV), *m/e*: 594.3721 (M′, calc. 594.3709 for $C_{40}H_{50}O_4$, 21 %), 579 (M′−15, 2 %), 576 (M′−18, 9 %), 565 (M−29, 1 %), 563 (M−31, 1 %), 558 (M′−18−18, 1 %), 530 (M′−64, 2 %), 502 (M′−92, 1 %), 488 (M′−106, 8 %), 470 (M′−124=M′−106−18, 5 %), 455 (M−139, 2 %), 428 (M′−166, 4 %), 425.2772 (calc. 425.2843 for $C_{31}H_{37}O$, 3 %), 410 (M−184, 2 %), 408.2707 (calc. 408.2664 for $C_{27}H_{35}O_3$, 2 %), 407 (2 %), 368.3369 (calc. 368.3389 for $C_{24}H_{32}O_3$, 3 %), 341 (3 %), 339 (3 %), 313 (2 %), 301 (2 %), 287.1680 (calc. 287.1648 for $C_{18}H_{25}O_3$, 4 %), 235 (17 %), 221 (1 %), 169.0870 (calc. 169.0864 for $C_9H_{13}O_3$, 16 %), 141.0930 (calc. 141.0915 for $C_8H_{13}O_2$, 55 %), 119 (45 %), 91 (100 %), 69 (60 %), 43 (100 %).

CD (MeOH): nm ($\Delta\varepsilon$) 240 (−3.1), 250 (−2.8), 258 (0), 290 (+10.2), 325 (0), 373 (−3.2).

For comparison capsorubin [26] had (MeOH): 240 (0), 249 (2.0), 299 (+9.9), 325 (0), 370 (−2.5).

R_F-value: TLC (SiO_2, 15 % MeOH−EtOAc) *ca.* 0.21; (SiO_2, 10 % MeOH−EtOAc) 0.14.

Electrophoretic behaviour. Cellulose acetate and Whatman papers were unsuitable due to irreversible pigment absorption. Acetate buffer, 0.05 M pH 7.0 containing 30 % isopropanol (for solubility reasons) was employed for (i) glass fiber sheets and (ii) polyacrylamide gel tubes at 2 m.a. Electrophoretic mobility (i) zeaxanthin 0.0 cm, zeaxanthin monosulfate 1.8 cm, bastaxanthin 2.0 cm, zeaxanthin disulfate 2.4 cm, (ii) zeaxanthin monosulfate 0.6 cm, bastaxanthin 0.7 cm, zeaxanthin disulfate 1.3 cm.

Solubility. Bastaxanthin dissolves well in MeOH, H_2O (if solid material first moistened with MeOH) and DMSO, is partly soluble in pyridine and EtOAc and badly soluble in tetrahydrofurane, ether and acetone.

Partition behaviour. Bastaxanthin was completely hypophasic when partitioned between hexane−50 % aq. MeOH.

Iodine catalyzed stereomutation in MeOH with traces of I_2 dissolved in benzene caused over longer periods no change in the electronic spectrum or formation of new zones on TLC (SiO_2).

Stereomutation by alkali treatment. Treatment of *1* (1 mg) with 5−10 % KOH in MeOH overnight caused no change in *vis.* absorption, ^{1}H NMR, MS or R_F-value (TLC, SiO_2).

Separation on Merck No. 5553 DC-Alufolien Kieselgel 60 (0.2 mm) in 15 % MeOH–EtOAc with prolonged development gave four zones:

All-*trans* bastaxanthin (*1*), major, λ_{max} (MeOH) 360, 474 nm, % D_B/D_{II}=16; MS (200 °C), *m/e* 594 (M′), 576 (M′−18), 565, 502 (M′−92), 488 (M′−106), 470 (M′−106−18), 141.

Neo A bastaxanthin (*1*, 9-*cis*?), major, λ_{max} (MeOH) 360, 470 nm, % D_B/D_{II}=20; MS (200 °C) *m/e* 594 (M′), 576 (M′−18), 488 (M′−106), 470 (M′−106−18), 141.

Neo B bastaxanthin (*1*, unspecified di-*cis*?), minor, λ_{max} (MeOH), 360, 471 nm, % D_B/D_{II}=20, MS (200 °C) *m/e* 594 (M′), 488 (M′−106), 470 (M′−106−18), 141.

Neo C bastaxanthin (*1*, unspecified mono-*cis*), major, R_F-value as bastaxanthin diacetate (*5*), λ_{max} (MeOH) 471 nm, % D_B/D_{II}=22; MS (200 °C) *m/e* 594 (M′?), spectrum contaminated with brominated metabolites; IR (KBr) 3400, 2930, 2860, 2170, 1735, (1660), 1635, 1575, 1470, 1375, 1240, 1090, 1050, 970, and 830 cm^{-1}. Acetylation provided a product with MS very similar to that of bastaxanthin diacetate (*5*) below. Prolonged treatment of the acetylated product with $NaBH_4$ in EtOH gave a reduced product λ_{max} (EtOH) 445 and 472 nm, inseparable from all-*trans* *8a* and *8b*, see below.

Slow reversible isomerization in 1 % KOH–MeOH (not readily in I_2-MeOH) of all-*trans* to Neo A, neo A to all-*trans*, neo B to neo A and of neo C to all-*trans* was demonstrated chromatographically.

Evidence for the presence of the 9-*cis* isomer in alkali-treated bastaxanthin (*1*) followed from ^{1}H NMR (CD_3OD): δ 4.18 s (=C−CH_2OD in 9-*cis*), up to 30 % of the δ 4.32 s (in all-*trans*) signal. Likewise Δ9-*cis* isomerization caused a shift of the δ 1.21 methyl signal to δ 1.30 and of the δ 1.92 methyl signal to δ 1.90, *cf.* Scheme 5.

Treatment with diazomethane. 1 (0.17 mg) in MeOH (1 ml) was treated with CH_2N_2 in ether (5 ml) for 5 min. No new products were formed according to *vis.* spectrum, MS and TLC (SiO_2).

Bastaxanthin, as acid (2). A solution of bastaxanthin (*1*, 0.95 mg) in MeOH/H_2O 1:1 was ion exchanged on a column (0.8×15 cm) packed with Dowex 50 (Fluka 44445). The eluate (10 ml) had pH 1.88. *2* had λ_{max} (MeOH) 474 nm, R_F (SiO_2, 15 % MeOH−EtOAc) 0.21 as *1*, was stable in dilute ether solution, but decolourized upon concentration.

Bastaxanthin methyl ester (3) was prepared from *2* (0.65 mg) and freshly prepared CH_2N_2 [29] in ether and isolated by TLC (SiO_2). *3* had R_F 1.00 (25 % MeOH−EtOAc), 0.24 (40 % acetone−hexane), λ_{max} (MeOH) 474 nm, MS (190 °C) *m/e* 706 (M<1 %), 676 (M−30, 5 %), 648 (3 %), 548 (5 %), 523 (6 %), 429 (40 %), 410 (60 %), 325 (7 %), 281 (12 %), 221 (18 %), 191 (24 %), 151 (50 %), 119 (21 %), 91 (60 %), 69 (65 %), 43 (100 %).

Acetylation of bastaxanthin (1). The acetylation of *1* (0.1 mg) in dry pyridine (1 ml) with acetic anhydride (0.1 ml) at 0 °C was monitored by TLC (SiO_2, 10 % MeOH−EtOAc). *1* (R_F=0.14) was converted *via* the monoacetate *4* (R_F=0.18) to the diacetate *5* (R_F=0.26). The following ratios were estimated: 5 min 50 % *1* +50 % *4*, 10 min. 20 % *1* +70 % *4* +10 % *5*, 15 min. 5 % *1* +80 % *4* +15 % *5*, 30 min, 0 % *1* +50 % *4* +50 % *5*, 45 min. 0 % *1* +30 % *4* +70 % *5*.

The mono- (*4*) and diacetate (*5*) were isolated in preparative experiments by acetylation of *1* (1−7 mg) at room temperature.

Bastaxanthin monoacetate (4), ca. 0.1 mg λ_{max} as *1*, was submitted to allylic oxidation with *p*-chloroanil [30] in EtOH for 1 h. No new, more pink products were formed judged by TLC.

Bastaxanthin diacetate (5), total yield *ca.* 10

mg; had λ_{max} (MeOH) 474 nm; IR (KBr) ν_{max} 3400 (s, OH), 3015 (w,=CH), 2960, 2910 and 2860 (s, CH), 2170 (w, C≡C), 1740 (s, acetate and 5-ring ketone), 1655 m (conj. C=O), 1555 (s), 1510 (m), 1460 (m), 1360 (m), 1230 (vs, S=O and ester), 1065 (s), 1040 (s, C–O), 970 (s, *trans*-CH=CH–), 835 (m, CR_2=CHR), and 795 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, after prolonged storage in CD_3OD, resulting in complete exchange of the four 2′,4′ protons): δ 1.04 s (3H, CH_3–16′), 1.19 s (3H) and 1.20 s (3H, CH_3–16,17), 1.47 s (3H, CH_3–18′), 1.92 s (3H, CH_3–18), 1.57 t (J=12 Hz, 1H, H-2_{ax}), 1.98 s (3H, CH_3–19′), 2.00 s (6H, CH_3–20,20′), 2.01 s (3H, OAc), 2.02 s (3H, allylic OAc), 2.21 dd (J_{gem}=18.5 Hz, $J_{ax,ax}$=10 Hz, 1H, H-4_{ax}), 2.61 dd (J_{gem}=18.5 Hz, $J_{eq,ax}$=5.5 Hz, 1H, 4-H_{eq}), 4.22 s (2H, H-17′), 4.9 s (=C–CH_2OAc), 6.3–6.9 m (olefinic H), 7.36 d (J=16 Hz, 1H, H-8′), irradiation at δ 7.34 caused the doublet at δ 6.65 (J=14.5 Hz) to collapse to a singlet; δ (100 MHz CD_3OD, fresh solution) exhibited extra CH_2 signals at *ca.* 2.35 m (*ca.* 2H, H-2,4) and 2.76 d (J=18 Hz, 1H, H-4); δ (pyridine-d_5) 1.11 s (3H, CH_3–16′), 1.25 s (lipid and CH_3–16,17), 1.46 s (3H, CH_3–18′), 1.95 s (*ca.* 3H, CH_3–18), 2.02 s (CH_3–20,19′,20′ and Ac), 2.2–3.4 m (CH_2), 4.40 s (2H, CH_2OAc), 5.12 s (=C–CH_2OAc), 6.4–7.5 m (olefinic H); MS (200 °C) *m/e* 678 (M′, 7 %), 663 (M′–15, <1 %), 618 (M′–60, 3 %), 572 (M′–106, 3 %), 512 (M–166, 1 %), 497 (2 %), 407 (2 %), 183 (8 %), 105 (90 %), 91 (90 %), 69 (49 %) and 43 (100 %); CD (MeOH) nm ($\Delta\varepsilon$) 232 (–3.8), 247 (–3.8), 258 (0), 290 (+12), 325 (0), 380 (–2.8).

Attempted silylation of bastaxanthin diacetate (5). *5* (0.15 mg) was submitted to standard silylation conditions [31] at room temperature. No new products were formed according to TLC.

Alkaline hydrolysis of bastaxanthin diacetate (5) at standard conditions [31] in 5 % KOH–methanol provided bastaxanthin (*1*) according to *vis.* spectrum, ^1H NMR, MS, and TLC (SiO_2).

Allylic oxidation of bastaxanthin (1). *1* (0.3 mg) in abs. EtOH (0.5 ml) and benzene (4 ml) was reacted with *p*-chloranil (1.5 mg) for 3 h. Additional *p*-chloranil (1.5 mg) and traces of I_2/benzene were added [30] and the reaction interrupted after 10 h at room temperature; pigment recovery 50 %. TLC revealed the formation of a slightly less polar, pink product *6*, R_F=0.22 (SiO_2, 15 %, MeOH–EtOAc); λ_{max} (MeOH) 495 nm; no MS could be obtained.

Acetylation of the allylic oxidation product 6 at 0 °C was monitored by TLC. *6* (0.1 mg) was converted to the monoacetate (*7*, R_F=0.29 on SiO_2, 15 % MeOH–EtOAc). The following ratios were estimated: 3 min 98 % *6* +2 % *7*, 10 min. 90 % *6* +10 % *7*, 15 min. 80 % *6* +20 % *7*, 30 min 70 % *6* +30 % *7*, and 4 h 100 % *7*.

$NaBH_4$ reduction of allylic oxidation product 6. Treatment of *6* (0.05 mg) with $NaBH_4$ in EtOH caused reduction to *8a+b* (1:1), λ_{max} (MeOH) (325), 338, (418), 443, and 471 nm, inseparable from *8a* and *8b* characterized below.

Complex metal hydride reduction of bastaxanthin (1). Treatment at 0 °C for 10 min of bastaxanthin (*1*, 0.1–0.5 mg aliquots) with (i) excess $NaBH_4$ in EtOH or (ii) $LiAlH_4$ in dry tetrahydrofurane or of bastaxanthin diacetate (*5*) with excess $NaBH_4$ in EtOH at room temperature gave the same reduction product *8*.

All-*trans* bastaxanthin (*1*, not previously alkali treated) gave on the commercial kieselgel plates two products (*8a* and *8b*) in 1:1 ratio, considered as all-*trans* C-6′ epimers.

Previously alkali-treated bastaxanthin gave four products, considered as mainly all-*trans* *8a* and *8b* (1:1) and mono-*cis* *8a* and *8b* (1:1), the latter pair being slightly more strongly adsorbed. Upon storage the mono-*cis* isomers were partly converted to the all-*trans* isomers. The isomerization occurred more rapidly in the presence of 5 % KOH in MeOH.

Reduced bastaxanthin 8. All-*trans* reduction product *8a+b*, R_F=0.18 (SiO_2, 15 % MeOH–EtOAc), had λ_{max} (MeOH) (325), 335, (418), 443, and 472 nm, % D_B/D_{II}=15 and % III/II=24. Mono-*cis* reduction product *8a+b* had R_F=0.22 (SiO_2, 15 % MeOH–EtOAc), λ_{max} (MeOH) (325), 335, (418), 443 and 472 nm, % D_B/D_{II}=16 and % III/II=24.

8 had λ_{max} (MeOH) (325) 338, (418), 443, and 471 nm % D_B/D_{II}=15, % III/II=22, IR (KBr) ν_{max} 3400 (s, OH), 3015 (w, =CH), 2960, 2920 and 2860 (s, CH), 2170 (w, C≡C), 1460 (m), 1230 (s, S=O), 1065 (m), 965 (s, *trans* CH=CH), and 835 (w, R_2C=CHR) cm^{-1}; ^1H NMR (CD_3OD) δ 0.95 s (3H, CH_3–16′), 1.19 s (3H) and 1.22 s (3H, CH_3–16,17), 1.28–1.6 (imp. and CH_3–18′), 1.95 s (6H, CH_3–18,19′), 1.98 (6 H, CH_3–20,20′), 3–4 (imp. and H-17′), 4.33 s (2H, =C–CH_2OH), and olefinic H; MS (220 °C) *m/e* 598 (M′, 1 %), 596 (M′–2, 1 %), 583 (M′–15, 5 %), 492 (M′–106, 1 %), 477 (M′–106–15, 2 %), 455 (2 %), 256 (19 %), 145 (16 %), 143 (24 %), 105 (40 %), 91 (60 %), 69 (50 %), 43 (100 %).

Standard acetylation of *8* (0.7 mg) provided after purification by TLC the tetraacetate *9* (0.5 mg).

Tetraacetate 9 *of reduced bastaxanthin.* *9* had R_F=0.67 (SiO_2, 25 % MeOH–EtOAc); λ_{max} (MeOH) 420, 444 and 470 nm, % III/II=23; ^1H NMR (CD_3OD) δ 1.09 s (lipid and CH_3-16′), 1.18 s (3H) and 1.20 s (3H, CH_3-16,17), 1.26 s

(lipid and CH_3-18'), 1.92 s (*ca.* 6H, CH_3-18,19'), 1.98 s (*ca.* 9H, CH_3-20,19',20'), 2,02 s (3H, Ac), 2.03 s (3H, Ac), 2.08 s (6H, two Ac), 2.1–3.0 m (CH_2), 4.07 d (J=11 Hz, 1H, H_a-17'), *ca.* 4.1 m (H-3), 4.23 d (J=11 Hz, 1H, H_b-17'), 4.83 s (2H, =C–CH_2OAc), and 6.2–6.8 m (olefinic H); MS (210 °C) *m/e* 776 (M', <1 %), 183 (13 %), 119 (41 %), 91 (67 %), 60 (80 %), 43 (100 %).

Semisynthetic bastaxanthol (*10*)

Enzymatic hydrolysis of bastaxanthin (1). Enzymes used were purchased from Sigma Chemical Company, St. Louis, Missouri, and were isolated from (i) *Helix pomatia* or (ii) *Patella vulgaris*. Equal weights of carotenoid and enzyme were used. Experiments carried out in 0.2 M acetate buffer or 0.2 % NaCl solution were unsuccessful due to salting out of the carotenoid.

Bastaxanthin (0.5–1 mg) was dissolved in 1 drop of MeOH, the solution diluted with 1.5 ml H_2O and treated with the enzyme at 37 °C for *ca.* 24 h. After transfer to EtOAc the pigment recovery was 90–100 % with 20–30 % conversion to bastaxanthol.

Bastaxanthol (10), total yield from enzymatic hydrolysis *ca.* 2 mg, R_F=0.87 (SiO_2, EtOAc), R_F=0.40 (SiO_2, 40 % acetone–hexane); λ_{max} (acetone) 362, 469, (495), (hexane) 360, 470 (495), (MeOH) 360, 470 and (benzene) 373, 486 nm; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.07 s (3H, CH_3-16'), 1.15 s (3H) and 1.22 s (3H, CH_3-16,17), 1.42 s (3H, CH_3-18'), 1.94 s (3H, CH_3-18), 1.96 s (9H, CH_3-20,19',20'), 2.13 d (J=18 Hz, 1H, H_a-2'), 2.26 d (J=18 Hz, 1H, H_b-2'), *ca.* 2.45 (H-4), 2.42 d (J=18 Hz, 1H, H_a-4'), 3.02 d (J=18 Hz, 1H, H_b-4'), 3.59 d (J=12 Hz, H_a-17'), 3.81 d (J=12 Hz, H_b-17'), 4.04 m (1H, H-3), 4.22 s (=C–CH_2OH, Δ 9-*cis*, *ca.* 35 % rel. *trans*), 4.38 s (=C–CH_2OH, *trans*), 6.2–6.8 m (olefinic H), 7.52 d (J=14 Hz, 1H, H-8'); δ (100 MHz, CD_3OD, protons at 2',4' partly exchanged) δ 0.97 s (*ca.* 3H, CH_3-16'), 1.19 s (*ca.* 3H) and 1.22 s (*ca.* 3H, CH_3-16,17), 1.48 s (*ca.* 3H, CH_3-18'), 1.92 s (*ca.* 3H, CH_3-18), 2.00 s (*ca.* 9H, CH_3-20,19',20'), 2.1–3.0 m (CH_2), 3.50 d (J=12 Hz, 1H, H_a-17'), 3.72 d (J=12 Hz, 1H, H_b-17'), 4.18 s (=C–CH_2OH, in Δ9-*cis*, *ca.* 40 % of *trans* signal), 4.36 (=C–CH_2OH, *trans*) 6.2–6.8 m (olefinic H), and 7.38 d (J=14 Hz, 1H, H-8'); δ ($CDCl_3$, 400 MHz) 1.10 s (*ca.* 3H, CH_3-16'), 1.15 s (CH_3-16 or 17 in all-*trans*), 1.20 s (*ca.* 3H, CH_3-17 or 16), 1.29 s (CH_3-16 or 17 in Δ9-*cis*), 1.35 s (3H, CH_3-18'), 1.46 t (J_{gem}=12 Hz, $J_{ax,ax}$=12 Hz, 1H, H-2_{ax}), 1.86 dd (J_{gem}=12 Hz, $J_{eq,ax}$=*ca.* 3 Hz, H-2_{eq}), 1.93 s (<3H, CH_3-18 in all-*trans*), 1.95 s (6H, CH_3-19,20'). 1.97 s (CH_3-18 in Δ9-*cis*), 1.99 s (3H, CH_3-19'), 2.13 d (J=18 Hz, 1H, H-2'$_a$), 2.26 d (J=18 Hz, 1H, H-2'$_b$) 2.41 d (J=18 Hz, H-4'$_a$), *ca.* 2.10 m (*ca.* 1H, H-4_{ax}),[21] *ca.* 2.45 m (*ca.* 1H, H-4_{eq}),[21] 3.03 d (J=18 Hz, 1H, H-4'$_b$), 3.49 dd (J=11 Hz, J_2=10 Hz, 1H, H_a in –CH_2OH at C-17'), 3.61 s broad (1H, OH), 3.80 d (J=11 Hz, 1H, H_b in CH_2OH at C-17'), 4.00 s broad (1H, H-3), 4.22 s (=C–CH_2OH, Δ 9-*cis*, *ca.* 40 % of *trans* signal) 4.38 s (=C–CH_2OH in all-*trans*), 4.42 s broad (1H, OH, signal disappears upon D_2O addition), 6.3–6.9 m (*ca.* 11 H, olefinic) with tentative assignments, 6.31 d (1H, J=9 Hz, H-10), 6.34 d (1H, J=12.5 Hz, H-7'), 6.39 d (1H, J=10 Hz, H-14), 6.41 d (1H, J=12.5 Hz, H-12), 6.46 d (1H, J=14 Hz, H-12'), 6.52 d (1H, J=9 Hz, H-14'), δ 6.58–6.73 m (*ca.* 5H, H-15,15',11,11',10'), 6.88 dd (<1H, J_1=8 Hz, J_2=13 Hz, H-11 in Δ 11 *cis*?); 7.52 d (J=15 Hz, 1H, H-8); signals ascribed to impurities δ 0.87 and 1.25 (lipid), 1.56 (H_2O), 2.59 dd (J_1=8 Hz, J_2=16 Hz, *ca.* 1H), 2.79 t (J=8 Hz, <1H), 2.86 t (J=8 Hz, <1H), 4.07 dd (J_1=8 Hz, J_2=16 Hz, *ca.* 1H), 5.05 s (<1H, signal remains after D_2O addition), 6.98 s (*ca.* 1H, not H-bonded OH since present also in CD_3OD spectrum of *10*) and signal did not disappear upon addition of NaOD/D_2O; MS (200 °C) *m/e* 612.3801 (calc. 612.3815 for $C_{40}H_{52}O_5$), 594 (M–18), 576 (M–18–18), 506 (M–106), 141, 105, 91, 69 and 43.

D-exchange of bastaxanthol (10). After D-exchange in NaOD/CD_3OD/D_2O followed by TLC (SiO_2) and elution with CH_3OH d_{1-4}-bastaxanthol (*10*) had MS (200 °C) corresponding to that of *10* with *m/e* 616, 615, 614, 613 (M) *etc.*

In a parallel experiment, capsanthinone (M=582) was treated in the same manner and showed for d_{1-4}-capsanthinone MS *m/e* 586, 585, 584, 583 (M).

Bastaxanthol triacetate (11), prepared by standard acetylation of bastaxanthol (*10*, 0.1 mg) had R_F=0.31 (SiO_2, 10 % acetone–hexane), R_F=0.87 (SiO_2, 40 % acetone in hexane); λ_{max} (hexane) 362, (445), 470 and 498 nm, % III/II=5, (MeOH) 360, 470 nm; MS (200 °C) *m/e* 738 (M), M–60, M–106, M–60–60, M–136, 143, 105, 91, 69, 43.

Allylic oxidation of bastaxanthol (10) was effected with *p*-chloranil [30] for 3 h and resulted in a deeper pink oxidation product (*12, ca.* 30 % of recovered pigment), which could not be properly separated from *10*. Acetylation gave the presumed diacetate *12b*, R_F=0.90 (SiO_2, EtOAc); λ_{max} (MeOH) 497 nm.

Acid hydrolysis of bastaxanthin (1). To bastaxanthin (*1*, 0.56 mg cryst.) in MeOH (3 ml) was added 0.3 N HCl in MeOH (1.5 ml). The mixture

was kept at 30–40 °C for 30 min, pigment recovery after transfer to EtOAc 0.4 mg (70 %). TLC (SiO_2, 10 % MeOH–EtOAc) revealed the presence of unreacted *1* (*ca.* 20 % of total) and in order of decreasing adsorption (SiO_2) the products *10* and *13* (together *ca.* 60 % of total) and *14, 15,* and *16* (together 20 %). The more polar products *10* and *13* were further characterized after standard acetylation.

Bastaxanthol (10) from acid hydrolysis, characterized as the triacetate *11* had R_F=0.31 (SiO_2, 10 % acetone–hexane) and was inseparable from the triacetate *11* derived from bastaxanthol (*10*) from the enzymatic hydrolysis, had λ_{max} (hexane) 472 and 500 nm, (acetone) 470 nm and (MeOH) 470 nm; MS (190 °C) *m/e* 738 (M), 678 (M–60), 632 (M–106), 618 (M–60–60), 572 (M–106–60), 512 (M–106–60–60), 452 (M–106–60–60–60), 407, 183, 141, 123, 105, 91, 69, 43.

Bastaxanthol dimethyl ketal (13) from the acid treatment, characterized as the triacetate *13b*, had λ_{max} (acetone) 467 nm; MS (200 °C) *m/e* 784 (M), 753 (M–31), 752 (M–32), 710 (M–32–42), 692 (M–92), 678 (M–106), 664 (M–60–60), 650 (M–32–42–60), 633 (M–60–60–31), 618 (M–106–60), 604 (M–60–60–60), 590, 558, 257, 197 (100 %), 137, 105 (100 %), 91 (100 %), 43 (100 %), 32 (100 %).

Bastaxanthol dimethyl ketal 19-methyl ether (14) from the acid treatment had λ_{max} (acetone) 466, (495) nm; MS (200 °C) *m/e* 672 (M), 641 (M–31), 640 (M–32), 622 (M–32–18), 577 (M–31–32–32), 566 (M–106), 215 (cleavage of Δ 7′), 155 (100 %), 138, 105 (100 %), 91 (100 %), 69 (100 %), 32 (100 %).

Bastaxanthol methyl ether (15) from the acid treatment had λ_{max} (acetone) 465, (493) nm; MS (200 °C) *m/e* 626 (M) 577, 551, 520 (M–106), 155 (100 %), 91 (100 %), 69 (100 %), 32 (100 %).

Bastaxanthol dimethyl ether (16) had λ_{max} 465, (493) nm, MS (200 °C) *m/e* 640 (M), 520 (M–106), 155 (100 %), 105, 91, 69, 55, 44, 43, 32 (all 100 %).

Capsanthinone dimethyl ketal-d_{3-4}. Capsanthinone-d_{3-4} (0.26 mg) was kept in 0.1 N HCl/MeOH (3 ml) at 30–40 °C for 1.5 h; pigment recovery 75 %. TLC (SiO_2, 40 % acetone in hexane) showed unreacted capsanthinone (80 % of total) and the less polar dimethyl ketal (15 %), λ_{max} (acetone) 358, 464 nm; MS *m/e* 631, 632 (d_{3-4}, M), M–32, 141, and 142 (strong, corresponding to *m/e* 155 for ketal *14* and *m/e* 197 for ketal *13b*).

Natural bastaxanthol (*10*)

Bastaxanthol (10), total yield *ca.* 1 mg, was found as a minor carotenoid amongst the non-polar carotenoid fractions of Batches 4 and 5. Natural *10* had R_F=0.87 (SiO_2, EtOAc), 0.40 (SiO_2, 40 % acetone in hexane); λ_{max} (acetone) (360), 468, (490) nm, (MeOH) 468, (490) nm; IR (KBr,weak) ν_{max} 3400 (vs, OH), 2900–3000 (m, CH) *ca.* 2100 w (C≡C), 1735 (m, 5-ring C=O), 1660 (s, conj. C=O), *ca.* 1550 (s, C=C), 1210 (m), 1120–1140 (m), *ca.* 1050 (m, C–O), 985 (m, *trans* CH=CH), 835 (w, CR_2=CHR), 700 (w) cm^{-1}; ^1H NMR (CD_3OD) δ 0.96 s (3H, CH_3–16′), 1.18 s (3H) and 1.23 s (3H, CH_3–16,17), 1.48 s (3H, CH_3–18′), 1.92 shoulder (CH_3–18), 1.96 s (*ca.* 3H, CH_3–19′), 2.00 s (*ca.* 6H, CH_3–20,20′), 2–3 (CH_2), 3–4.2 (imp. and H-17′) 4.32 s (=C–CH_2OH), 6.3–6.8 m (olefinic H) and 7.32 d (*J*=14 Hz, 1H, H-8′); MS (190 °C) *m/e* 612 (M), 594 (M–18), 576 (M–18–18), 506 (M–106), 488 (M–106–18), 141; CD (MeOH) nm Δε 220 (–9), 260 (0) 287 (+2.5), 310 (0).

Acetylation of natural 10. The acetylation at standard conditions at 0 °C, monitored by TLC (SiO_2), showed three intermediary acetates, presumably the allylic monoacetate (A), two allylic diacetates (B and C) and a final triacetate (*11*). The following ratios were estimated 5 min. 50 % *10* +50 % *A; 10 min. 40 % 10,* 40 % A and 20 % *B,* 15 min. 30 % *10,* 50 % A and 20 % B, 30 min. 0 % *10,* 50 % A, 25 % B, 25 % C; 45 min. 0 % *10,* 20 % A, 50 % B, 20 % C and 10 % *11*, 90 min. 0 %*10,* 10 % A, 40 % B, 10 % C and 40 % *11*.

Bastaxanthol triacetate (11) had R_F=0.31 (SiO_2, 10 % acetone in hexane) and λ_{max} (MeOH) as *10*; MS (200 °C) *m/e* 738 (M), 678 (M–60), 576, 572 (M–60–106), 183.

$NaBH_4$-reduction of natural bastaxanthol (10). Reduction of *10* (0.1 mg) in MeOH with $NaBH_4$ gave the presumed pentol *17* as two epimers (*a* and *b*) with R_F=*ca.* 0.5 (SiO_2, 10 % MeOH–EtOAc), each with λ_{max} (MeOH) (415), 441 and 469 nm, % III/II=28. Standard acetylation of *17* (0.05 mg) provided the less polar presumed pentaacetate of unchanged *vis.* spectrum; MS (210 °C) *m/e* 766 (M–60), 660 (M–60–106).

Artefact bastaxanthol dimethyl ketal (13). *13,* yield *ca.* 0.3 mg, was isolated from Batch 4+5. *13,* R_F=0.6 (SiO_2, 40 % acetone in hexane), less strongly adsorbed than bastaxanthol (*10*) had λ_{max} (MeOH) 470 (490) nm; MS (190 °C) *m/e* 626.3971 (calc. 626.3941 for $C_{41}H_{54}O_5$, M–32), 608.3866 (calc. 608.3837 for $C_{41}H_{52}O_4$, M–32–H_2O), 594 (M–32–32), 520

(M−32−106), 155, 141, 91, 69, 43, 32. Acetylation provided the triacetate *13b* R_F=0.5 (SiO_2, 10 % acetone in hexane), VIS λ_{max} (acetone), 470 nm; MS (200 °C) *m/e* 784 (M), 752 (M−32), 740 (M−44), 724 (M−60), 710 (M−32−42), 678 (M−106), 660 (M−60−32−32), 197 strong, 91, 69, 60, 43, 32.

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