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Carotenoid Sulfates. 4.* Syntheses and Properties of Carotenoid Sulfates

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Carotenoid sulfates have been prepared from 14 selected carotenols for spectroscopic characterization, studies of their stability in solution and their water solubility.

Carotenoids containing *sec* non-allylic hydroxy groups provided sulfates stable in methanol solution, exemplified by zeaxanthin mono- and disulfate, alloxanthin mono- and disulfate, fucoxanthin monosulfate, peridinin monosulfate, capsorubin mono- and disulfate and astaxanthin mono- and disulfate.

Acid catalyzed methanolysis of zeaxanthin disulfate gave zeaxanthin with complete retention of configuration. Enzymatic hydrolysis of alloxanthin monosulfate is reported.

Less stable sulfates were obtained from *sec vic* diol type-, phenolic and *tert*-carotenols; caloxanthin, nostoxanthin, 3-hydroxyisorenieratene, 3,3′-dihydroxyisorenieratene, rhodovibrin, di-OH-lycopene and OH-chlorobactene.

Acid catalyzed methanolysis of the *tert* carotenols proceeded via carbocations, judged by the solvolysis products characterized.

Characteristic spectroscopic properties of carotenoid sulfates are pointed out.

Water solubilities were studied.

Recently the structural elucidations of the first carotenoid sulfate, bastaxanthin,[1] and naturally occurring derivatives thereof,[2] have been published. For this work zeaxanthin disulfate and lycoxanthin sulfate were prepared as models.[3]

Since carotenoid sulfates represent a unique class of water soluble carotenoids[4,5] mono- and disulfates have been prepared for a series of selected carotenols for spectroscopic and chemical characterization, including stability studies in solution, and for estimation of water solubility. Trivial names are used for the carotenoids. Semirational IUPAC names[6] are stated in Experimental.

RESULTS AND DISCUSSION

Stable carotenoid sulfates. Sulfate formation was in general effected by treatment with a pyridine-SO_3 complex prepared from chlorosulfonic acid and pyridine.[3,7] The presumed mechanism leads to S–O bond formation with retention of configuration of the C–O bond of the initial carotenol,[4] Scheme 3a. The reaction was quenched with water and the

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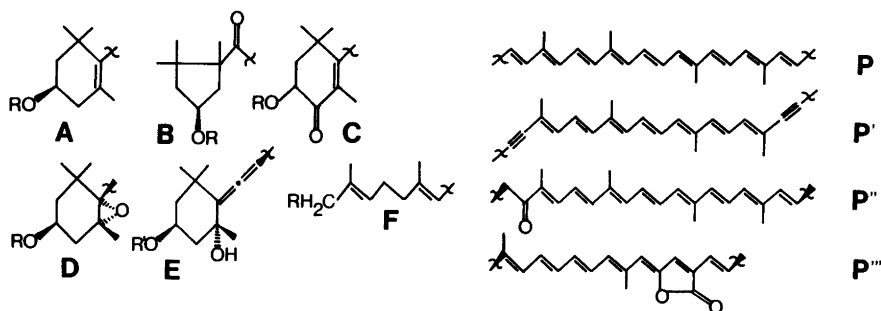


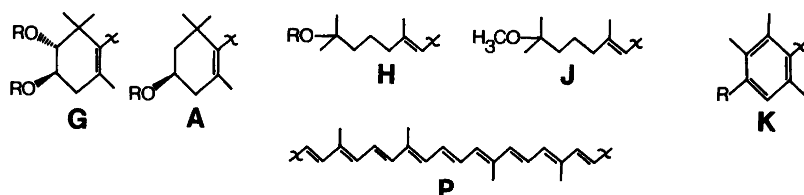
Scheme 1. Carotenoids forming stable sulfates.

Zeaxanthin	*1*	A–P–A,	R=H
	1a		$R=SO_3^-$, R=H
	1b		$R=SO_3^-$
Alloxanthin	*2*	A–P'–A,	R=H
	2a		$R=SO_3^-$, R=H
	2b		$R=SO_3^-$
Fucoxanthin	*3*	D–P''–E	R=H, R'=Ac
	3a		$R=SO_3^-$, R'=Ac
Peridinin	*4*	E–P'''–D	R=H, R'=Ac
	4a		$R=SO_3^-$, R'=Ac
Capsorubin	*5*	B–P–B	R=H
	5a		$R=SO_3^-$, R=H
	5b		$R=SO_3^-$
Astaxanthin	*6*	C–P–C	R=H
	6a		$R=SO_3^-$, R=H
	6b		$R=SO_3^-$
Lycoxanthin	*7*	F–P–F	R=OH, $R=CH_3$
	7a		$R=OSO_3^-$, $R=CH_3$

pyridinium salt of the carotenoid sulfate converted to the alkali salt with NaOH (for alkali stable carotenoids) or NaCl.

Carotenoid sulfates that could be stored unchanged in methanol solution for at least 3 months at −10 °C are considered as stable. Carotenols forming stable sulfates are listed in Scheme 1. Zeaxanthin (*1*), alloxanthin (*2*), fucoxanthin (*3*), peridinin (*4*) and capsorubin (*5*) all possess *sec* non-allylic hydroxy groups in 6- or 5-membered rings. The natural bastaxanthins[1,2] belong to this category. Also the *sec* bis-*α*-ketol astaxanthin (*6*) and lycoxanthin (*7*) with a *prim* hydroxy group allylic to one double bond, formed rather stable sulfates.

(3*R*,3'*R*)-Zeaxanthin (*1*) provided a monosulfate (*1a*) and a disulfate (*1b*) of unchanged Cotton effect, consistent with retention of configuration at C-3,3'. Acid catalyzed methanolysis (0.1 N HCl) of *1b* resulted in the formation of partly *cis*-isomerized zeaxanthin (*1*) with CD properties compatible with gross retention of configuration at C-3,3', see Scheme 3b. Mono-*cis* isomers of zeaxanthin have a reverted Cotton effect.[8] The optical purity of product *1* (~100 % 3*R*,3'*R*) was eventually proved by the recent carbamate method.[9] No methyl ethers of zeaxanthin were formed upon methanolysis of the sulfates (*1a*,*1b*).



Scheme 2. Carotenoids forming less stable sulfates.

Caloxanthin	*8*	G–P–A	R=H
	8a		R=SO_3^-, (R=H)$_2$
	8b		(R=SO_3^-)$_2$, R=H
	8c		R=SO_3
Nostoxanthin	*9*	G–P–G	R=H
	9a		R=SO_3^-, (R=H)$_3$
	9b		(R=SO_3^-)$_2$ (R=H)$_2$
	9c		(R=SO_3^-)$_3$ R–H
	9d		R=SO_3^-
Rhodovibrin	*10*	H–P–J	R=H
	10a		R=SO_3^-
Di–OH–lycopene	*11*	H–P–*H*,	R=H
	11a		R=SO_3^-, R=H
	11b		R=SO_3^-
OH-Chlorobactene	*12*	K–P–*H*,	R=H
	12a		K(R=H), *H*(R=SO_3^-)
3-Hydroxyisorenieratene	*13*	K–P–K,	R=OH, R=H
	13a		R=OSO_3^-, R=H
3,3′-Dihydroisorenieratene	*14*	K–P–K,	R=OH
	14a		R=OSO_3^-, R=OH
	14b		R=OSO_3^-

(3*R*,3′*R*)-Alloxanthin (*2*) formed a monosulfate (*2a*) and a disulfate (*2b*). The monosulfate (*2a*) was hydrolyzed enzymatically in low yield by a sulfatase to alloxanthin.

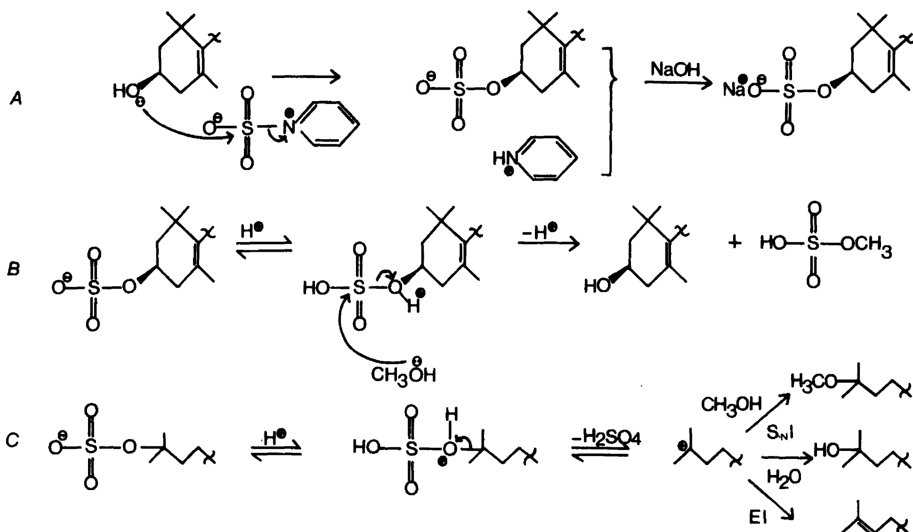
The alkali-labile fucoxanthin (*3*) and peridinin (*4*) each afforded monosulfates (*3a,4a*) in good yield. The *tert* hydroxy group was left intact, as demonstrated by subsequent formation of trimethylsilyl ethers.

Capsorubin (*5*) gave smooth formation of the monosulfate (*5a*) and disulfate (*5b*). (3*S*,3′*S*)-Astaxanthin (*6*) provided a monosulfate (*6a*) and disulfate (*6b*). Upon acid catalyzed methanolysis the disulfate *6b* was converted to astaxanthin (*6*, chirality of product not examined). Lycoxanthin monosulfate (*7a*) has been characterized previously.[3]

Less stable carotenoid sulfates. Carotenols forming less stable sulfates which upon storage in methanol was partly converted to less polar solvolysis products are listed in Scheme 2.

Only small amounts of the triol caloxanthin (*8*) and the tetrol nostoxanthin (*9*) with non-allylic *sec* α-glycol end groups were available. Apparently they underwent normal sulfate formation. However, the sulfates (*8 a–c; 9 a–d*) were unstable and only a presumed nostoxanthin monosulfate (*9a*) was partly characterized.

The *tert* carotenols rhodovibrin (*10*), di-OH-lycopene (*11*) and OH-chlorobactene (*12*), which cannot be acetylated under standard conditions,[10] but form trimethylsilyl ethers,[11] all



Scheme 3. Presumed mechanisms for the formation and acid catalyzed solvolysis of carotenoid sulfates.

provided sulfates (*10a, 11a,b, 12a*). Storage in water or methanol resulted in solvolysis of the sulfates. The *tert* monosulfate *12a* upon treatment with 0.1 n HCl in aqueous methanol was fast and quantitatively converted to the parent carotenol (*12*), its methyl ether (*12c*) and the aryl carotene chlorobactene (*12b*) with an isopropylidene end group.[12] The result is compatible with S_N1 and E1 reactions via the *tert*-carbocation, Scheme 3c.

The phenolic 3-hydroxyisorenieratene (*13*) and 3,3′-dihydroxyisorenieratene (*14*) underwent slow sulfate formation, affording the expected mono- (*13a, 14a*) and disulfate (*14b*). In case of the diol *14* a blue oxidation product was also partly characterized, *cf.* Ref. 13. The noted instability of these aryl carotenoid sulfates upon storage in methanol may be rationalized by solvolysis *via* resonance stabilized carbocations, allowing charge delocalization on the polyene chain. Simple phenolic sulfates are stable.[14]

Spectroscopic properties. As previously found[1-3] carotenoid sulfates did not form molecular ions on electron impact, but M′ ions of the presumed thermal elimination products obtained by loss of $NaHSO_4$ in the ion source prior to ionization.

IR-Spectra of the carotenoid sulfates had diagnostic, strong S=O absorption around 1240 cm^{-1}.

The sulfate function had little influence on the ^1H NMR spectra except for a downfield shift of the methine proton of *sec* sulfates of *ca.* 0.6 ppm relative to the parent carotenol. By ^{13}C NMR the signal of the carbon carrying the sulfate function was shifted downfield *ca.* 9 ppm on sulfate formation.

Electronic spectra in organic solvents were hardly influenced by the sulfate function, and the same qualitative Cotton effects were also recorded. However, absorption spectra of aqueous, centrifuged solutions were significantly hypsochromically shifted, see published spectra[4] for capsorubin (*5*), astaxanthin (*6*) and their sulfates. The CD spectrum of zeaxanthin disulfate (*1b*) recorded in water also differed considerably from that in organic solvent.

Water solubility. The water solubility of the stable carotenoid sulfates depended not only on the number of sulfate functions present, but on the total carotenoid structure.

Monosulfates could be transferred from an aqueous hypophase to ethyl acetate, whereas disulfates required chloroform–methanol mixtures. Most sulfates were readily soluble in methanol, and moistening the dry sulfate with methanol brought the sulfate more easily into aqueous solution. The water solubility was reduced in the presence of salts, and complete precipitation of *e.g.* the sulfates of fucoxanthin (*3a*) and peridinin (*4a*) from an aqueous solution was effected with NaCl.

The following water solubilities were measured in mg/ml OH-chlorobactene sulfate (*14a*)$\geq$0.01, astaxanthin disulfate (*6b*)$\geq$0.02, zeaxanthin disulfate (*1b*)$\geq$0.05, capsorubin disulfate (*5b*)$\geq$0.14, fucoxanthin sulfate (*3a*)$\geq$0.20 and peridinin sulfate (*4a*)$\geq$0.36.

EXPERIMENTAL

General. General precautions for work with carotenoids were taken.[10,11] Further details may be found elsewhere.[15]

Spectroscopy. The terms % III/II and D_B/D_{II} used to describe electronic spectra are defined elsewhere.[16] For MS diagnostically useful peaks only are cited. Mass spectra were recorded at 70 eV with a direct inlet system at the temperatures cited. M' for the carotenoid sulfates refer to ($M-NaHSO_4$) for monosulfates and ($M-2NaHSO_4$) for disulfates. CD spectra were recorded with a Roussel Jouane Dichrograph.

Chromatography. Column chromatography of carotenoid sulfates was carried out on kieselgel using MeOH in EtOAc for development. In some cases ion exchange chromatography[17] was employed. Preparative TLC (SiO_2, 1 mm) was used in the purification procedure and analytical TLC (Merck No. 5553 DC-Alufolien Kieselgel 60, 0.2 mm) for monitoring sulfate formation *etc.* R_F-values refer to 15 % MeOH in EtOAc if not otherwise specified. HPLC of *cis–trans* isomerized zeaxanthin (*1*) was carried out as described elsewhere.[18]

Sulfate formation. For 0.5–10 mg substrate the following procedure was generally used: The pyridine $-SO_3$ complex was prepared by dropwise addition of chlorosulfonic acid (4–6 drops) with stirring to dry (dried over BaO) pyridine (1–2 ml) at −10 °C. The respective carotenol (specified amount) in dry pyridine (1–2 ml, −10 °C) was added and the mixture left at room temperature for the specified time. The reaction was monitored by TLC and quenched by the addition of 10 % KOH in H_2O to pH *ca.* 9. The carotenoids were extracted with EtOAc from an aqueous hypophase. In cases of base labile carotenoids (*e.g. 3, 4* and *6*) NaCl was added instead of NaOH. Excess NaCl was avoided in order to prevent precipitation of the sulfates. Carotenoid sulfates which could not be transferred from H_2O to EtOAc were extracted with $CHCl_3$–MeOH mixtures. Yields refer to recovered carotenoids transferred to the organic solvent as determined spectrophotometrically. The organic extract was concentrated to dryness *in vacuo* and the carotenoids dissolved in MeOH prior to chromatographic purification.

Water solubility. A spectrophotometrically determined amount of carotenoid sulfate in MeOH was taken to dryness and dissolved in an inadequate quantity of H_2O (*ca.* 5 ml) at room temperature. The aqueous solution was centrifuged (9750 rpm for 20 min), decanted and excess carotenoid dissolved in MeOH for spectrophotometric determination. The difference between the two calculated amounts was used to estimate the carotenoid concentration in H_2O.

(3R,3'R)-Zeaxanthin (*1*, β,β-carotene-3,3'-diol), *ex Flavobacterium* sp.[19] or synthetic.[20] *1* (5–80 mg) was sulfated in 7 separate experiments with 37–85 % pigment recovery: unreacted *1* (0–25 % of recovered carotenoid), monosulfate *1a* (minor) and disulfate *1b* (major). A *ca.* 20 × excess of chlorosulfonic acid was preferably used.

Zeaxanthin monosulfate. (*1a*), R_F=0.8, VIS λ_{max} nm (MeOH) (425), 446 and 472, % III/II=1; ^{1}H NMR (MeOD) δ 1.08 s (3H, Me-1), 1.12 s (9H, Me-1,1'), 1.74 s (Me-5,5'), 1.89 s?, 1.97 s (Me-9, 13,9',13'), 6.1–6.8 m (olefinic H), MS (205 °C) *m/z* 550 (M'), 532 (M'−18), 458 (M'−92), 444 (M'−106); CD (MeOH) nm ($\Delta\varepsilon$) 225 (−1.6), 251 (+2), 290 (−2.2).

1a (0.12 mg) was treated with 0.1 N HCl in MeOH (3 ml) for 35 min at 40 °C; pigment recovery 50 %, consisting of unreacted *1a* (50 % of recovered) and *1* (50 %). Product zeaxanthin (*1*) was identified by R_F (co-chromatography) and VIS λ_{max}.

Zeaxanthin disulfate (1b), cf. previous characterization,[3] R_F=0.2, VIS λ_{max} nm (MeOH) (425), 448 and 476, % III/II=30, (EtOAc) 340, (425), 452 and 478, (H_2O before centrifugation) 340 and 390, (H_2O, after centrifugation 20 min 9750 rpm) 335 and 400; ^{1}H NMR (MeOD) δ 1.07 s (6H, Me-1,1′), 1.18 (6H, Me-1,1′), 1.74 s (6H, Me-5,5′), 1.97 s (12H, Me-9,13,9′,13′), 4.48–4.68 m (2H, H-3,3′), 6.13 s (4H, H-7,8,7′,8′), 6.16–6.72 m (10H, olefinic); ^{13}C NMR (CH_3OH) δ, values in parenthesis refer to zeaxanthin (*1*)[21] in $CDCl_3$, 29.0 (CH_3-1,1′; 28.7), 30.7 (CH_3-1,1′; 30.2) 74.0 (C-3,3′; 65.1), 40.6 (C-4,4′; 42.4), 21.7 (CH_3-5,5′; 21.6), 12.8 (CH_3-9,13,9′,13′; 12.7), C-2 obscured by CH_3OH; MS (210 °C) *m/z* 532 (M′, 100 %), 440 (M′−92, 10 %), 426 (M′−106, 2 %); CD (MeOH) nm ($\Delta\varepsilon$) 225 (−6.8), 251 (+3.4), 290 (16.5), CD (H_2O) 230 (−1), 280 (−1.5), 385 (−13), 400 (0), 405 (2.5); water solubility ≥0.05 mg/ml.

1b (1.3 mg) was treated with 0.1 N HCl (5 ml) for 6 h at 40 °C; pigment recovery 50 %: monosulfate *1b* (minor) and *1* (major). In a separate experiment 35 min treatment resulted in 52 % pigment recovery: unreacted *1b* (34 % of recovered), monosulfate *1a* (55 %) and zeaxanthin (*1*, 11 %). The product monosulfate *1b* had R_F and VIS λ_{max} as above; MS (205 °C) *m/z* 550 (M′, 100 %), 458 (M′−92, 12 %); CD (MeOH) nm ($\Delta\varepsilon$) 225 (−2), 242 (+2), 250 (0), 285 (−4.7), 330 (−2.4), 365 (0). Product zeaxanthin (*1*) had R_F as authentic *1*; MS (205 °C) *m/z* 568 (M, 100 %), 476 (M−92, 18 %); VIS λ_{max} nm (acetone) (425), 450 and 476, % III/II=7, % D_B/D_{II}=10, compared with all-*trans 1* (425), 452 and 480, % III/II=25, % D_B/D_{II}=10 and I_2-catalyzed stereomutation mixture of all-*trans 1* (425), 448 and 474, % III/II=5, % D_B/D_{II}=22. HPLC of the solvolysis product *1*: neo A (9 % of total), all-*trans* (75 %) and neo U (16 %) compared with I_2-catalyzed stereomutation mixture[22] all-*trans* (66 % of total); CD (EPA) nm ($\Delta\varepsilon$) 225 (−7.7), 250 (+3.8), 260 (0), 280 (−11.5), 340 (0) in comparison with I_2-catalyzed stereomutation mixture of all-*trans 1* 225 (−3), 250 (−1.5), 280 (−4), 320 (−1.9), 360 (0) and all-*trans 1* (CH_2Cl_2)[23] 225 (−7), 251 (+8.1), 290 (−14.6), 348 (+3.7).

The optical purity of product zeaxanthin (*1*, 0.1 mg) obtained by the acid methanolysis of *1a*+*1b* was examined by the carbamate method.[9] HPLC in system 1[9] of the dicarbamate in direct comparison with that of authentic, partly *cis*-isomerized *1* (3*R*,3′*R*) dicarbamate revealed ~100 % optical purity of the test sample: R_T=23.51 *cis* (12 % of total) and R_T=28.15 *trans* (88 %). The dicarbamates of the enantiomeric (3*S*,3′*S*) and meso (3*R*,3′*S*) zeaxanthin have lower R_T, see Ref. 9.

(3R,3′R)-Alloxanthin (*2*, 7,8,7′,8′-Tetradehydro-β,β-carotene-3,3′-diol). Synthetic all-*trans 2*[24] (0.36 mg), 25 min reaction time, provided 0.26 mg (72 %) recovered carotenoids; unreacted *2* (30 % of recovered), monosulfate *2a* (54 %) and disulfate *2b* (16 %).

Alloxanthin monosulfate (2a); R_F=0.5; VIS λ_{max} nm (MeOH), (417), 440 and 472, (H_2O) 372, (420), (440), (475); MS (205 °C) *m/z* 546 (M′, 68 %), 531 (M′−15, 5 %), 528 (M′−18, 5 %), 95 (100 %). *2a* was readily soluble in MeOH, partly soluble in H_2O.

2a (0.2 mg) was dissolved in MeOH (2 drops). The solution was diluted with H_2O (2 ml) and treated with arylsulfatase *ex. Helix pomatia* at 37 °C for 24 h as described elsewhere;[1] pigment recovery 65 % after transfer to EtOAc. Alloxanthin (*2*, 10 % of recovered) was identified by VIS λ_{max} and co-chromatography.

Alloxanthin disulfate (2b), available 0.04 mg, R_F=0.2; VIS λ_{max} (MeOH) (417), 440 and 468 nm, (H_2O), 382, (445), (475) nm; MS (205 °C) *m/z* 528 (M′). *2b* appeared to be readily soluble in MeOH and H_2O.

Fucoxanthin (*3*,(3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*R*)-5,6-Epoxy-3,3′,5′-trihydroxy-6′,7′-didehydro-5,6,7,8,5′,6′-hexahydro-β,β-caroten-8-one) *ex Fucus vesiculosus*. *3* (6 mg), 1 h reaction time, provided 4.8 mg (80 %) recovered carotenoid; unreacted *3* (20 %) and the monosulfate *3a* (80 %).

Fucoxanthin 3-sulfate (3a). R_F=0.6; VIS λ_{max} nm (MeOH) (330), 444 and (465), (H_2O) 405–420 (broad); IR (KBr) ν_{max} (KBr)cm^{-1} 3430 (vs, OH), 2960, 2930, 2880 (s, CH), 1940 (w, allene), 1740 (s, C=O), 1660 (s, conj. C=O), 1610 w, 1570 and 1530 (w, C=C); 1460 (w, CH_2), 1365 (s, CH_3), 1250 (vs, S=O and acetate), 1070, 1030, 1010, 970 (vs, *trans* CH=CH), 920 (w) and 835 (w, CR_2=CHR); ^{1}H NMR (CD_3OD) δ values in bracket refer to fucoxanthin (*3*) 0.96 s (0.94; 3H, Me-16), 1.07 s (1.02, 6H, Me-16′,17), 1.20 s (1.19; 3H,

Me-18), 1.32 s (1.32; 3H, CH_3-17'), 1.38 s (1.38; 3H, Me-18'), 1.83 s, 1.89 s, 1.93 s and 2.01 s (1.84, 1.95 and 2.01; 12H, Me-19,19',20,20'), 2.01 s (2.01; 3H, acetate), *ca.* 4.5 (3.81; 1H, H-3), *ca.* 5.4 (5.4; 1H, H-3') and 6.03–6.78 (6.08–6.78; olefinic H); MS (200 °C) *m/z* 640 (M', 7 %), 622 (M'−18, 7 %), 562 (M'−60−18, 3 %), 43 (100 %). *3a* had solubility in $H_2O \geq 0.2$ mg/ml. Complete precipitation of *3a* (1 mg) from H_2O (5 ml) was effected upon addition of aqueous NaCl (50 %, 5 ml), resulting in a colourless solution after centrifugation.

Fucoxanthin 3-sulfate (3a) *5'-trimethylsilyl ether.* *3a* was submitted to standard silylation[10] providing *3a*-TMS ether with the same VIS λ_{max} and inseparable from *3a* upon TLC; MS (205 °C) *m/z* 712 (M'), 694 (M'−18), 620 (M'−92), 606 (M'−106).

Peridinin (*4,* 3*S*,5*R*,6*S*,3'*S*,5'*R*,6'*R*)-5',6'-Epoxy-3,5,3'-trihydroxy-6,7'-didehydro-5,6,5',6'-tetrahydro-10,11,20-trinor-*β*,*β*-caroten-19',11'-olide 3-acetate) *ex* dinoflagellates.[25] *4* (4.9 mg), 2 h reaction time, provided 3.7 mg (75 %) recovered carotenoid; unreacted *4* (20 % of recovered) and the monosulfate *4a* (80 %).

Peridinin 3'-sulfate (*4a*). R_F=0.53; VIS λ_{max} nm (MeOH) 460, (EtOAc) 455, (H_2O) 408; IR (KBr) ν_{max} cm^{-1} 1245 (vs, S=O and acetate), 1065 (s), 970 (s, *trans* CH=CH); ^{1}H NMR (CD_3OD) *δ*, values in brackets refer to peridinin (*4*), 0.99 s (0.97; 3 H, Me-16'), 1.07 s (1.07; 3H, Me-17), 1.20 s (1.19, 1.20; 6H, Me-17',18'), 1.31 s (1.35; 3H; Me-16), 1.38 s (1.38; 3H, Me-18), 1.83 s, 2.21 s (1.83, 2.21; 6H, Me-19,20'), 2.01 s (2.02; 3H, acetate), 4.6 broad (3.8; 1H, H-3'), 5.4 broad (5.4; 1H, H-3), 5.9–6.61 (5.9–6.6; olefinic H), 7.12 d (7.18; 1H, H-7'), 7.39 (7.37; 1H, H-10'); MS (215 °C) *m/z* 612 (M', 16 %), 594 (M'−18, 24 %), 552 (M'−60, 5 %), 534 (M'−60−18, 6 %), 520 (M'−92, 3 %), 43 (100 %).

4a was readily soluble in MeOH, solubility $H_2O \geq 0.36$ mg/ml. *4a* was precipitated from H_2O with NaCl, *cf.* *3a* above.

4a give no new acetylated product at standard acetylation conditions.

Peridinin 3'-sulfate (4a) *3-trimethylsilyl ether.* *4a* was submitted to standard silylation providing *4a*-TMS ether of unchanged VIS λ_{max} and inseparable from *4a* upon TLC; MS (205 °C) *m/z* 684 (M').

Capsorubin (*5*; (3*S*,5*R*,3'*S*,5'*R*)-3,3'-Dihydroxy-*κ*,*κ*-carotene-6,6'-dione). Synthetic *5*[26] (3 mg), 1 h reaction time, provided 2.1 mg (70 %) recovered carotenoid; unreacted *5* (10 % of recovered), monosulfate *5a* (15 %) and disulfate *5b* (75 %).

Capsorubin monosulfate (5a). R_F=0.87; VIS λ_{max} nm (MeOH) 480 and (510), (H_2O) 403; MS (200 °C) *m/z* 592 (M'); CD (MeOH) nm (Δε) 232 (+2.9), 239 (0), 250 (−3.0), 264 (0), 298 (+8.7), 325 (0), CD (H_2O) 250 (−2.1), 260 (0), 270 (+4.6), 275 (0), 290 (−18.4), 305 (0), 320 (+3.5), 345 (0), 365 (−13.4), 390 (0). Capsorubin (*5*) had CD (MeOH) nm (Δε) 240 (0), 250 (−2.0), 261 (0), 298 (+9.9), 325 (0).

Capsorubin disulfate (*5b*), available in total 3.5 mg; R_F=0.2; VIS λ_{max} nm (MeOH) 480, (510), (H_2O) 406; IR (KBr) λ_{max} 3400 (s); 2960, 2920 and 2860 (s, CH), 1735 (imp.), 1670 (s, conj. C=O), 1560 (s), 1450 (s), 1385 (m), 1235 (vs, S=O), 1050 (s), 1010 (m), 970 (s, *trans* CH=CH), 780 (m); ^{1}H NMR (CD_3OD) *δ*, values in bkackets refer to capsorubin (*5*), 0.85 s (0.83; 6H, Me-16,16'), 1.19 s (1.21; 6H, Me-17,17'), 1.35 s (1.36; Me-18,18'), 1.99 s (12H, Me-19,19', 20,20'), 4.9 broad (4.4; 2H,H-3,3'), 7.30 d (2H, H-8,8') and 6.2–6.8 conj. olefinic H. ^{13}C NMR (CD_3OD) *δ* 12.8 (C-19,19',20,20'), 21.2 (C-18,18'), 25.3, 26.0 (C-16,16',17,17'), 42.8 (C-1,1'), 60 (C-5,5'), 78.7 (C-3,3'; *cfr.* 70.3 for *5*), 122.3 (C-7,7'), 125.8 (C-11,11'), 132.7 (C-15,15'), 135.3 (C-9,9'), 136.6 (C-14,14'), 138.4 (C-13,14'), 142.4 (C-10,10'), 143.1 (C-12,12'), 148.4 (C-8,8'), 205.0 (C-6,6'); *cf.* assignments for *5*[27]; MS (205 °C) *m/z* 564 (M', 25 %), 472 (M'−92, <1 %), 43 (100 %); CD (MeOH) nm (Δε) 244 (0), 253 (−2.5), 265 (0), 298 (+7.3), 325 (0), CD (H_2O) 270 (+8.3), 280 (0), 290 (−11.3), 310 (0), 335 (+5.3), 355 (0), 360 (−1.5), 370 (0), 385 (+4.5), 400 (+3.8).

5b was readily soluble in MeOH, partly soluble in pyridine and in H_2O solubility ≥0.14 mg/ml.

(3*S*,3'*S*)-*Astaxanthin* (*6,* 3,3'-Dihydroxy-*β*,*β*-carotene-4,4'-dione). Synthetic *6*[28] (3 mg), 2.5 h reaction time, TLC revealed unreacted *6* (*ca.* 15 % of recovered), monosulfate *6a* (5 %) and disulfate (80 %). *Ca.* 25 % of the pigments could be transferred to EtOAc from H_2O; the rest was extracted with $CHCl_3$−MeOH; total recovery *ca.* 70 %.

Astaxanthin disulfate (*6b*) R_F=0.25; VIS λ_{max} nm (MeOH) 475, (H_2O) 405; IR (KBr) λ_{max} 3350 (s); 2970, 2940 and 2880 (s, CH), 1740 (imp.), 1675 (w, conj.C=O), 1575, 1430 (w), 1390 (w), 1250 (vs, S=O), 1150 (m), 1070 (w), 1020 (s), 990 (w), 965 (s, *trans*

CH=CH), 930 w, 830 (w, CR_2=CHR), 770; ^{1}H NMR (DMSO-d_6) δ, values in brackets refer to *6*, 1.16 s, 1.27 s (1.15, 1.28; 12H, Me-16,16′, 17,17′), 1.80 s (1.81; 6H, Me-18,18′), 1.97 s (1.97; 6H, Me-20,20′), 1.99 s (1.99; 6H, Me-19,19′), 6.18−6.8 (olefinic H); MS unsuccessful.

6b (dry) was practically insoluble in most organic solvents, partly soluble in DMSO and DMF, solubility in H_2O *ca.* 0.02 mg/ml.

6b (0.15 mg) was hydrolyzed as zeaxanthin disulfate (*1b*) above with 89 % pigment recovery; unreacted *6b* (10 % of total) and astaxanthin(*6*, 90 %). Product astaxanthin (*6*) had VIS λ_{max} and R_F as authentic *6*; MS (205 °C) *m/z* 596 (M, 100 %), 584 (M−2, 50 %), 504 (M−92, 17 %), 502 (M−92, 8 %), 490 (M−106, 33 %), 488 (M−2−106, 17 %).

Lycoxanthin (*7*, ψ,ψ-caroten-16-ol), synthetic[29] (0.4 mg) reaction period 1 h, provided 0.3 mg (75 %); unreacted *7* (50 %), and the sulfate *7a* (50 %).

Lycoxanthin 16- or 17-sulfate (*7a*). R_F=0.21 (10 % MeOH in EtOAc); VIS λ_{max} nm (MeOH) 360, 442, 468 and 498; see previous characterization.[3]

Stability test for sulfates of 1−7. Aliquots (*ca.* 0.1 mg of these sulfates in MeOH (1−2 ml) at −20 °C for 3 months showed no formation of less polar products by TLC.

Caloxanthin (*8*, 2*R*,3*R*,3′*R*)-β,β-Carotene-2,3,3′-triol) *ex Anacystis nidulans.*[30] *8* (0.5 mg); 3 h reaction period; by TLC (SiO_2, 25 % MeOH-EtOAc) unreacted *8* (5 % of recovered), monosulfates *8a* (30 %), disulfates *8b* (50 %, same R_F as *1b*) and trisulfates *8c* (20 %). After extractive isolation pigment recovery was 0.43 mg (85 %). TLC now revealed two products (*ca.* 40 %) less polar than *8*, *8* (*ca.* 20 %) and one product (*ca.* 40 %) slightly less polar than zeaxanthin disulfate (*1b*) which decomposed upon further TLC.

Nostoxanthin (*9*, 2*R*,3*R*,2′*R*,3′*R*)-β,β-Carotene-2,3,2′,3′-tetrol) *ex Anacystis nidulans.*[30] *9* (0.5 mg), 2 h reaction period; by TLC (SiO_2, 25 % MeOH−EtOAc) unreacted *9* 10 % of recovered), monosulfates *9a* (30 %), disulfates *9b* (30 %), tri/tetrasulfates *9c,d* (30 %). After extractive isolation the pigment recovery was 0.27 mg (54 %). TLC now showed only nostoxanthin (*9*, identified by R_F and MS) and a presumed monosulfate *9a*.

Nostoxanthin monosulfate (*9a*). R_F=0.23, less polar than zeaxanthin disulfate (*1b*); VIS λ_{max} nm (acetone) (425), 448 and 425; IR (KBr) λ_{max} 1250 (s, S=O), 960 (s, *trans* CH=CH); MS (205 °C) *m/z* 582 (M′), 564 (M′−18), 546 (M′−18−18).

Rhodovibrin (*10*, 1′-Methoxy-3′,4′-didehydro-1,2,1′,2′-tetrahydro-ψ,ψ-caroten-1-ol), synthetic.[31] *10* (10 mg), 2 h reaction period, pigment recovery 8.4 mg (84 %): unreacted *10* (40 % of recovered) and monosulfate *10a* (60 %).

Rhodovibrin sulfate (*10a*). R_F=0.52; VIS λ_{max} nm (MeOH) (355), 372, (455), 478 and 510, % D_B/D_{II}=30 (*cis*-isomerized), (H_2O) 385; IR (KBr) ν_{max} 1220, 1130 and 1030 (vs, S=O, OMe), 965 (vs, *trans* CH=CH); MS (210 °C) *m/z* 566 (M′, 30 %), 460 (M′−106, 12 %), 91 (100 %).

10a was soluble in MeOH and $CHCl_3$, badly soluble in EtOAc and H_2O.

10a gave in moist $CHCl_3$ solution rhodovibrin (*10*, by TLC). Storage in MeOH and H_2O resulted in unpolar products with shorter chromophore.

Di-OH-Lycopene (*11*, 1,2,1′,2′-Tetrahydrolycopene-1,1′-diol), synthetic.[32] 11 (10 mg), 2 h reaction period. Products could not be transferred to EtOAc and required large volumes of $CDCl_3$ for extraction from H_2O, pigment recovery 2.5 mg (25 %). TLC revealed two sulfates: Unidentified and disulfate *11b*. The unidentified sulfate had R_F 0.6; VIS λ_{max} nm (MeOH) 450; IR (KBr) λ_{max} 1240 (vs, S=O), 965 (s, *trans* CH=CH); MS (200 °C) 530 (100 %).

Di-OH-Lycopene disulfate (*11b*) R_F=0.1 (SiO_2, 5 % MeOH−EtOAc); VIS λ_{max} nm (MeOH) (345), 360, (440), 465 and 495, % D_B/D_{II}=39, (H_2O) 395; MS (210 °C) *m/z* (536 (M′, 25 %), 444 (M′−92, 8 %), 430 (M′−106), 91 (100 %).

11b was readily soluble in MeOH, partly soluble in ($CHCl_3$), relatively low solubility in H_2O.

OH-Chlorobactene (*12*, 1′,2′-dihydro-ψ-ψ-caroten-1-ol), synthetic.[33] *12* (2 mg), 45 min reaction period, provided 1.8 mg (90 %) pigment recovery: unreacted *12* (10 %) and monosulfate *12a* (90 %).

OH-Chlorobactene sulfate (*12a*) R_F=0.3; VIS λ_{max} nm (MeOH) (430) 458 and 485, (acetone) (445), 458 and 485; MS (210 °C) *m/z* 532 (M′, 100 %), 420 (M′−92, 12 %), 426 (M′−106, 6 %).

12a was readily transferred to EtOAc from H_2O; solubility in H_2O 0.01 mg/ml.

12a was stable in MeOH at −10 °C for one week. Acid catalyzed solvolysis of *12a* (0.54 mg) in 0.1 n HCl/MeOH containing some water as for *1b* for 1 h gave 96 % pigment recovery: chlorobactene (*12b,* 7 % of recovered, *12*-methyl ether (*12c,* 57 %) and *12* (36 %). Chlorobactene was identified upon co-chromatography with an authentic sample,[12] VIS λ_{max} nm (acetone) 348, (335), 458 and 487; MS (210 °C) *m/z* 532 (M, 100 %). The methyl ether *12c* was characterized by VIS λ_{max} nm (acetone) 348, (435), 458 and 487; MS (210 °C), *m/z* 564 (M, 100 %), 532 (M−32, 6 %), 472 (M−92, 9 %), 458 (M−106, 12 %). Product *12* was inseparable from authentic *12*; VIS λ_{max} nm (acetone) 348, (430) 358, and 485; MS (210 °C) *m/z* 550 (M, 100 %), 458 (M−92, 21 %), 444 (M−106, 32 %).

3-Hydroxyisorenieratene (*13,* ϕ,ϕ-caroten-3-ol) *ex Streptomyces mediolani.*[34] *13* (2.5 mg), 6 h reaction period, provided 2.3 mg (88 %) recovered pigment: unreacted *13* (80 % of recovered) and monosulfate *8a* (20 %).

3-Hydroxyisorenieratene sulfate (*13a*). R_F=0.45 (SiO_2, 10 % MeOH−EtOAc); VIS λ_{max} nm (MeOH) 453 and (480); MS not achieved. *13a* gave no colour reaction with bisdiazobenzidin for fenols[35,36] at conditions where *13* turned brown. *13a* was readily soluble in MeOH and acetone.

3,3'-Dihydroxyisorenieratene (*14,* ϕ,ϕ-carotene-3,3'-diol) *ex Streptomyces mediolani.*[34] *14* (10 mg), 3 h reaction period, provided 8 mg (80 %) recovered pigment: unreacted *14* (60 %), monosulfate *14a* (30 %) and disulfate *14b* (10 %). The reaction mixture in addition, repeatedly, contained a blue, unstable product more strongly adsorbed than *14* and less strongly than *14a, cf.*[13] This product had VIS λ_{max} nm ($CDCl_3$) 600, (acetone) 570; IR (KBr) ν_{max} 3400 (s, OH), 2960, 2930 and 2860 (s) (CH), 1625 (conj. C=O?), 1580, 1515, 1435, 1375, 1340 and 1260 (all m-w), 1120, 980 and 950 (m, *trans* CH=CH, retro?) and 875 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ, values in brackets refer to *14,* 2.03 s (1.99 and 2.06; *ca.* 12H, Me-19,19',20,20'). 2.15 s, 2.27 s, 2.29 s, 2.44 s (2.17 and 2.24; *ca.* 18H, aromatic Me?), 6.25−6.99 (6.13−6.69; olefinic H); MS (200 °C) *m/z* 570, 560, 538, 468, 452, 449 and 410.

3,3'-Dihydroxyisorenieratene monosulfate (*14a*). R_F=0.45 (SiO_2, 10 % MeOH−EtOAc), inseparable from *8a*, VIS λ_{max} nm (MeOH) 453, (H_2O) 400 nm; IR (KBr) cm^{-1} 1230 (S=O), 960 (*trans* CH=CH); MS not achieved. Bisdiazobenzidin[35] caused a grey-brown colour with *14a*; with *14* a strong blue-brown colour.

14a was readily soluble in MeOH and acetone.

Storage in moist $CDCl_3$ containing traces of HCl caused quantitative conversion to *14,* shown by R_F, and MS.

3,3'-Dihydroxyisorenieratene disulfate 14b. R_F=0.2 (SiO_2, 10 % MeOH−EtOAc); VIS λ_{max} nm (MeOH) 450. *14b* was irreversibly adsorbed to cellulose. *14b* decomposed readily.

Stability test for sulfates of carotenols 8−14. Upon manipulation with these carotenoids in solution unpolar derivatives were formed. None of these sulfates were completely intact upon storage in MeOH at −20 °C for 3 months.

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