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Carotenoid Sulfates. 5.* Preparation and Solvolytic Reactions of Unstable Carotenoid Sulfates

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***tert*-Carotenoid C-5 sulfates obtained from azafrin and its methyl ester, *sec* allylic sulfates of lutein, lactucaxanthin, isozeaxanthin and β,β-carotene-3,4,3′,4′-tetrol and the *prim* allylic sulfate of β-apo-2′-carotenol were too unstable in solution for practical application.**

Product analyses from the methanolysis of such unstable carotenoid sulfates support solvolysis *via tert* or resonance stabilized carbocations.

Sulfates as leaving groups, *e.g.* in the azafrin series, may be synthetically useful.

In the previous paper of this series[1] we have described the partial syntheses and properties of i) carotenoid sulfates stable in methanol solution and ii) less stable carotenoid sulfates undergoing relatively slow solvolysis in methanol or aqueous solution.

We now report the preparation of carotenoid sulfates undergoing fast solvolysis. Product analysis support solvolysis *via* tertiary or resonance stabilized carbocations.

Trivial names are used, but semirational IUPAC names[2] are included in the Experimental part.

RESULTS AND DISCUSSION

Seven carotenols providing such unstable carotenoid sulfates are listed in Scheme 1. Included are the *tert* carotenols azafrin (*1*) and methyl azafrin (*2*), carotenols with *sec* allylic hydroxy groups in ε-rings such as lutein (*3*) and lactucaxanthin (*4*), carotenols containing *sec* hydroxy groups allylic to the polyene chain exemplified by isozeaxanthin (*5*) and 3,4,3′,3′-tetrahydroxy-β,β-carotene (*6*), and finally β-apo-2′-carotenol (*7*) with *prim* hydroxy group allylic to the polyene chain.

Whereas the *tert* hydroxy group of the allenic end group in fucoxanthin and peridinin provided no sulfate under the conditions used,[1] carotenoids containing the diol end group A formed monosulfates slowly. Thus azafrin (*1*) and its methyl ester (*2*) each afforded monosulfates (*1a* and *2a*), Scheme 1. Upon silylation azafrin (*1*) gave a di-trimethylsilyl derivative (*1b*), accompanied by a bathochromic shift in the visible absorption spectrum ascribed to silyl ester formation. Azafrin methyl ester (*2*), consistent with previous results,[3–5] gave a mono-trimethylsilyl ether (*2b*) only, Scheme 2. It may be inferred that the *tert*

* No. 4. See Ref. 1.

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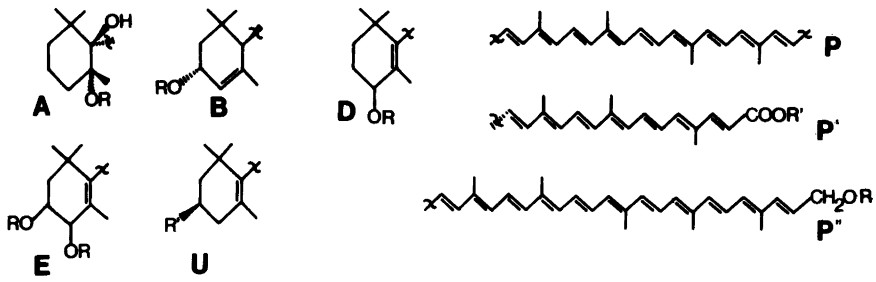
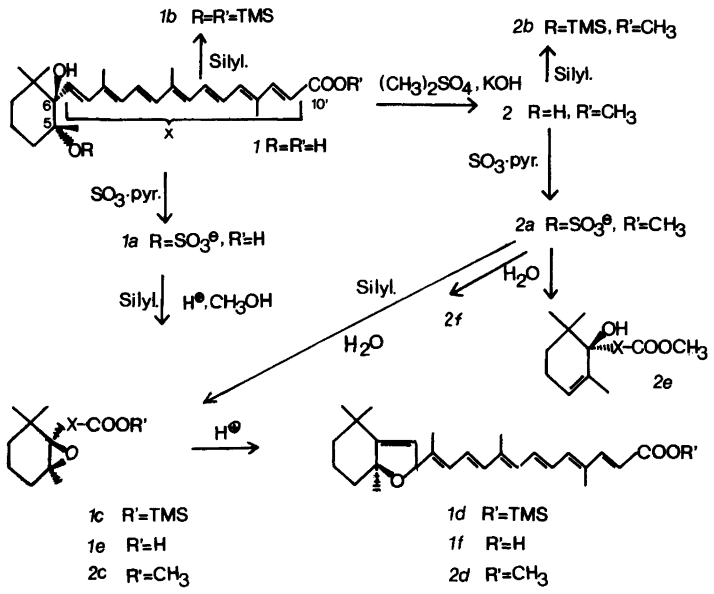


Azafrin	*1* A–P′	R=H, R′=H
	1a	R=SO_3^-, R′=H
Azafrin methyl ester	*2* A–P′	R=H, R′=CH_3
	2a	R=SO_3^-, R′=CH_3
Lutein	*3* U–P–B	R=H, R′=OH
	3a	R′=OSO_3^-, R=H
	3b	R′=OH, R=SO_3^-
	3c	R′=OSO_3^-, R=SO_3^-
Lactucaxanthin	*4* B–P–B	R=H
	4a	R=SO_3^-, R=H
	4b	R=SO_3^-
Isozeaxanthin	*5* D–P–D	R=H
	5a	R=SO_3^-,R=H
	5b	R=SO_3^-
	5c	R=CH_3,R=H
	5d	R=CH_3
3,4,3′,4′-Tetrahydroxy-β,β-carotene	*6* E–P–E	R=H
	6a	R=SO_3^-, (R=H)$_3$
	6b	(R=SO_3^-)$_2$, (R=H)$_2$
β-Apo-2′-carotenol	*7* U–P′	R′=H, R=H
	7a	R′=H, R=SO_3^-

Scheme 1. Carotenols forming unstable sulfates.

hydroxy group at C-6 is sterically inaccessible for silylation[3–5] and sulfate formation. Supporting evidence for allocation of the sulfate to C-5 in *1a* and *2a* was sought by silylation. However, unlike the *sec* sulfates of fucoxanthin and peridinin with *tert* hydroxy groups available for silylation,[1] the *tert* monosulfates (*1a, 2a*) did not survive the silylation process including extractive isolation, and resulted in desulfated products (*1c, 1d, 2c,* Scheme 2), presumably formed *via* C-5 carbocations, Scheme 5a. The epoxide *2c,* previously obtained by treatment of *2* with a sulfurane,[4] and *1c* had characteristic VIS, and MS properties, and the azafrin derivative *1c* was readily rearranged to the furanoid derivative *1d.* The chiralities assumed for *1c* and *2c* would result from nucleophilic attack of the C-6 hydroxy group of a C-5 cation, and that of *1d* (predicted two C-8 epimers) from the known retention of configuration at C-5 of epoxides upon furanoid rearrangement.[6] Other furanoid derivatives of azafrin methyl ester (*2*) have been prepared by treatment of *2* with $TiCl_4$ in benzene.[4] Acid catalyzed methanolysis of azafrin monosulfate (*1a*) also resulted in furanoid products (*1f*), presumably formed *via* the epoxide *1e.*

Azafrin-5-monosulfate (*1a*) in water behaved as a carotenoid soap with foaming. However, the *tert* sulfates *1a* and *2a* were unstable in aqueous solution and provided less polar hydrolysis products. Thus azafrin methyl ester monosulfate (*2a*) provided these



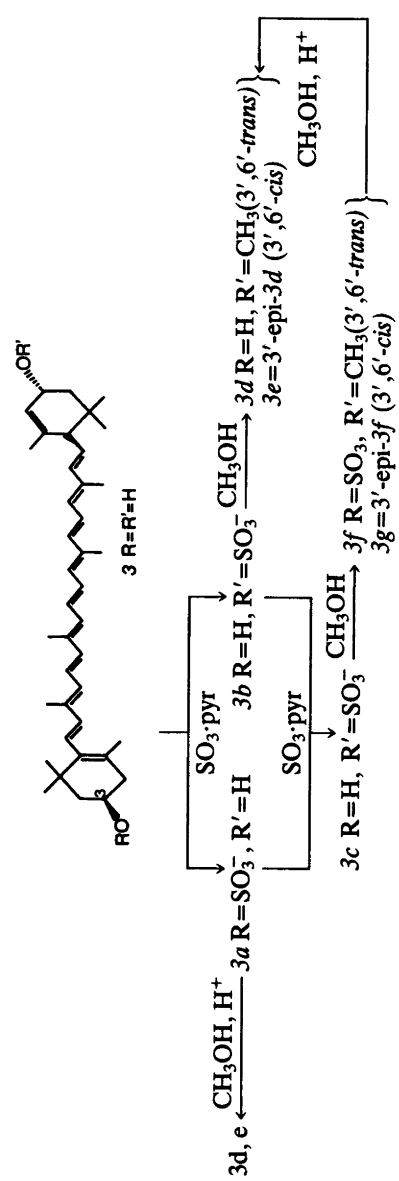
Scheme 2. Formation and reactions of the *tert*-5-monosulfates of azafrin (*1*) and its methyl ester (*2*).

products: The epoxide *2c*, which was furanoid rearranged to *2d* upon subsequent acid treatment; the bisallylic *tert* carotenol *2e*, which responded to conditions for allylic rearrangement upon subsequent acid treatment, and an unidentified product *2f* with prolonged chromophore. The MS fragmentation pattern of *2f* did not lend support to a pinacolic rearrangement 6-one product. However, products *2c* and *2e* offer circumstantial evidence for hydrolysis *via* the C-5 cation.

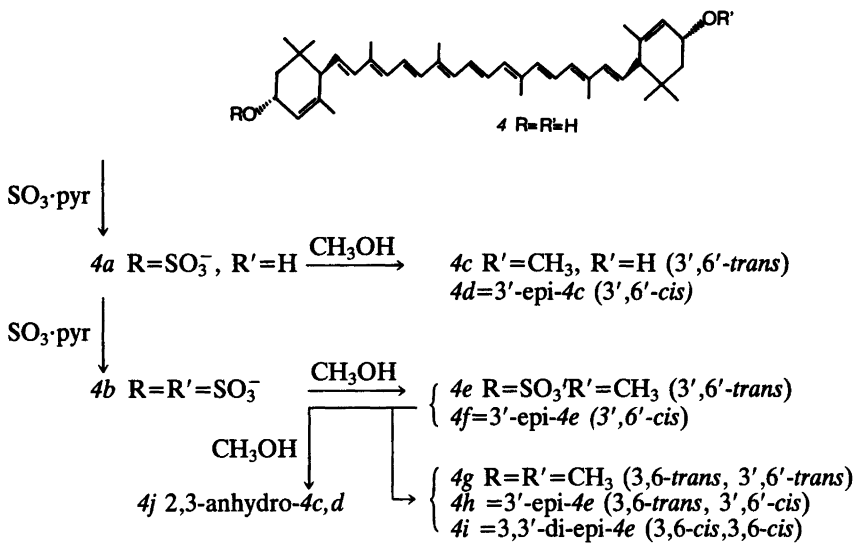
A priori lutein (*3*) was expected to form a disulfate (*3c*, Scheme 1). However, the initially formed disulfate *3c* underwent fast methanolysis, providing stable monosulfates *3f, g*, possessing allylic methoxy groups, Scheme 3. Whereas the non-allylic 3-monosulfate *3a* was stable, formation of the allylic 3'-monosulfate *3b* was inferred from the methanolysis products, the lutein 3'-methyl ethers (*3d,e*). The formation of both the 3',6'-*trans* (*3d, 3f*) and 3',6'-*cis* (*3e, 3g*) isomers support cationic intermediates in the solvolysis, see Scheme 5b. The diastereomeric methyl ethers *3f* (3',6'-*trans*) and *3g* (3',6'-*cis*) as well as the diastereomeric methyl ethers *3d* (3',6'-*trans*) and *3e* (3',6'-*cis*), were formed in 1.7:1 ratio. The configuration of the dominant 3',6'-*trans* isomers allows quasiequatorial conformation of both the methoxy group and the polyene chain.

The monosulfates *3a* and *3f,g* could not be separated quantitatively by TLC, but relative quantities could be estimated by ^{1}H NMR (400 MHz).[8,9] Unexpectedly, product analysis of the sulfatation mixture after access to methanol suggested that the non-allylic 3-hydroxy group of lutein (*3*) underwent faster sulfate formation than the allylic one. Thus the composition of a reaction mixture prior to methanolysis of unreacted lutein (*3*, 50 % of total), non-allylic monosulfate *3a* (32 %), allylic monosulfate (*3b*, 13 %) and disulfate (*3c*, 5 %), could be estimated.

The allylic diol lactucaxanthin (*4*) with two identical 3-hydroxy-ε-end groups identical to the one in lutein (*3*) gave a very unstable monosulfate (*4a*) and disulfate (*4b*), Scheme 4,



Scheme 3. Sulfate formation and subsequent methanolysis of lutein (*3*).



Scheme 4. Formation and methanolysis of lactucaxanthin (*4*) mono- and disulfates.

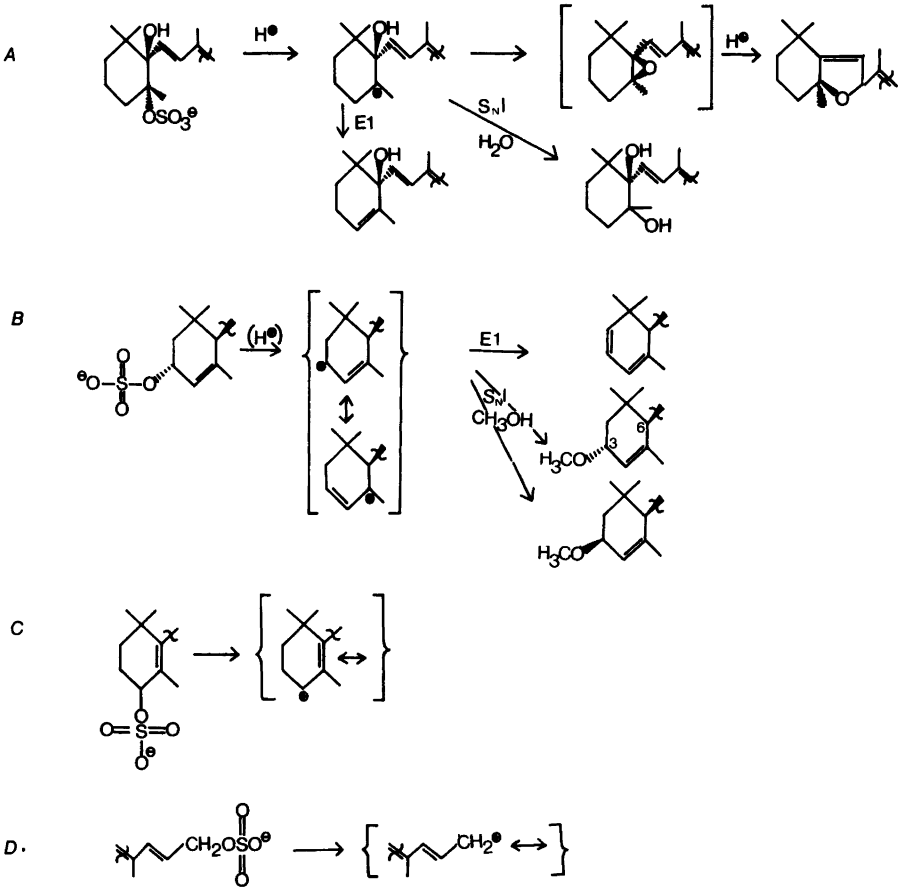
which were only partly characterized. These carotenoid sulfates underwent fast solvolysis to the corresponding methyl ethers. Product lactucaxanthin (*4*) and its monomethyl ethers (*4c,d*) were considered derived from the monosulfate (*4a*) and the dimethyl ethers (*4g,h,i*) from the disulfate (*4b*), the monosulfate monomethyl ethers *4e,f* presumably representing intermediates in the methanolysis of the disulfate *4b*. Methanolysis *via* allylic C-3 cation is supported by the racemization of the previously sulfated sites, as demonstrated by ^{1}H NMR evidence. As for the lutein (*3*) case the ratio of 3,6(3',6') *trans*:3,6(3',6') *cis* methoxylated ε-end groups of the methanolysis products was 1.7:1. A solvolysis product with properties compatible with 2,3-double bond (*4j*, Scheme 4) is consistent with a C-3 cation intermediate, Scheme 5. The chirality of the minor product lactucaxanthin was not investigated. Solvolysis products compatible with the C-5 cation intermediate, Scheme *5b*, were not detected.

The diol isozeaxanthin (*5*) with hydroxy groups allylic to the polyene chain formed a partly characterized disulfate *5a*, characteristically strongly adsorbed to SiO_2. Isozeaxanthin monomethyl ether (*5c*) and dimethyl ether (*5d*), isolated from the sulfatation mixture after access to methanol are considered methanolysis products of the monosulfate *5a* and disulfate *5b*, respectively, formed *via* the allylic carbocation, Scheme 5c.

For the tetrol *6*, Scheme 1, a complex product mixture was obtained. A presumed mono-(*6a*) and disulfate (*6b*) were partly characterized.

Finally β-apo-2'-carotenol (*7*) provided a partly characterized, allylic sulfate (*7a*), Scheme 1. Product *7a* was strongly adsorbed to SiO_2 and underwent solvolysis to less polar products, presumably *via* a resonance stabilized carbocation, Scheme 4d.

In conclusion the *tert* sulfates obtained from azafrin (*1*) and its methyl ester (*2*), the *sec* allylic sulfates of lutein (*3*), lactucaxanthin (*4*), isozeaxanthin (*5*) and the tetrol *6*, as well as the *prim* allylic sulfate of β-apo-2'-carotenol (*7*) were too unstable in solution for practical application. This includes azafrin 5-monosulfate (*1a*) which had foaming properties in water as a real soap.



Scheme 5. Solvolysis reactions of unstable carotenoid sulfates.

However, the use of sulfates as leaving groups, *e.g.* in the azafrin series, may on a preparative scale lead to derivatives so far not readily available.

EXPERIMENTAL

General. General precautions, spectroscopy, chromatography and the general procedure for sulfate formation are given in the previous paper of this series.[1]

Azafrin (*1*, (5*R*,6*R*)-5,6-Dihydroxy-5,6-dihydro-10′-apo-β-caroten-10′-oic acid) *ex Escobedia scabrifolia, cf.* Ref. 10. R_F=0.55 (SiO_2 Alufolien 5 % MeOH in EtOAc; System 2) *1* (10 mg), 5 h reaction period; pigment recovery *ca.* 50 %: unpolar product (5 % of recovered), unreacted *1* (65 %) and monosulfate *1a* (30 %) judged by TLC of the reaction mixture prior to transfer to $CHCl_3$ from an aqueous hypophase.

Azafrin 5-monosulfate (*1a*), R_F=0.42; R_F=0.12 (System 2); VIS λ_{max} nm (MeOH) (385), 407 and 429, (H_2O) (388), 410 and 433; MS (210 °C) *m/z* 408 (M′, 100 %). Solubility in $H_2O \geq 0.3$ mg/ml with characteristic foaming.

Upon distribution between EtOAc: H_2O *1a* had partition ratio 7:3 at pH 10 and 10:0 at pH 3. However, unpolar hydrolysis products were formed during this test.

Solvolysis of *1a* was effected in i) H_2O and ii) acidified MeOH. Hydrolysis in H_2O was relatively fast (in one experiment measured 30 % conversion after 1 h) providing an azafrin-like product; R_F=0.75 (SiO_2, 50 % acetone in hexane; C-5 epimers are not expected to separate in this system), inseparable from authentic *1*; VIS λ_{max} nm (acetone) 412 and (433); MS (205 °C) *m/z* 426 (M, 3 %) 408 (M−18, 10 %), 173 (100 %) and less polar products.

1a (0.72 mg) was treated with 0.1 N HCl in MeOH for 10 min by the previous procedure;[1] pigment recovery 90 %: furanoid *1f* (95 % of total) and *1e* (5 %).

Product *1f* had R_F=0.80 (SiO_2, EtOAc), compared with 0.64 for *1*; R_F=0.49 (System 2); VIS λ_{max} nm (MeOH) (365), 383 and 404, % III/II=66; MS (205 °C) *m/z* 408 (M, 100 %), 302 (M−106, 14 %), 205 (32 % homopyrylium), 165 (45 %, pyrylium). The less strongly adsorbed product *1e* had R_F=0.88 (System 2); VIS λ_{max} nm (acetone) 395; MS (205 °C) 422 (M).

Silylation of *1a* (0.5 mg) in dry pyridine (1 ml) was effected with hexamethylsilazane (0.2 ml) and trimethylchlorosilane (0.1 mg); pigment recovery 30 %: *1c* (70 % of recovered) and *1e* (30 %). Product *1c*, R_F=0.61 (System 2); VIS λ_{max} nm (acetone) (390), 413 and 435; MS (205 °C) *m/z* 480 (M, 5 %), 408 (M−72, 100 %), 205 (31 %, homopyrylium), 165 (40 %, pyrylium). After storage λ_{max} shifted to 394 and 410 nm (MeOH) upon conversion of *1c* to *1d*. Product *1e*, R_F=0.65 (System 2); VIS λ_{max} nm (acetone) 390 and 408; MS (205 °C) *m/z* 408 (M−72, 13 %), 382 (100 %), 205 (50 %, homopyrylium), 165 (40 %, pyrylium).

Azafrin 5,10′-ditrimethylsilyl ether (*1b*). Standard silylation as for *1a* above for 3 h gave 90 % pigment recovery. *1b* (60 % of recovered) had R_F=0.65 (System 2); VIS λ_{max} nm (acetone) 415 and 435; MS (205 °C) *m/z* 570 (M, 36 %), 498 (M−72, 100 %).

Azafrin methyl ester (*2*, methyl (5*R*,6*R*)-5,6-dihydroxy-5,6-dihydro-10′-apo-β-caroten-10′-oate) from *1*, *cf.*,[11] R_F=0.83 (System 2). *2* (16.8 mg), 5 h reaction period; pigment recovery 8.5 mg (51 %): unreacted *2* (78 %) of recovered and *2a* (22 %).

Azafrin methyl ester 5-monosulfate (*2a*), R_F=0.13 (System 2); VIS *m/z* nm (MeOH) 415 and (435), (H_2O) 433; MS (205 °C) *m/z* 422 (M′, 100 %), 404 (M′−18, 72 %), 442 (M′−80, 17 %), 205 (100 %, homopyrylium), 165 (12 %, pyrylium).

2a was readily soluble in H_2O (≥ 0.13 mg/ml), but hydrolyzed fast. After 1 h in H_2O 30−75 % conversion to less polar products were observed by TLC. In one experiment was isolated an azafrin methyl ester-like product inseparable from authentic *2*, R_F=0.51 (SiO_2, 50 % acetone−hexane; C-5 epimers may not separate in this system), VIS λ_{max} nm (MeOH) 415; MS (205 °C) *m/z* 440 (M, 100 %), 422 (M−18, 12 %).

Product *2c* had R_F=0.69 (SiO_2, EtOAc), VIS λ_{max} nm (acetone) (395), 415 (435); ($CHCl_3$) 428, (445); MS (200 °C) *m/z* 422 (M, 100 %), 407 (M−15, 8 %), 391 (M−31, 8 %), 342 (M−80, 17 %), 205 (homopyrylium, 42 %), 165 (pyrylium, 33 %). Treatment of *2c* with 0.03 N HCl in $CHCl_3$ caused transformation to *2d*; VIS *m/z* nm ($CHCl_3$) 405 (425).

Product *2e* had R_F=0.73 (SiO_2, EtOAc); VIS λ_{max} nm (acetone) (395) 415, (435), ($CHCl_3$) 428, 445; MS (200 °C) *m/z* 422 (M, 50 %), 404 (M-18, 38 %), 149 (100 %), no *m/z* 205 or 165 ions. Treatment of *2e* with 0.03 N HCl in $CHCl_3$ caused a bathochromic shift to 443 nm (round shaped).

Product *2* had R_F=0.71 (SiO_2, EtOAc); VIS λ_{max} nm (408) 428, 452, % III/II=5; MS (200 °C) *m/z* 422 (M, 62 %), 391 (M−31, 5 %), 149 (100 %). *2f* could not be acetylated (R_F and MS unchanged) and gave no products with longer chromophore upon treatment with acidified chloroform.

Silylation of *2a* was carried out by the standard procedure for 4 h. After extractive work up followed by TLC *2c*, was isolated: R_F=0.61 (SiO_2, EtOAc) relative to R_F=0.36 for *2* and R_F=0 for *2a*; VIS λ_{max} nm (acetone) (395), 412. MS (205 °C) *m/z* 422 (M, 100 %), 407 (M−15, 11 %), 391 (M−31, 11 %), 342 (M−80, 27 %), 205 (36 %, homopyrylium), 165 (31 %, pyrylium).

Azafrin methyl ester 5-trimethylsilyl ether (*2b*), prepared from *2* by standard procedure, R_F=0.69 (SiO_2, EtOAc), R_F=0.86 (System 2); VIS λ_{max} nm (acetone) 415 and 435; MS (205 °C) 512 (100 %, M), 422 (M−18−72, 7 %).

Lutein (*3*, 3*R*,3′*R*,6′*R*-β,ε-carotene-3,3′-diol) *ex Medicago sativa*, National Chlorophyll Co. *Ca* 10 % zeaxanthin was removed from this sample by TLC on special plates[12] (*3*, R_F=0.56, zeaxanthin R_F=0.26). Of 8 sulfatation experiments on the 1−10 mg scale a typical one is cited: *3* (10.4 mg), 1 h reaction period, reaction mixture prior to work up showed by

TLC unreacted *3* (50 % of total), monosulfates *3a,b* (30 %) and disulfate *3c* (20 %); pigment recovery after work up 93 %. Longer reaction periods gave lower pigment recovery and higher proportion of sulfates. The monosulfate(s) was formed relatively fast, on the 1 mg scale after 30 min *ca.* 50 % conversion. In one experiment (9.5 mg *3*, 1 h reaction period, work up in the presence of MeOH), product analysis including ^{1}H NMR showed lutein (*3*, 50 % of recovered), lutein 3-sulfate (*3a*, 32 %), lutein 3'-methyl ether *3d,e*, 13 %) and lutein 3-sulfate-3'-methyl ether (*3f,g*, 5 %). When the reaction mixture was worked up in the absence of MeOH, using EtOAc/DMF as specified for lactucaxanthin (*4*) below, no methylated products were formed. However, the disulfate *3c* hydrolyzed in contact with H_2O.

Lutein 3-monosulfate (*3a*), R_F=0.38 (SiO_2, 15 % MeOH–EtOAc) VIS λ_{max} nm (MeOH) 443 and 468, % III/II=60; ^{1}H NMR (CD_3OD, 400 MHz), obtained for a mixture with *3f, g*; assignments for *3a* δ 0.85 s and 1.00 s (Me-1'), 1.08 s and 1.11 s (Me-1), 1.63 s (Me-5'), 1.73 s (Me-5), 1.91 s (Me-9'), 1.96 s (Me-9,13.13'), 2.42 d (J=9 Hz, H-6'), 4.2 m (H-3'), 4.65 m (H-3), 5.5 d (H-4') and 6.1–6.8 m (conj. olefinic H); ^{13}C NMR (CD_3OD δ 12.8 (C-19,20,20'), 13.0 (C-19'), 21.8 (C-18), 23.1 (C-18'), 24.0 and 30.1 or 30.7 (C-16',17'), 29.0 and 30.7 or 30.1 (C-16,17), 40.6 (C-4), 66.4 (C-3'), 74.0 (C-3), 126.9 (C-4'?); MS (200 °C) *m/z* 500 (M'), 532 (M'–92); CD (MeOH) nm ($\Delta\varepsilon$) 222 (+3.6), 240 (+7.0), 280 (0), 286 (–0.3), 305 (0), 338 (–0.8), 350 (0); water solubility ≥0.03 mg/ml.

When the sulfatation mixture was worked up in the presence of MeOH *3a* was obtained in mixture with *3f,g*. Separation by TLC was incomplete. MS revealed the presence of *3f,g* and ^{1}H NMR (400 MHz) established the relative amounts of *3a:3g*. From one experiment 45 % *3a*+34 % *3f*+21 % *3g* was estimated from the intensities of the two OMe and skeletal Me signals.

Acid methanolysis of *3a* (0.5 mg) in 0.1 n HCl–MeOH for 30 min[1] gave 90 % pigment recovery: *3a,f,g* (74 %), *3d* (16 %), and *3e* (10 %).

Allylic methylation of *3a* (4 mg) with 0.03 n HCl–MeOH for 60 min provided *3f,g* judged by MS.

Enzymatic hydrolysis, *cfr.* alloxanthin monosulfate,[1] of *3a* was unsuccessful.

Lutein disulfate (*3c*), R_F=0.18 (SiO_2, 15 % MeOH–EtOAc); VIS λ_{max} nm (acetone) 442 and 468, % III/II=40, MS (205 °C) *m/z* 532 (M'), 440 (M'-92), 426 (M'-106). Storage in MeOH resulted in conversion to *3,3f,g* and *3d,e*.

Lutein 3'-methyl ether (*3d,e*), R_F=*ca.* 0.53 (SiO_2, hexane); VIS λ_{max} nm (MeOH) 442 and 468, % III/II=52; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.84 s (3H, Me-1') 0.94 and 0.97 s (3H, Me-1'), 1.07 s (6H, Me-1), 1.64 s and 1.62 s (3H, Me-5'), 1.73 s (3H, Me-5), 1.92 s (3H, Me-9'), 1.97 s (9H, Me-9,13,13').

Lactucaxanthin (*4*, (3*R*,6*R*,3'*R*,6'*R*)-ε,ε-carotene-3,3'-diol), synthetic.[13] In 5 experiments *4* (1–2 mg), *ca.* 1 h reaction period, reaction mixture revealed by TLC unreacted *4* (*ca.* 20 % of total), monosulfate *4a* (50–70 %) and disulfate *4b* (10–30 %). Standard work up showed 80–90 % pigment recovery.

The sulfates were isolated from experiments where contact with MeOH and H_2O was avoided and DMF–EtOAc used for extraction and chromatography (SiO_2, 20 % DMF–EtOAc) and were partly characterized. Otherwise, secondary, methylated products were obtained.

Lactucaxanthin monosulfate (*4a*), R_F=0.33 (SiO_2, 15 % MeOH–EtOAc); VIS λ_{max} nm (acetone) (390), 413, 438 and 468, % III/II=67. Storage in moist MeOH resulted in conversion to a lactucaxanthin-like product and mainly the monomethyl ethers (*4c,d*) as revealed by R_F, VIS and MS.

Lactucaxanthin disulfate (*4b*), R_F=0.11 (SiO_2, 15 % MeOH–EtOAc); VIS λ_{max} nm (acetone) (390), 413, 438 and 468; % III/II=69. Storage in moist MeOH caused conversion to a lactucaxanthin-like product and the monomethyl ethers *4c,4d*, judged by R_F, VIS and MS. Upon storage of *4b* in DMF/EtOAc a lactucaxanthin-like product was formed, judged by R_F and VIS.

Lactucaxanthin monomethyl ethers (*4c, d*), isolated after work up in the presence of MeOH, less polar than *4*; VIS λ_{max} as *4*; MS (205 °C) *m/z* 582 (M, 6 %), 564 (M–16, 34 %), 532 (M–18–32, 8 %), 444 (M–32–106, 6 %), 43 (100 %).

Lactucaxanthin monosulfate monomethyl ethers (*4e,f*), isolated after work up in the presence of MeOH, R_F and VIS λ_{max} as *4a*; MS (205 °C) *m/z* 564 (M', 35 %), 532 (M'-32,

35 %), 440 (M'−32−92, 1 %), 426 (M'−32−106, 3 %), 265 (100 %).

Storage in moist MeOH provided the methyl ethers (*4c,d, 4g,h,i*) according to R_F, VIS and MS.

Lactucaxanthin dimethyl ethers (*4g,h,i*), isolated after work up in the presence of MeOH; R_F=0.5 (SiO_2, 7 % acetone-hexane), compared with R_F=0.2 for *4*; VIS λ_{max} nm (acetone) 415, 438 and 468, % III/II=90, ^{1}H NMR (400 MHz) $CDCl_3$ δ, *cis* refers to 3,6(3',6') *cis* and *trans* to 3,6(3',6') *trans* configuration: 0.84 s (Me-1,1'), 0.94 s (Me-1,1' *cis*), 0.97 s (Me-1,1', *trans*), 1.62 s (Me-5,5', *trans*), 1.64 s (Me-5,5', *cis*), 1.91 s (Me-9,9'), 1.96 (Me-13,13'), 2.15 d (*J*=9 Hz, H-6-6', *cis*), 2.42 d (*J*=9 Hz, H-6,6', *trans*), 3.36 s (OMe, *trans*), 3.38 s (OMe, *cis*), 3.82 m (H-3,3', *cis*?), 4.05 m (H-3,3', *trans*?), *ca.* 5.5 m (H-4,4'), 5.6−6.7 m (conj. olefinic H), 3,6(3',6') *trans*: 3,6(3',6') *cis* ratio 1,76, *c.f.* assignments for related end groups,[7,8] MS (200 °C) *m/z* 596 (M, 100 %), 564 (M−32, 23 %), 532 (M−32−32, 20 %).

2',3'-Anhydrolactucaxanthin 3-methyl ether (*4j*). This product comprised 7 % of the total recovered carotenoid in one experiment where the reaction mixture was worked up in the presence of MeOH; R_F=0.9 (SiO_2, 7 % acetone−hexane); VIS λ_{max} nm (acetone) 414, 438 and 468; MS (200 °C) *m/z* 564 (M, 100 %), 532 (M−32, 23 %), 458 (M−106, <1 %).

Isozeaxanthin (*5*, (4*RS*,4'*RS*)-β,β-carotene-4,4'-diol) was prepared by $LiAlH_4$ reduction in ether of synthetic canthaxanthin[14] *5* (3.5 mg), 1 h reaction period, pigment recovery 77 % after standard work up. Unpolar products and sulfated, strongly adsorbed products (SiO_2, 60 % MeOH−EtOAc; only partly eluted with MeOH) were observed by TLC. Attempted purification by ion exchange chromatography resulted in decomposition.

Isozeaxanthin disulfate (*5b*) R_F=0.5 (TLC cellulose, 10 % MeOH−EtOAc); VIS λ_{max} nm (MeOH) (420), 446 and 473; MS (200 °C) *m/z* 532 (M', 45 %), 440 (M'−92, 6 %), 91 (100 %). Storage in MeOH resulted in less polar products.

Isozeaxanthin monomethyl ether (*5c*), isolated after work up in the presence of MeOH, R_F=0.4 (SiO_2, 20 % acetone−hexane); VIS λ_{max} nm (MeOH) (425), 448 and 475; MS (200 °C) *m/z* 582 (M, 100 %), 566 (M−16, 20 %), 564 (M−18, 33 %), 550 (M−32, 13 %), 534 (M−16−32, 10 %), 532 (M−18−32, 8 %), 490 (M−92, 6 %).

Isozeaxanthin dimethyl ether (*5d*), isolated after work up in the presence of MeOH; R_F=0.75 (SiO_2, 20 % acetone−hexane); VIS λ_{max} as for *5b*; MS (200 °C) *m/z* 596 (M, 100 %), 564 (M−32, 22 %), 532 (M−32−32, 7 %), 504 (M−92, 5 %).

5c gave a positive test for allylic ether[15] by treatment with 0.03 N HCl in $CHCl_3$. The presumed 3,4,3',4'-tetradehydro-β,β-carotene products had VIS λ_{max} nm (acetone) 455 (broad).

3,4,3',4'-Tetrahydroxy-β,β-carotene (*6*,3*RS*,4'*RS*,3'*RS*, 4'*RS*-β,β-carotene-3,4,3'-4'-tetrol) prepared by standard $NaBH_4$-reduction[14] of synthetic astacene[17] in EtOH. *6* (1 mg), 2 h reaction period. TLC showed before work up unreacted *6* (20 %), and 5 more polar products; pigment recovery 70 %.

Tetrahydroxy-β,β-carotene monosulfate (*6a*), adsorptivity between *6* and *6b*, R_F=*ca.* 0.5 (SiO_2, 10 % MeOH−EtOAc); VIS λ_{max} nm (MeOH) (420), 446 and 472, (H_2O) 385, (440); MS unsuccessful. *6a* was stable in MeOH.

Tetrahydroxy-β,β-carotene disulfate (*6b*), R_F=*ca.* 0.2 (SiO_2, 15 % MeOH−EtOAc, inseparable from zeaxanthin disulfate), VIS λ_{max} nm (MeOH) (420), 445 and 470, (H_2O) 392 (440); MS unsuccessful. *6b* was fairly stable in MeOH.

β-Apo-2'-carotenol (*7*), prepared by $LiAlH_4$-reduction in ether of synthetic β-apo-2'-carotenal.[18] *7* (3.8 mg), 30 min reaction period. TLC revealed unreacted *7* (50 %) and a more polar product *7a* (50 %).

β-Apo-2'-carotenol sulfate (*7a*), R_F=0.24 (SiO_2, 10 % MeOH−EtOAc; could only be partly eluted with MeOH); VIS λ_{max} nm (MeOH) (435), 462 and 490, % III/II=12; IR (KBr) ν_{max} cm^{-1} 1249 w (S=O), 975 (*trans* CH=CH); MS unsuccessful.

Storage in MeOH resulted in less polar, unidentified products.

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