Synthetic Sulfur Carotenoids: 3′-Thiolutein

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Partial syntheses of the title compound [3′-thiolutein; (3R, 3′S, 6′R)-3′-mercapto-β,β-caroten-3-ol] via its S-acetyl derivative and of the S-acetyl derivative of (4′RS)-4′-mercapto-β,β-caroten-4-one are described.

Naturally occurring carotenoids containing elements other than hydrogen and oxygen attached to the carbon skeleton have not been encountered. A C19H24O(OCH3)S-carotenoid extracted from sulfur bacteria is now considered to be an artifact, and in natural carotenoid sulfates, the sulfur is not directly attached to the carbon skeleton. A carotenoid-like heterocoumpound, a nitrogen caroviolagen, has recently been synthesized. However, the incorporation of sulfur into carotenoids has either not been reported or did not ultimately succeed. Synthetic sulfur compounds are known within series of other common natural products, e.g. thiocarbohydrates, thioesters, thioflavonoids, and antibiotics, including thiolutin, homonomically related to the present work.

Sulfur compounds are important in metabolic processes or conversely may act as antimetabolites. The question arising from all this is: are there no naturally occurring sulfur carotenoids?

Results and discussion

Among the naturally occurring organic sulfur compounds, thiols are common and the thiol group is frequently connected with biological activity. We therefore aimed at the preparation of carotene thios.

The total synthesis of nearly any carotenoid is now possible, but reactions with preformed carotenoids are hampered by their instability. Conditions including light, heat, acid or oxygen cannot be tolerated, thus eliminating most of the classical synthetic methods. However, a recently described soft synthesis for thiols, starting from primary, tertiary or allylic alcohols was successfully applied to the preparation of the first carotenethiol.

Lutein (1) reacted with thiocetic acid in the presence of catalytic amounts of dry ZnCl2 or ZnI2 at room temperature to provide two isomeric thiocetates in 67% yield. With ZnI2 the reaction proceeded faster, but formation of by-products diminished the yield of the thiocetate. The results obtained may be rationalized according to Scheme 1, consistent with the previous assumption of an Sα1 type mechanism involving a carboxation. Thus (3R, 3′R, 6′R)-lutein (1) afforded a non-separable mixture of C-3′ epimeric thiocetates 2a and 2b under conditions where the non-allylic C-3 hydroxy function did not react. According to 1H NMR data, Scheme 2, the 3′,6′-trans epimer 2a was dominant after crystallization from benzene–hexane. Identification of the 3′,6′-trans and 3′,6′-cis isomers was mainly based on the chemical shift of the H-6′ methine proton. Similar mixtures of 3′,6′-trans and 3′,6′-cis C-3′ methyl ethers have been encountered, e.g. by methanalysis of lutein (1) disulfate, providing a 1:6:1 mixture of the 3′,6′-trans and 3′,6′-cis C-3′ methyl ether. The 3′,6′-trans configuration allows both the C-3′ and C-6′ substituents to occupy quasi-equatorial positions. In our case the relatively higher occurrence (3:1) of the 3′,6′-trans epimer may be due to the crystallization prior to 1H NMR measurements.

The thioacetates 2a and 2b had 1H NMR properties compatible with the structures assigned. Tentative 13C NMR assignments to the thioacetates 2a,b were based on comparison with reported values. The absorption spectrum of the thioacetates 2a,b were unchanged relative to lutein (1). In the IR spectrum, absorption at 1685 cm⁻¹ (SCOCH3) was observed. The mass spectrum showed characteristic fragment ions: M−34 (H2S), M−76 (CH3COSH), M−18 (H2O) and M−92 (toluene).

Hydrolysis of the thioacetates 2a,b with methanolic KOH, which is expected to proceed with retention of configuration (Scheme 1), afforded the stable monothiol 3 as a non-separable C-3′ epimeric mixture 3a,b in 29% (ZnCl2) and 21% overall yield. Again, after crystallization 3′,6′-trans-3a was the major epimer according to 1H NMR data. Substitution of the C-3′ hydroxy function with the thiol group caused only small changes in the chemical shifts of the methyl groups of the e-ring, but significant upfield shifts of the C-3′ methine proton. The expected molecular ion was observed on electron impact together with other diagnostic fragment ions M−18 (H2O), M−34 (H2S), M−52 (H2S+H2O) and M−92 (toluene). No absorption attributable to C–S or S–H bonds were noted in the IR spectrum. The absorption spectrum in visible light corresponded to that of lutein. However, the thiol 3a,b had a lower Rf-value on silica plates than lutein.
Reduction of the thioacetate 2a,b with LAH\textsuperscript{23} and subsequent isolation\textsuperscript{29} gave a lower yield of the thiol 3a,b than alkaline hydrolysis.

The allylic diol (4RS,4'SR)-isozeaxanthin (5), Scheme 3, prepared by LAH-reduction of canthaxanthin (4), reacted less readily under conditions for thioacetate formation. A dithioacetate 6 was characterized by MS. In addition to a strong molecular ion, fragment ions were observed corresponding to the elimination of CH\textsubscript{3}COS, CH\textsubscript{2}COSH, (CH\textsubscript{3})\textsubscript{2}OS, and 2 \times CH\textsubscript{3}COSH.

The main product was identified as the monothioacetate 7. A bathochromic shift of the absorption spectrum relative to isozeaxanthin (5) was compatible with autoxidation of the 4-hydroxy function, a process known to occur readily.\textsuperscript{30} \textsuperscript{1}H NMR assignments are included on structure 7, Scheme 3, with reference to data for canthaxanthin (4), isozeaxanthin (5) and its diacetate.\textsuperscript{23} The monothioacetate 7 exhibited the molecular ion and fragment ions as observed for the dithioacetate 6 above. However, attempts to isolate the corresponding allylic thiols of 6 and 7 after LAH reduction failed.

As a third category of activated carotenols providing carbocations, the tertiary alcohol 1,2,1',2'-tetrahydrolycopene-1,1'-diol (8) was tested. Treatment under conditions for thioacetate formation did not provide the expected product. Other competitive carbocation reactions are likely to have occurred in this case.

**Experimental**

*General methods.* General methods and instrumentations were as normally employed in our laboratory.\textsuperscript{31}}
precautions for work with carotenoids were taken. Spectral fine structure of VIS spectra are expressed as % III/II. Only prominent or diagnostically useful peaks in the mass spectra are reported.

The S-acetyl derivative of (3R,3'SR,6'R)-3′-mercapto-β-e-caroten-3-ol (2a,b). (3R,3'SR,6'R)-Lutein (1, 25 mg, 0.44 μmol ex. alfalfa, National Chorophyll Co.) was dissolved in CH₂Cl₂ (3 ml). Thioacetic acid (13 μl, 0.18 μmol) was added by a syringe followed by dry ZnCl₂ (6 mg), cf. Ref. 21. After being stirred for 90 min at 25 °C, the reaction mixture was diluted with CH₂Cl₂ and treated with water until neutral. The dried (Na₂SO₄) extract provided, after purification by vacuum liquid chromatography (VLC) and TLC (SiO₂, hexane–acetone mixtures), 17.3 mg (67%) of the pure (98%, HPLC, CN column) monothioacetates 2a,b.

In two subsequent parallel experiments 1 (25 mg) was treated under the same conditions as described above with dry ZnCl₂ (6 mg) and dry ZnI₂ (14 mg). (ZnI₂ was prepared by reaction of Zn with HI, extraction with ether and drying at 10 Torr, 25 °C). The reaction was monitored by TLC. ZnI₂ caused a more rapid reaction with 1, but more by-products were observed.
Scheme 3. Partial synthesis of the S,S-diacyl derivative of β,β-carotene-4,4'-dithiol (6) and the S-acetyl derivative of 4'-mercapto-
β,β-caroten-4-one (7) from isoezaexanthin (5).

2a,b had the following properties: $R_p = 0.37$ (TLC, SiO$_2$,
30% acetone in hexane, cf. 1 ($R_p = 0.24$); $t_R = 2.9$ min
(HPLC, CN-column, 76% hexane – 17% isopropyl acetate – 7% acetone – 0.1% MeOH, cf. 1 ($t_R = 4.8$ min); VIS
acetone) 424, 444 and 470 nm, % III/II = 33; IR (KBr)
3440 (OH), 3030, 1735, 1685 (COCH$_3$), 965 (trans di-
substituted double bonds) cm$^{-1}$; $^1$H NMR (500 MHz,
CDCl$_3$) after crystallization from benzene–hexane: see the
assignments given in Scheme 2; $^{13}$C NMR (125 MHz,
CDCl$_3$): signals were compatible with reported values for
lutein (1)$^{23}$ with diagnostic differences caused by the C-3'
thioacetate function: δ 195.2 (C=O), 30.6 (CH$_3$-C=O), no C-3'-OH signal at 65.9,$^{23}$ C-3'-S tentatively 28.94 ($^3_0$$^3_0$trans) and 29.93 ($^3_0$$^3_0$-cis); MS [IP 70 eV; m/z (% rel.
int.): 626 (33, M), 550 (100, [M–CH$_3$COSH], 532 (4,
[M–CH$_3$COSH$\cdot$H$_2$O]), 458 (4, [M–CH$_3$COSH–tolu-
ene]), 444 (4, [M–CH$_3$COSH–xylene]). For comparison,
lutein (1) monoacetate, prepared by acetylation of 1 with
an equimolar amount of acetic anhydride in pyridine, gave,
under identical mass spectrometric conditions, m/z (% rel.
int.): 610 (100, M), 592 (13, [M–H$_2$O]), 550 (63,
[M–CH$_3$COOH]), 532 (11, [M–CH$_3$COOH–H$_2$O]).

(3R,3'SR,6'R)-3'-mercapto-β,ε-caroten-3-ol (3a,b). Portions of crude 2 from the two parallel experiments de-
scribed above, were dissolved separately in ether (5 ml)
and hydrolyzed with 10% methanolic KOH (1.5 ml) over-
night at 25°C. After neutralization the pigments were ex-
tracted with ether, and the dried (Na2SO4) extracts were subjected to VLC and TLC, to afford 7.4 mg (29% overall yield; ZnCl2) and 5.4 mg (21% overall yield; ZnI2) of the monothiols 3a, b.

3a, b had the following properties: Rf = 0.46 (SiO2, 40% acetone in hexane) in comparison with lutein (1) Rf = 0.55; ta = 7.3 min (conditions as for 2a, b); VIS (nm, acetone) as for 1 and 2a, b; MS [IP 70 eV; m/z (% rel. int.):] 584 (11, M), 566 (5, [M-H2O]), 550 (100, [M-H2S]), 532 (5, [M-H2S-H2O]), 458 (4, [M-H2S-H2O]), 444 (2, [M-H2S-toluene]). IR (KBr): 3440 (OH), 3040, 965 (trans, substituted double bond) cm⁻¹; 1H NMR (500 MHz, CDCl3) after crystallization from benzene–hexane: see Scheme 2.

(4RS,4'R,RS)-β,β'-Carotene-4,4'-diol (5) (isoeoxanthin).

This was prepared by standard procedures by LAH reduction in dry ether or NaBH4 reduction in methanol of 50 mg synthetic canthaxanthin (4, Hoffmann–La Roche). Rf = 0.05 (SiO2, 5% acetone in hexane), VIS (acetone): 432, 450 and 477 nm.

To 5 (20 mg, 0.035 mmol) in CH2Cl2 (7 ml) was added ZnCl2 (3 mg), ZnI2 (7 mg) and thiaoacetic acid (13 μl, 0.18 mol). The mixture was stirred for 24 h at 25°C. TLC revealed, after work-up as described for 1 above, the presence of nine products. The four major products were separated by VLC and preparative TLC.

The S,S-diacetyl derivative of (4RS,4'R,RS)-β,β'-carotene-4,4'-diol (6). This was obtained as a mixture (< 10% by 1H NMR spectroscopy) with the S-acetyl derivative of (4'R,RS)-4'-mercapto-β,β'-carotene-4-one (7), yield 0.8 mg (35%). Rf (mixture) = 0.32 (SiO2, 5% acetone in hexane), 3f (Rf = 0.05. MS [IP 70 eV; m/z (% rel. int.)] 684 (100, M), 624 (59, M-), 609 (9, [M-CH2CO]), 608 (67, [M-CH2CO2H]), 548 (46, [M-CH2CO2H]), 523 (66, [M-CH2CO2H]), 523 (61, [M-2×CH2CO2H]); M refers to 6 and M- to 7. VIS (acetone) 457 nm rounded peak. IR (KBr) 1690 (SCOC2H5) 1670 (C=O), 970 (trans substituted double bonds) cm⁻¹. 1H NMR (500 MHz, CDCl3) after crystallization from benzene–hexane: see the assignments in Scheme 3.

After treatment of 6 and 7 (0.8 mg) with LAH in dry ether and the described work up, 0 thiol could be isolated.

1,2,1',2'-Tetrahydrolycopene-1',4'-diol (8). Synthetic 8 (52 mg, 0.09 mmol, Hoffmann–La Roche, Nutley) was dissolved in CH2Cl2 (15 ml). ZnI2 (29 mg) and thiaoacetic acid (31 μg, 0.4 mmol) were added. After being stirred at 25°C overnight the dark blue solution was washed with water until neutral whereupon it turned red. TLC gave two products, less polar than 8, in insufficient quantity for further characterization.

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References


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