Synthetic Sulfur Carotenoids: 3′-Thiolutein

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Partial syntheses of the title compound [3′-thiolutein; (3*R*, 3′*RS*, 6′*R*)-3′-mercapto-β,ε-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 $C_{40}H_{47}O(OCH_3)S$-carotenoid extracted from sulfur bacteria[1] is now considered to be an artifact,[2] and in natural carotenoid sulfates, the sulfur is not directly attached to the carbon skeleton.[3,4] A carotenoid-like heterocompound, a nitrogen caroviologen, has recently been synthesized.[5] However, the incorporation of sulfur into carotenoids has either not been reported or did not ultimately succeed.[6] Synthetic sulfur compounds are known within series of other common natural products, e.g. thiocarbohydrates,[7,8] thiosteroids,[9] thioflavonoids[10] and antibiotics, including thiolutin,[11,12] homonymically related to the present work.

Sulfur compounds are important in metabolic processes[13] or conversely may act as antimetabolites.[8,14] 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.[13,15–17] We therefore aimed at the preparation of carotene thiols.[18]

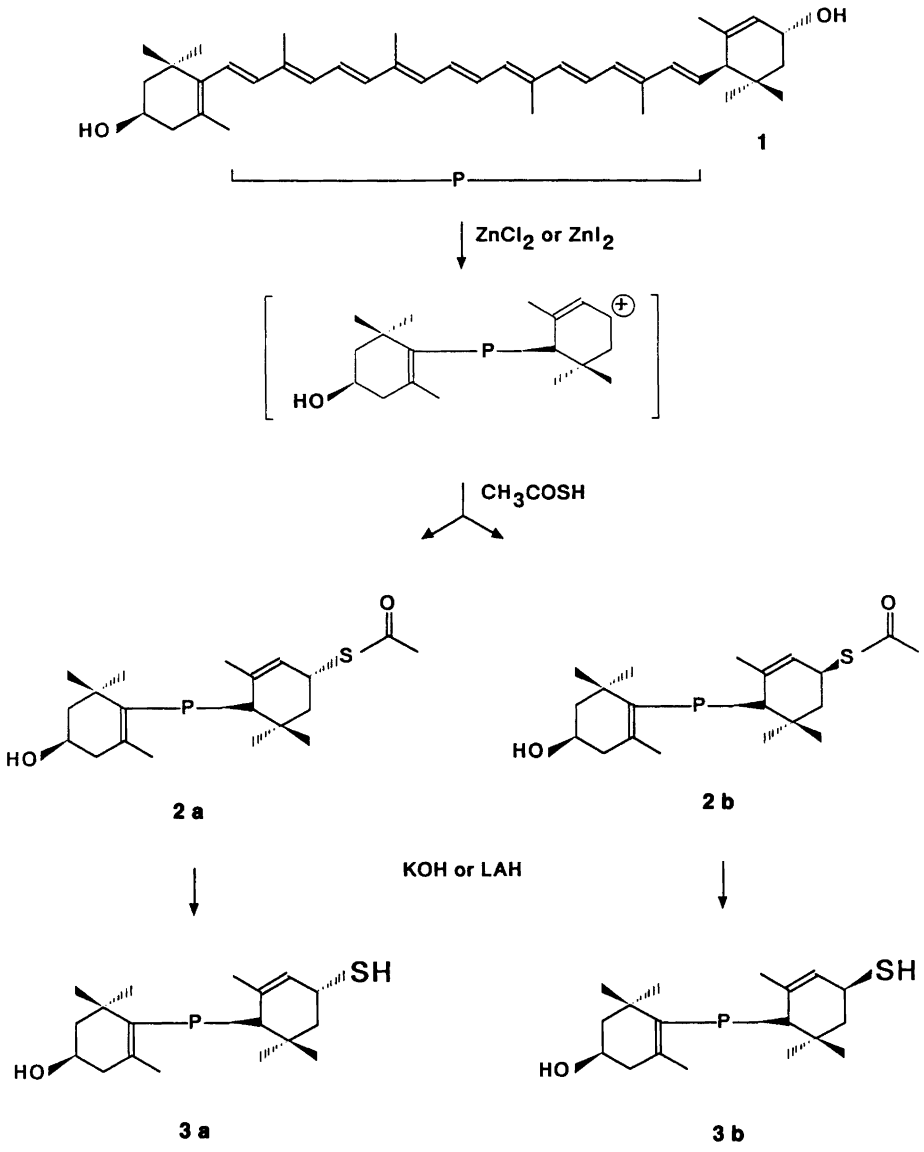
The total synthesis of nearly any carotenoid is now possible,[19,20] but reactions with preformed carotenoids are hampered by their instability. Conditions including light, heat, acid or oxygen cannot be tolerated, thus eliminating most of the classical synthetic methods. However, a recently described soft synthesis for thiols, starting from primary, tertiary or allylic alcohols[21] was successfully applied to the preparation of the first carotenethiol.

Lutein (**1**) reacted with thioacetic acid in the presence of catalytic amounts of dry $ZnCl_2$ or ZnI_2 at room temperature to provide two isomeric thioacetates in 67 % yield. With ZnI_2 the reaction proceeded faster, but formation of by-products diminished the yield of the thioacetate. The results obtained may be rationalized according to Scheme 1, consistent with the previous assumption of an S_N1 type mechanism involving a carbocation.[21] Thus (3*R*, 3′*R*, 6′*R*)-lutein (**1**) afforded a non-separable mixture of C-3′ epimeric thioacetates **2a** and **2b** under conditions where the non-allylic C-3 hydroxy function did not react. According to ^{1}H 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.[22,23] Similar mixtures of 3′,6′-*trans* and 3′,6′-*cis* C-3′ methyl ethers have been encountered, e.g. by methanolysis of lutein (**1**) disulfate, providing a 1.6:1 mixture of the 3′,6′-*trans* and 3′,6′-*cis* C-3′ methyl ether.[24] 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 ^{1}HNMR measurements.

The thioacetates **2a** and **2b** had ^{1}H NMR properties compatible with the structures assigned. Tentative ^{13}C NMR assignments to the thioacetates **2a,b** were based on comparison with reported values:[25,26] δ ca. 29 (C-3′), 30.6 (CH_3–C=O) and 195.2 (S–C=O).

As expected the absorption spectrum of the thioacetates **2a,b** were unchanged relative to lutein (**1**). In the IR spectrum, absorption at 1685 cm^{-1} ($SCOCH_3$)[27] was observed. The mass spectrum showed characteristic fragment ions: $M-34$ (H_2S), $M-76$ (CH_3COSH), $M-18$ (H_2O) 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 % ($ZnCl_2$) and 21 % overall yield (ZnI_2). Again, after crystallization 3′,6′-*trans*-**3a** was the major epimer according to ^{1}H NMR data, Scheme 2. 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 ε-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 $M-18$ (H_2O), $M-34$ (H_2S), $M-52$ (H_2S+H_2O) 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 R_F-value on silica plates than lutein.



Scheme 1. Partial synthesis of 3′-thioluteins (**3a,b**) via their 3′-*S*-acetyl derivatives (**2a,b**).

Reduction of the thioacetate **2a,b** with LAH[28] and subsequent isolation[29] gave a lower yield of the thiol **3a,b** than alkaline hydrolysis.

The allylic diol (4*RS*,4′*RS*)-isozeaxanthin (**5**), Scheme 3, prepared by LAH-reduction of canthaxathin (**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_3COS, CH_3COSH, (CH_3COS + CH_3COSH) and 2×CH_3COSH.

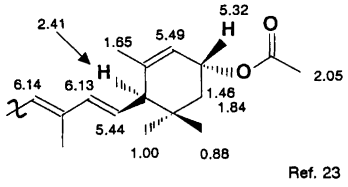
The main product was identified as the monothioacetate **7**. A bathochromic shift of the absorption spectrum relative to isozeaxanthin (**5**) was compatible with autoxidation of the 4-hydroxy function, a process known to occur readily.[30] ^{1}H NMR assignments are included on structure **7**, Scheme 3, with reference to data for canthaxanthin (**4**), isozeaxanthin (**5**) and its diacetate.[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.

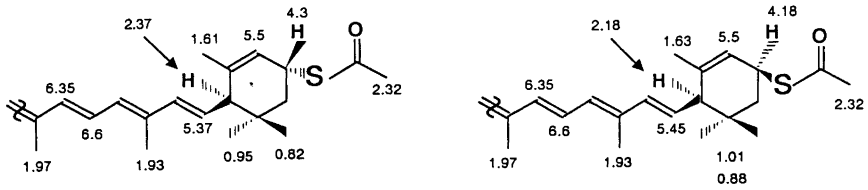
Experimental

General methods. General methods and instrumentations were as normally employed in our laboratory.[31] General

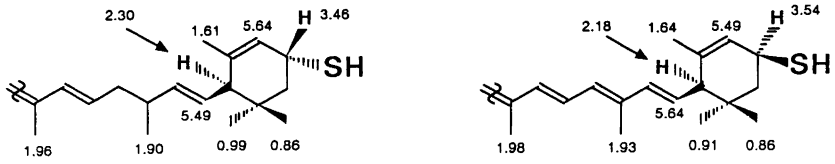
3′,6′-*trans* lutein 3′,6′-*cis*



3′,6′-*trans*- lutein acetate



2 a **2 b**



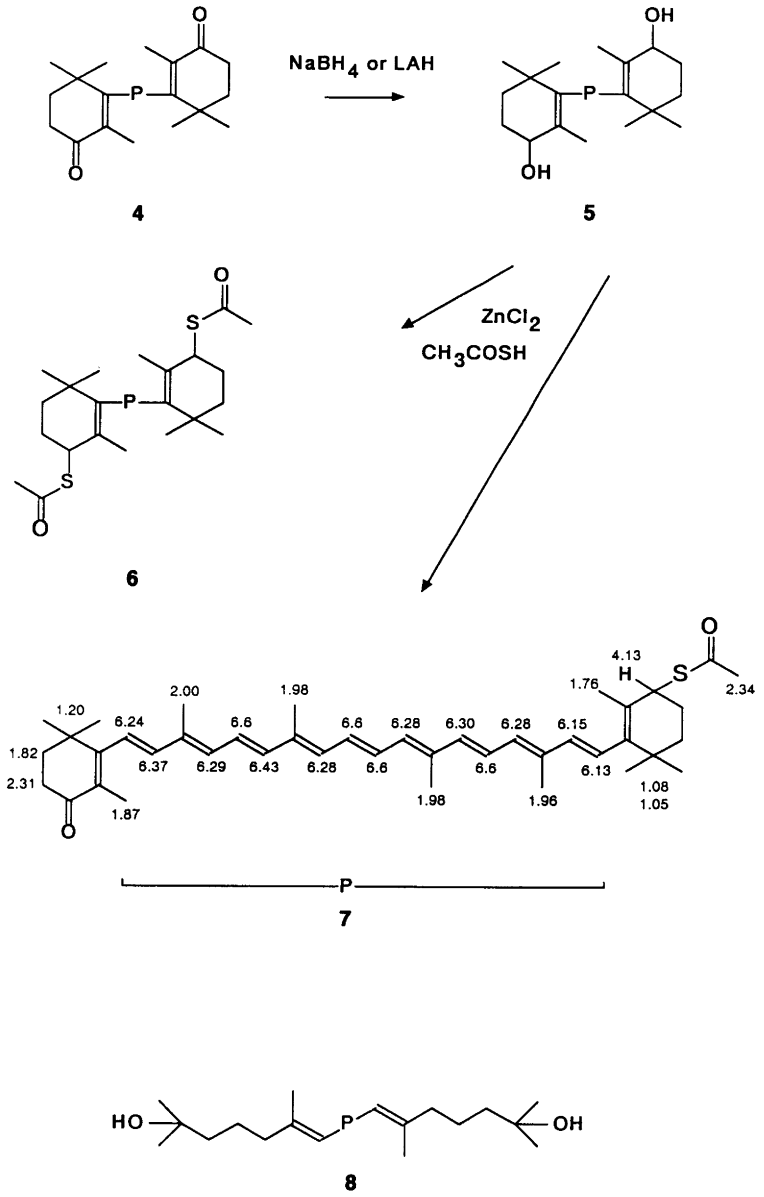
3 a **3 b**

Scheme 2. ^{1}H NMR assignments of 3′,6′-*trans* and 3′,6′-*cis* 3′-epimeric *S*-acetyl derivatives of 3′-thiolutein (**2a,b**) and 3′-thioluteins (**3a,b**) with reference to model compounds.[23]

precautions for work with carotenoids were taken. Spectral fine structure of VIS spectra are expressed as % III/II.[32] Only prominent or diagnostically useful peaks in the mass spectra are reported.

The S-*acetyl derivative of (3*R,3′RS,6′R*)-3′-mercapto-β,ε-caroten-3-ol* (**2a,b**). (3*R*,3′*R*,6′*R*)-Lutein (**1**, 25 mg, 0.44 μmol *ex.* alfalfa, National Chlorophyll Co.) was dissolved in CH_2Cl_2 (3 ml). Thioacetic acid (13 μl, 0.18 μmol) was added by a syringe followed by dry $ZnCl_2$ (6 mg), cf. Ref. 21. After being stirred for 90 min at 25 °C, the reaction mixture was diluted with CH_2Cl_2 and treated with water until neutral. The dried (Na_2SO_4) extract provided, after purification by vacuum liquid chromatography (VLC)[33,34] and TLC (SiO_2, 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 $ZnCl_2$ (6 mg) and dry ZnI_2 (14 mg). (ZnI_2 was prepared by reaction of Zn with HI,[35] extraction with ether and drying at 10 Torr, 25 °C). The reaction was monitored by TLC. ZnI_2 caused a more rapid reaction with **1**, but more by-products were observed.



Scheme 3. Partial synthesis of the *S,S*-diacetyl derivative of β,β-carotene-4,4′-dithiol (**6**) and the *S*-acetyl derivative of 4′-mercapto-β,β-caroten-4-one (**7**) from isozeaxanthin (**5**).

2a,b had the following properties: R_F = 0.37 (TLC, SiO_2, 30% acetone in hexane, cf. **1** (R_F = 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 ($SCOCH_3$), 965 (*trans* disubstituted double bonds) cm^{-1}; ^1H 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′,6′-*trans*) and 29.93 (3′,6′-*cis*); MS [IP 70 eV; *m/z* (% rel. int.)]: 626 (33, *M*), 550 (100, [*M*−CH_3COSH], 532 (4, [*M*−$CH_3COSH-H_2O$]), 458 (4, [*M*−CH_3COSH−toluene]), 444 (4, [*M*−CH_3COSH−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_2O]), 550 (63, [*M*−CH_3COOH]), 532 (11, [*M*−$CH_3COOH-H_2O$]).

*(3*R,*3′*RS,*6′*R*)-3′-mercapto-β,ε-caroten-3-ol* (**3a,b**). Portions of crude **2** from the two parallel experiments described above, were dissolved separately in ether (5 ml) and hydrolyzed with 10% methanolic KOH (1.5 ml) overnight at 25°C. After neutralization the pigments were ex-

tracted with ether, and the dried (Na_2SO_4) extracts were subjected to VLC and TLC, to afford 7.4 mg (29 % overall yield; $ZnCl_2$) and 5.4 mg (21 % overall yield; ZnI_2) of the monothiols **3a,b**.

3a,b had the following properties: R_F = 0.46 (SiO_2, 40 % acetone in hexane) in comparison with lutein (**1**) R_F = 0.55; t_R = 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*−H_2O]), 550 (100, [*M*−H_2S]), 532 (5, [*M*−H_2S−H_2O]), 458 (4, [*M*−H_2S−toluene]), 444 (2, [*M*−H_2S−toluene]). IR (KBr): 3440 (OH), 3040, 965 (*trans*, disubstituted double bond) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) after crystallization from benzene–hexane: see Scheme 2.

(4RS,4'RS)-β,β-Carotene-4,4'-diol (**5**) (*isozeaxanthin*). This was prepared by standard procedures by LAH reduction in dry ether or $NaBH_4$ reduction in methanol of 50 mg synthetic canthaxanthin (**4**, Hoffmann–La Roche). R_F = 0.05 (SiO_2, 5 % acetone in hexane), VIS (acetone): 432, 450 and 477 nm.

To **5** (20 mg, 0.035 mmol) in CH_2Cl_2 (7 ml) was added $ZnCl_2$ (3 mg), ZnI_2 (7 mg) and thioacetic 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'RS)-β,β-carotene-4,4'-dithiol (**6**). This was obtained as a mixture (< 10 % by ^1H NMR spectroscopy) with the *S*-acetyl derivative of (4'*RS*)-4'-mercapto-β,β-carotene-4-one (**7**), yield 0.8 mg (35 %). R_F (mixture) = 0.32 (SiO_2, 5 % acetone in hexane), cf. **5** (R_F = 0.05. MS [IP 70 eV; *m/z* (% rel. int.)] 684 (100, *M*), 624 (59, *M'*), 609 (9, [*M*−CH_3COS]), 608 (67, [*M*−CH_3COSH]), 548 (46, [*M'*−CH_3COSH]), 523 (66, [*M*−CH_3COS−CH_3COSH]), 532 (61, [*M'*−2×CH_3COSH]); *M* refers to **6** and *M'* to **7**. VIS (acetone) 457 nm rounded peak. IR (KBr) 1690 ($SCOCH_3$) 1670 (C=O), 970 (*trans* disubstituted double bonds) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) after crystallization from benzene–hexane: see the assignments given in Scheme 3.

After treatment of **6** and **7** (0.8 mg) with LAH in dry ether and the described work up,[29] no thiol could be isolated.

1,2,1',2'-Tetrahydrolycopene-1,1'-diol (**8**). Synthetic **8** (52 mg, 0.09 mmol, Hoffmann–La Roche, Nutley) was dissolved in CH_2Cl_2 (15 ml). ZnI_2 (29 mg) and thioacetic 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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