Tetrahedron: Asymmetry Vol. 4, No. 3, pp. 361-368, 1993
Printed in Great Britain

0957-4166/93 $6.00+.00

Synthetic Sulfur Carotenoids II[1]: Optically Active Carotenoid Thiols

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(*Received in UK* 30 *November* 1992; *accepted* 12 *January* 1993)

Dedicated to the memory of Günther Snatzke

Abstract: (3*R*,3′*R*)-Zeaxanthin was reacted with thioacetic acid or with thiocyano acid in a Mitsunobu reaction to provide the corresponding thioacetates and thiocyanates with inverted configuration. Reduction or hydrolysis of these intermediates provided (3*R*,3′*S*)-3′-mercapto-β,β-caroten-3-ol and (3*S*)-2′,3′-didehydro-β,β-carotene-3-thiol whose CD spectra are discussed.

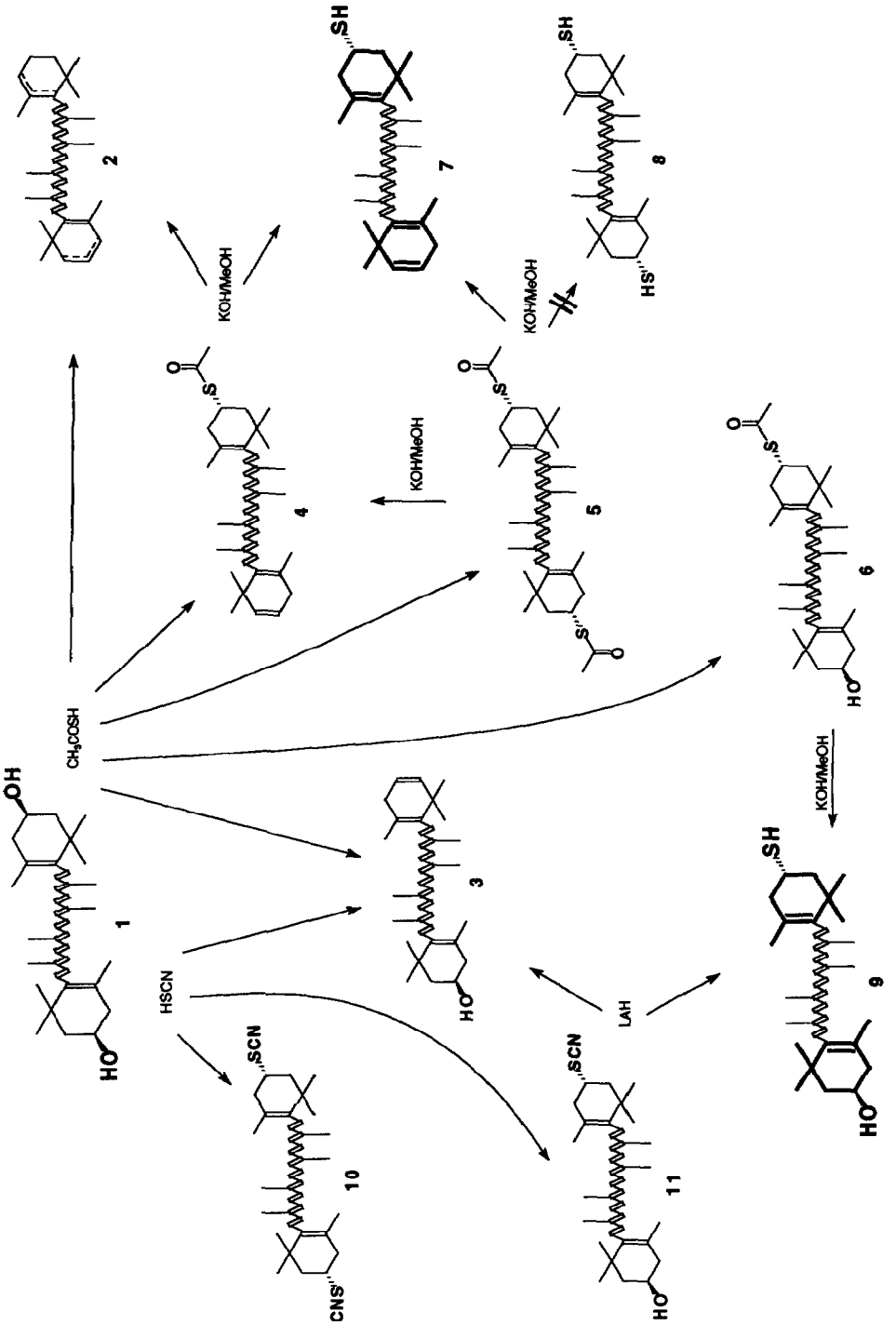
Introduction

Phototropic sulfur bacteria biosynthesize carotenoids[1], but no naturally occuring carotenoids with sulfur directly bound to the carbon skeleton have been encounterd[2]. Also in sulfur rich fossil fuels where hydrogenated carotenoids are assumed to be connected to each other and to steroids and hopanoids by (poly)sulfide linkages, only sulfur-free perhydrocarotenes have been detected[3].

The first synthesis of a sulfur containing carotenoid was described in 1959 by Martin and Karrer[4], followed by other examples in 1981 by Yamaguchi and co-workers[5] and in 1988 by Lehn and co-workers[6]. Today, sulfur carotenoids may be prepared on an industrial scale as intermediates for the commercial synthesis of sulfur-free carotenoids[7,8]. However, in these carotenoid derivatives the sulfur atom is connected to an aromatic ring[4-9], a structural feature not expected to be encountered in natural carotenoids. On the other hand, the thiol group is a biologically important moiety.

There has been an increasing interest in carotenoids as potential nonlinear optical materials[10,11] and organic conductors[12-15]. Carotenoid thiols may facilitate the preparation of monolayers on metal surfaces of Au, Ag, or Cu *etc.* for investigation of the optical and electrical properties of carotenoids[16-18]. Thiols are known to be radioprotectives[19,20] and chemotherapeutic agents[21]. Carotenoids also exhibit radioprotective[22] and other medicinal[23] effects. The individual prophylactic functions might possibly increase in carotenoid thiols.

1) Part I: Ref. 29



Scheme 1
Synthesis of homochiral carotenoid thiols via the S-acetyl or thiocyano derivatives

Thiols and carotenoids[24] are both known to participate in the biological antioxidant defense system[25,26]. Carotenoid thiols might therefore function as combined antioxidants[27].

The first racemic carotenoid thiol was synthesized a few years ago in our laboratory[28,29]. In view of the potential physical and biological properties, the synthesis of optically active carotenoid thiols was of interest. Optically active carotenoids substituted with functional groups other than -OR (R = H, CH_3, Ac, Glyc. *etc.*) have not been synthesized before.

Results and Discussion

The first unsuccesful attempts to prepare optically active carotenoid thiols were based on mesylate intermediates. However, reaction with both thiourea[30] and cesium thioacetate[31] did not provide the desired products from the dimesylate of (3*R*,3′*R*)-zeaxanthin (**1**).

The Mitsunobu reaction[32], successfully applied for the preparation of lutein diastereoisomers and zeaxanthin enantiomers[33], were found to be an effective route also for the synthesis of carotenoid thiols, see Scheme 1. (3*R*,3′*R*)-Zeaxanthin (**1**) gave in reaction with thioacetic acid, triphenylphosphine and azodicarboxylate[34,35] considerable amounts of the elimination products **2**[30] and, to a lesser extent, the configurationally unchanged (*R*)-hydroxy mono elimination product **3**. The (*S*)-monothioacetate **4** was obtained as the main sulfur containing product, accompanied in much lower quantities by the (*R,S*)-hydroxythioacetate **6** and the (*S,S*)-dithioacetate **5**. The yield of sulfur containing carotenoids comprised 39% of the total recovered carotenoid.

Hydrolysis or reduction of the thioacetates **4** and **5** both provided the (*S*)-monothiol **7**, whereas the expected dithiol **8** was not formed. Similary, the thioacetate **6** afforded the (*R,S*)-mercaptoalcohol **9**.
CD-spectroscopy confirmed the stereospecific formation of the thiol products. The Cotton effect shown in Fig. 2 demonstrated the inverted configuration of the thiol **7** compared to that of carotenol **3**, the signs of the CD bands of **3** being identical to that of (3*R*,3′*R*)-zeaxanthin (**1**) (Fig. 1) and (3*R*)-β,β-caroten-3-ol (cryptoxanthin)[36].

The hypothesis was first advanced by Snatzke and co-workers[37] that the signs of the CD bands of carotenoids with β-rings are determined solely by the helicity created by the cyclohexene ring and the C7-C8 double bond, regardless of the type of substituent attached to the ring. The nearly mirror like Cotton effects of the (*R*)-carotenol **3** and the (*S*)-thiol **7** seem to confirm this hypothesis (Fig. 2). On the other hand it is well established that chiral perturbed sulfides exhibit weak Cotton effects (Δε: 0.3 - 2.5) between 235 nm and 255 nm[38-40]. The hardly detectable inflection at 240 nm and the shift of the minimum from 250 to 255 nm in the CD spectrum of the thiol **7** may possibly originate from a contribution of the C-S $n \rightarrow \sigma^*$ transition to the Cotton effect. The CD spectrum of the *pseudo meso* (*R,S*)-mercaptoalcohol **9** (Fig. 3) did not exhibit distinct Cotton effects in the range 210 nm to 450 nm, confirming the predominant influence of the cyclohexene ring conformation on the electronic optical activity. However, a weak plateau, again at 240 nm, may arise from the (*S*)-configurated thiol group.

Thiocyanates represent other potential substrates for thiol synthesis. Changing thioacetic acid with thiocyano acid[41] (for a modified synthesis see Ref. 42), the Mitsunobu reaction gave the elimination product **3** and the thiocyanates **10** and **11**, albeit in much lower yields than for the corresponding

thioacetates. Reduction of the thiocyanate **11** with LAH provided again the carotenol **3** and the mercaptoalcohol (*R,S*)-**9**, identical with the compounds obtained above.

The *sec.* C3-hydroxy group of zeaxanthin (**1**), prone to substitution and to elimination, decreased considerably the yield of thiols obtained by the described, though not optimized, reactions: thiol **7** (2.4% yield), mercaptoalcohol **9** (0.2%), dithiol **8** (0%). However, the ease of the experiment, based on the availability of preformed optically active zeaxanthin (**1**), offers, as a partial synthesis, advantages until a total synthesis is available for carotenoid thiols.

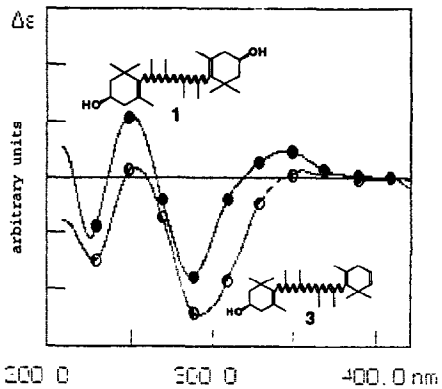


Figure 1
CD spectra of (3*R*,3′*R*)-zeaxanthin (**1**) ● and (3*R*)-2′,3′-didehydro-β,β-caroten-3-ol (**3**) ◑ in EPA

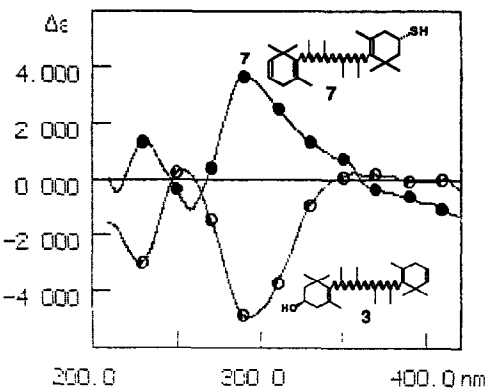


Figure 2
CD spectra of (3*R*)-2′,3′-didehydro-β,β-caroten-3-ol (**3**) ◑ and (3*S*)-2′-3′-didehydro-β,β-carotene-3-thiol (**7**) ● in EPA

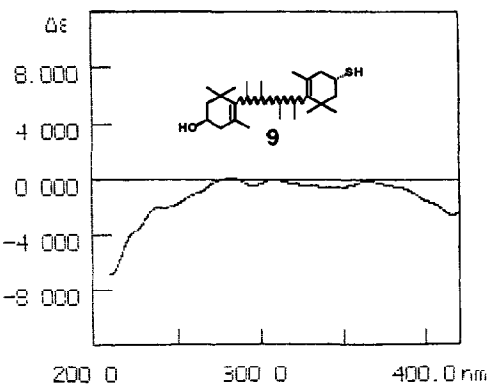


Figure 3
CD spectra of (3*R*, 3′*S*)-3′-mercapto-β,β-caroten-3-ol (**9**) in EPA

Experimental

General methods. General precautions for work with carotenoids were taken[43]. After reaction the products were adsorbed on silicagel, dried *in vacuo* and separated by flash chromatography (silicagel 60, Merck), followed by further purification on preparative TLC plates (silicagel 60 G, Merck) with heptane-acetone mixtures. R_F-values were determined on TLC aluminium sheets (silicagel 60, Merck). For the mass spectra (IP 70 eV, 210°), only prominent or diagnostically useful peaks are reported. The VIS spectra refers to λ_{max} (nm) in CH_2Cl_2, the CD spectra were measured in EPA (ethanol/isopentane/ether 2:5:5)[36]. ^1H-(400 MHz) and ^{13}C-(100 MHz) NMR spectra were recorded in $CDCl_3$. The NMR spectra were interpretated by comparison with data of similar products[44,45].

Reaction of zeaxanthin (1) with thioacetic acid

Triphenylphosphine (369 mg, 1.4 mmol) was dissolved in dry THF (2.5 ml). At 0° diisopropylazodicarboxylate (277 µl, 1.4 mmol) was added with a syringe. After formation of a white precipitate, the solution was stirred for 30 min. Zeaxanthin (1) (200 mg, 0.35 mmol) dissolved in THF (10 ml) was added with a syringe followed after cooling to -20° by thioacetic acid (100 µl, 1.4 mmol), *cf.* Ref. 34. Stirring was continued at -20° until most of **1** had reacted (45 min). Higher temperatures (0°) decreased considerably the yield of sulfur containing carotenoids. Chromatographic work-up gave:

Tetradehydro-β,β–carotene (2)[30]

Yield 32.6 mg; R_F = 0.74 (**1** R_F = 0.17, 30% v/v acetone-heptane); VIS: 465 nm (round), (**1**: 458, 486 nm); MS (m/z): 532.

(3*R*)-2′,3′-Didehydro-β,β-caroten-3-ol (3)

Yield 17 mg; R_F = 0.28; VIS: 458, 483 nm; MS (m/z): 550 (*M*), 532 (*M*-H_2O); CD: see Fig. 1; NMR: see Scheme 2: A,B and D,E.

(3′*S*)-3′-Methylthiocarboxyl-2,3-didehydro-β,β-carotene (4)

Yield 71 mg, 33%; R_F = 0.48; VIS: 460, 480 nm; MS (m/z): 608 (*M*), 532 (*M*- CH_3COSH), 516 (*M*-toluene), *cf.* Ref. 29.

(3*S*,3′*S*)-3,3′-Di(methylthiocarbonyl)-β,β-carotene (5)

Yield 5.3 mg, 2%; R_F = 0.44; VIS: 458, 483 nm; MS (m/z): 684 (*M*), 608 (*M*-CH_3COSH), 592 (*M*-toluene), 532 (*M*-CH_3COSH), 516 (608-toluene).

(3*R*,3′*S*)-3′-Methylthiocarbonyl-β,β-caroten-3-ol (6)

Yield 8.7 mg, 4%; R_F = 0.26; VIS: 456, 484 nm; MS (m/z): 626 (*M*), 608 (*M*-H_2O), 532 (608-CH_3COSH).

Formation of thiols

The methylthiocarboxylates **4**, **5**, and **6**, dissolved in THF-MeOH, were hydrolyzed with KOH (10% in MeOH) and stirred overnight at 40°, or reduced with LAH in dry THF at 40° (*cf.* Ref. 29) with usually lower yields. Chromatographic work-up gave:

(3*S*)-2′,3′-Didehydro-β,β-carotene-3-thiol (7)

Obtained from **4**: yield 4.8 mg, 7%; R_F = 0.39 (**3** R_F= 0.28, **1** R_F = 0.17, 30% v/v acetone-heptane); VIS: 460, 480 nm (**1**: 458, 486 nm); MS (m/z): 566 (*M*), 532 (*M*-H_2S); CD: see Fig. 2; NMR: see Scheme 2: A,C and D,F. Another product from the hydrolysis of **4** was identified as the elimination product **2**.

(3*R*,3′*S*)-3′-Mercapto-β,β-caroten-3-ol (9)

Obtained from **6**: yield 0.4 mg, 5%; R_F = 0.25; VIS: 456, 482 nm; MS (m/z): 584 (*M*), 566 (*M*-H_2O), 550 (*M*-H_2S), 532 (550-H_2O), 458 (550-toluene); NMR: see Scheme 2: B,C.

Hydrolysis of the dithioacetate **5** gave four products in amounts too low for identification. By MS and co-chromatography two of the products appeared to be identical with **4** and **7**.

Reaction of zeaxanthin (1) with thiocyano acid

Zeaxanthin (**1**) (113.6 mg, 0.2 mmol) and triphenylphosphine (115.3 mg, 0.44 mmol) were dissolved in benzene. A HSCN-solution (*ca.* 10% in benzene, 1.5 ml) and diethyl azodicarboxylate (69.3 µl, 0.4 mmol) were added, *cf.* Ref. 41. After stirring overnight at 35°, chromatographic work-up gave five products in low amounts of which three were identified as:

2′,3′-Didehydro-β,β-caroten-3-ol (3)

Identical by MS and co-chromatography with the product described above.

β,β-Carotene-3,3′-di(thiocyanate) (10)

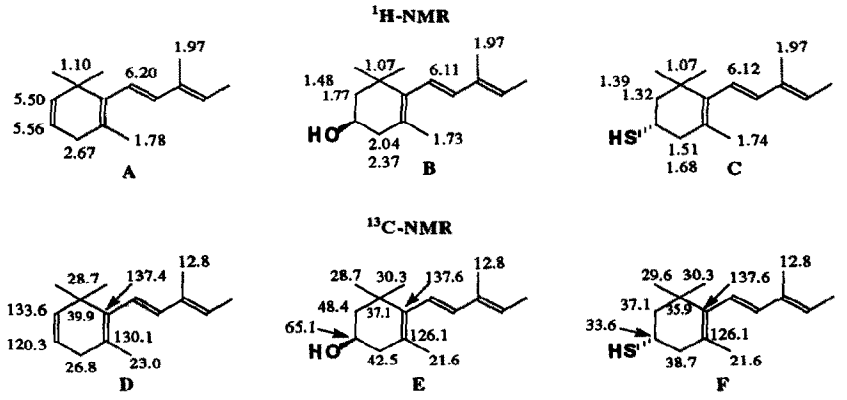
R_F = 0.48 (**1** R_F = 0.17, 30% v/v acetone-heptane); MS (m/z): 650 (*M*), 591 (*M*-HSCN), 558 (*M*-toluene), 532 (591-HSCN).

3′-Thiocyano-β,β-caroten-3-ol (11)

Main product; R_F = 0.32; MS (m/z): 609 (*M*), 550 (*M*-HSCN), 517 (*M*-toluene), 503 (*M*-xylene), 541 (*M*-158).

Formation of thiols

Thiocyanate **11** was dissolved in dry ether and stirred with LAH for 5 h, *cf.* Ref. 46. After usual work-up, two products were isolated by prep. TLC and identified by MS and co-chromatography as the alcohol **3** and mercaptoalcohol **9**.



Scheme 2

^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR ($CDCl_3$) data of carotenoid thiols (tentative assignments)

Reaction of zeaxanthin(1)dimesylate with cesium thioacetate

The dimesylate of **1**[30] (64 mg) was reacted with cesium thioacetate during 20 h at 22°, *cf.* Refs. 31, 47. Chromatographic separation of the intermediates and subsequent reduction with LAH gave products in insufficient quantities for further caracterization.

Acknowledgements

We thank Dr. H. Mayer (Hoffmann-La Roche, Basel) for a sample of synthetic (*R,R*)-zeaxanthin and B. Olsrød for the mass spectra. H.-R. S was partly supported by a research grant from Hoffmann-La Roche, to S.L.-J.

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