Short Communication

Bacterial Carotenoids 56.* On the Spirilloxanthin Stereoisomeric Set

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Spirilloxanthin (1, Scheme 1) is an aliphatic tridecaene carotenoid characteristic of phototrophic bacteria of the families Rhodospirillaceae and Chromatiaceae.[1] Studies on geometrical isomerism in spirilloxanthin (1), previously referred to as rhodoviolascin,[2,3] started long ago.[3] (All-E)-1 was characterized as remarkably labile. Stereoisomerization upon standing or refluxing in benzene, or by iodine catalysis in light caused the formation of an unidentified neo A isomer (ca. 40% of total carotenoid).[3,4] Improved separation of the iodine-catalyzed quasiequilibrium mixture into four zones by circular paper chromatography (PC) was later achieved.[5,6]

The early work was based on simple chromatographic systems [column chromatography (CC), PC] and inadequate identification criteria for Z-isomers (VIS spectra). The recent availability of analytical and semipreparative HPLC with concomitant recording of VIS spectra for the difficult separation of unstable compounds[7] and of ^{1}H NMR spectroscopy for the unequivocal identification of geometrical isomers,[8] now facilitates studies on geometrical isomerism in the carotenoid series.[9,10]

Recently we have studied in detail the natural occurrence of Z-isomers and the composition of the iodine-catalyzed stereomutation mixture of $(2S,2'S)$-bacterioruberin (2),[11] a related tridecaene C_{50}-carotenoid encountered in halophilic bacteria.[12]

Spirilloxanthin (1) and bacterioruberin (2) possess the same chromophoric system, but differ in the structure of the aliphatic end groups. It was of interest to carry out a quantitative and qualitative comparison of the iodine-catalyzed equilibrium mixture of 1 for comparative purposes.

Results and discussion

Synthetic spirilloxanthin (1)[6] was available. Iodine-catalyzed stereomutation in benzene solution was carried out

by the usual procedure.[4] The stereoisomeric mixture was separated by HPLC, see Fig. 1, in a system resolving eight stereoisomers, judged by their t_R-values. VIS spectra including the location of the cis-peak[4] and subsequent reversible isomerization of individual isomers. Since extinction coefficients in visible light for the individual Z-isomers are unknown and lower than for the (all-E)-isomer,[4] the quantification (Table 1) was based on identical extinction values at λ_{max}. This means that the (all-E)-isomer represented less than 37% of total spirilloxanthin, a remarkably low content, cf. e.g. Refs. 9–11.

Semipreparative HPLC was employed for sample collection for ^{1}H NMR spectra and reversible isomerization tests. Structures were assigned (Table 1) to the individual isomers on the basis of (i) λ_{max} shifts relative to the (all-E)-isomer [2–7 nm for (mono-Z)-isomers and 8–12 nm for (di-Z)-isomers], (ii) %D_B/D_{II}-values[13] as a measure of cis-peak intensity, reflecting the bent shape of the molecule, with larger values for central Z-bonds,[4] (iii) reversible isomerization tests monitored by HPLC and (iv) ^{1}H NMR data in comparison with the isomerization shift values established by Englert[8] for Z-bonds in different positions. Sterically hindered Z-bonds in $\Delta 3$, $\Delta 7$ and $\Delta 11$ positions are not formed under these conditions and may be disregarded.[4,9–11] ^{1}H NMR assignments of the (all-E)-, (5Z)- and (9Z)-isomers are given in Table 2. Our spectrum for (all-E)-1 showed higher resolution than a reported spectrum in benzene,[14] both at 400 MHz.

The (13Z)-isomer with the highest cis-peak intensity, was extremely labile and isomerized to the (all-E)-isomer during ^{1}H NMR analysis at room temperature. The assignment was based on comparison of VIS data for (13Z)-2[11,15] including the high %D_B/D_{II}-value and relative abundance.[11]

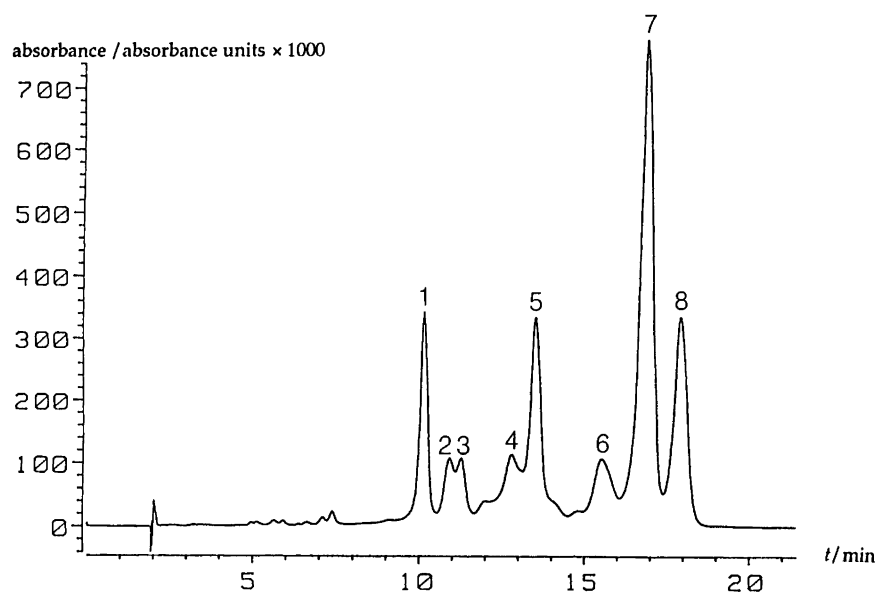The result (Table 1) supports a thermodynamic
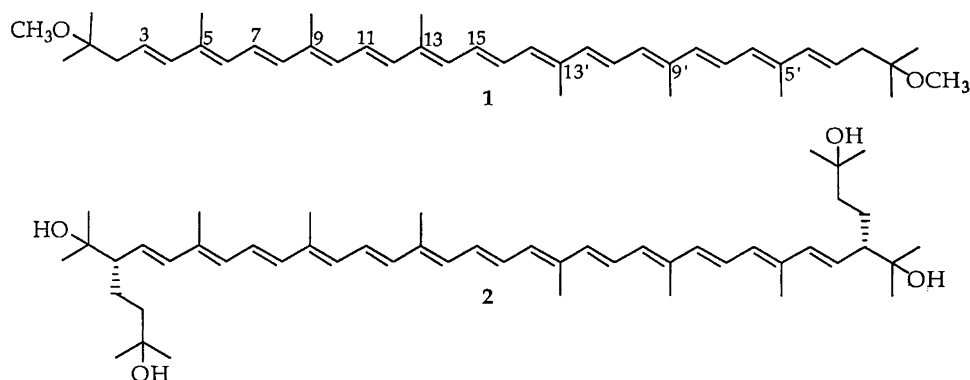
Scheme 1.

Fig. 1. HPLC chromatogram of the iodine-catalyzed stereomutation mixture of spirilloxanthin, cf. Table 1.

Table 1. Composition of the iodine-catalyzed stereomutation mixture of spirilloxanthin (1). Data for (2S,2'S)-bacterioruberin (2)[12] are included for comparison.

Isomer of 1	t_T/min^a	$\lambda_{max}/\text{nm}^b$	%III/II	%D_B/D_{II}	% of total 1	2, % of total
1 13Z	10.2	369, 386, 459, *487*, 520	51	77	10	9
2 5Z,13'Z	11.0	368, 384, 455, *482*, 515	47	47	4	—
3 5Z,13Z	11.3	368, 384, 456, *483*, 515	50	46	4	—
4 5Z,9'Z	12.9	368, 384, 457, *483*, 516	50	19	7	5
5 9Z	13.5	370, 386, 459, *487*, 521	66	21	14	15
6 5Z,9Z	15.6	384, 459, *486*, 518	62	14	7	—
7 all-E	17.0	388, 466, *494*, 528	73	8	37	54
8 5Z	18.2	386, 463, *492*, 525	71	8	16	17

[a]Spheri-5 silica; hexane–ethylisopropylamine–acetone 98.5:0.05:1.5; flow 1.5 ml min^{-1}. [b]In HPLC eluent. Highest value is italicised.

stability order of all-E > $5Z$ > $9Z$ > $13Z$ for spirilloxanthin (1), consistent with the trend previously observed for bacterioruberin (2). However, the lower relative content of (all-E)-1 in the quasiequilibrium mixture was noted and compensated by the formation of more (di-Z)-isomers. This phenomenon must be ascribed to the different size and branching of the end groups in 1 and 2.

The ($5Z,9Z$)- and ($5Z,9'Z$)-isomers were identified by VIS and ^{1}H NMR data, whereas the tentative identification of the less abundant ($5Z,13Z$)- and ($5Z,13'Z$)-isomers rested on VIS data including high cis-peaks, reversible isomerization to the entire stereochemical set, and selective isomerization of the purified ($5Z$)-isomer to the assumed ($5Z,13Z$)- and ($5Z,13'Z$)-isomers.

Table 2. Chemical shifts (δ-values) of some geometrical isomers of spirilloxanthin (1) in CDCl₃.

Proton	(all-E)-1	(5Z)-1	(9Z)-1
H-2	2.32	2.38	2.33
H-3	5.72	5.77	5.75
H-4	6.16	6.70ᵃ	6.18
H-5	6.11	6.01ᵃ	6.19ᵃ
H-7	6.60	6.74ᵃ	6.61
H-8	6.35	6.28ᵃ	6.89ᵃ
H-10	6.23	6.22	6.08ᵃ
H-11	6.65	6.65	6.80ᵃ
H-12	6.38	6.38	6.31ᵃ
H-14	6.27	6.27	6.26
H-15	6.65	6.65	6.65
H-15'	6.65	6.65	6.65
H-14'	6.27	6.27	6.27
H-12'	6.38	6.38	6.38
H-11'	6.65	6.65	6.65
H-10'	6.23	6.23	6.23
H-8'	6.35	6.35	6.35
H-7'	6.60	6.60	6.60
H-6'	6.11	6.11	6.11
H-4'	6.16	6.16	6.16
H-3'	5.72	5.72	5.72
H-2'	2.32	2.32	2.32
CH₃-16,17	1.16	1.16	1.16
CH₃-18	1.93	1.93	1.93
CH₃-19	1.97	1.99ᵃ	1.98ᵃ
CH₃-20	1.98	1.98	1.98
CH₃-16',17'	1.16	1.16	1.16
CH₃-18'	1.93	1.93	1.93
CH₃-19'	1.97	1.97	1.97
CH₃-20'	1.98	1.98	1.98
OCH₃	3.23	3.23	3.23

ᵃProtons with isomerization shifts relative to (all-E)-1 are marked.

In the normal-phase HPLC system employed the three strongly bent (13Z)-bond-containing isomers were eluted first, followed by the three (9Z)-bond-containing ones, the (all-E)-isomer and the most strongly retained (5Z)-isomer.

The (15Z)-isomer with the highest predicted *cis*-peak[4,14] was not isolated, which is compatible with the observed instability of the (13Z)-isomer and a similar situation for bacterioruberin (2).[12]

It is interesting to note that the very unstable (15Z)-isomer of spirilloxanthin (1) has recently been shown to be bound to the photoprotective reaction center in *Rhodospirillum rubrum*, whereas the (all-E)-iosmer is bound to the light harvesting complex and the (13Z)-isomer is additionally present in the chromatophore membrane.[14]

Experimental

General methods and instruments were as commonly employed.[9] ¹H NMR spectra were recorded with a Jeol EX 400 FT instrument (400 MHz, CDCl₃).

Iodine-catalyzed stereomutation. The isomerization of synthetic **1** (0.04–12 mg) was carried out in benzene

containing 2% iodine in indirect sunlight. The reaction was monitored by VIS spectroscopy and HPLC. Quasiequilibrium was reached within 45 min; VIS λ_{max} changed from 389, 466, *494*, 528 nm, %III/II[13] = 68, %D_B/D_{II} = 8 [(all-E)-1] to 393, (477), *503*, 538 nm, %III/II = 32 and %D_B/D_{II} = 19 in benzene. The isomerization mixture was examined by HPLC (Spherie-5, Silica 220 × 4.6 nm; hexane–ethyldiisopropylamine–acetone 98.5 : 0.05 : 1.5; flow 1.5 ml min⁻¹; detection wavelength 490 nm), see Table 1.

Reversible isomerization of individual isomers. HPLC-purified individual isomers were kept in benzene in darkness at room temperature for up to 24 h. The reaction was monitored by HPLC.

Individual isomers. These were isolated by preparative HPLC (Chromosphere SI-5, 250 × 10 mm; solvent as above; flow 3 ml min⁻¹; 60 injections) providing eight fractions, t_R 20–50 min, purity test by analytical HPLC; t_R values (analytical column) and spectroscopic criteria are given in Table 1.

(all-E)-1 (Isomer 7). Cryst., synthetic **1**, 95% pure (all-E)-isomer by ¹H NMR. VIS λ_{max} (acetone): 369, 387, 465, *493*, 525 nm, %III/II = 47, %D_B/D_{II} = 8. MS m/z (rel. int.): 596 (M, 100%). 564 (M − 32, 12%), 523 (M − 73, 14%), 504 (M − 92, 3%), 490 (M − 106, 60%), 73 (> 100%). IR (KBr) 3033–2824 (CH), 1450 (CH₂), 1379, 1365 (*gem*-CH₃), 1083 (OCH₃) 972 (E-disubstituted C=C). ¹H NMR, Table 2, $J_{2,3}$ = 7.3 Hz, $J_{3,4}$ = 15.6 Hz, $J_{6,7}$ = 11.2 Hz, $J_{7,8}$ = 14.7 Hz, $J_{10,11}$ = 11.2 Hz, $J_{11,12}$ = 14.8 Hz, $J_{14,15}$ = 10.3 Hz. Coupled protons were confirmed by a 2D ¹H–¹H COSY spectrum.

(5Z)-1 (Isomer 8). Available 0.5 mg, t_R, VIS data Table 1. ¹H NMR Table 2. The olefinic region was consistent with data for (5Z)-2.[11] HPLC-purified (5Z)-1 was isomerized in darkness in benzene for 24 h, providing (5Z,13Z)-1 (10%), (5Z,13'Z)-1 (10%) all (all-E)-1 (7%) as new products.

(9Z)-1 (Isomer 5). Available 0.4 mg, t_R, VIS data Table 1. ¹H NMR Table 2. The olefinic region was consistent with data for (9Z)-2.[11] HPLC-purified (9Z)-1 was isomerized in darkness in benzene for 24 h, providing (all-E)-1 (13%), and other Z-isomers (total 12%).

(13Z)-1 (Isomer 1). Available 0.2 mg, t_R, VIS data Table 1. During ¹H NMR measurement (2 h, RT) isomerization to mainly (all-E)-1 (90% of total) occurred, cf. published ¹H NMR data.[14] Selective isomerization of (13Z)-1 to the (all-E)-isomer (73% of total) in darkness at room temperature for 24 h, was demonstrated as monitored by HPLC.[15]

(5Z,9Z)-1 (Isomer 6). Available < 0.1 mg, t_R, VIS data Table 1. The ¹H NMR spectrum[15] was consistent with half an (all-E)-1 structure, and the (5Z,9Z)-assignment was compatible with the isomerization shifts of the

related $(9Z,13Z)$-system published by Englert[8] causing downfield shifts of H-4 to 6.68 (d, $J = 15$ Hz) and H-8 to ca. 6.8 ppm.

(5Z,9'Z)-1 (Isomer 4). Available ca. 0.1 mg, t_R, VIS data Table 1. By ^{1}H NMR the olefinic region was compatible with that of $(5Z,9'Z)$-2.[11] Reversible isomerization to $(9Z)$-1 and (all-E)-1 was observed by HPLC.

(5Z,13Z)-1 (Isomer 3). Available <0.1 mg, t_R, VIS data Table 1. Selective reversible isomerization to $(5Z)$-1 and (all-E)-1 was observed by HPLC analysis. During ^{1}H NMR measurement extensive isomerization to the entire stereochemical set occurred. $(5Z)$-1 provided this isomer upon isomerization, see above.

(5Z,13'Z)-1 (Isomer 2). Available <0.1 mg, t_R, VIS data Table 1. Selective reversible isomerization to $(5Z)$-1 and (all-E)-1 was observed by HPLC analysis. Extensive isomerization to the entire stereochemical set occurred. $(5Z)$-1 provided this isomer upon isomerization, see above.

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