**Bacterial Carotenoids. LIII. C_{50}\text{-Carotenoids. 19.}^{*}** Absolute Configuration of Sarcinaxanthin and Sarcinaxanthin mono-\(\beta\)-D-glucoside. Isolation of Sarcinaxanthin Diglycoside

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By improved \(^1\)H NMR data sarcinaxanthin is shown to be centrosymmetric with terminal methylene groups in substituted \(\gamma\)-rings, consistent with IR and MS data. \((2R,6R,2'R,6'S)\)-chirality for sarcinaxanthin (4) follows from CD and \(^1\)H NMR data in comparison with appropriate models.

Evidence is presented that sarcinaxanthin occurs in *Sarcina lutea* at least partly esterified with a C_{6}H_{12}COOH acid.

A previously characterized C_{50}-carotenoid \(\beta\)-D-glucoside is by \(^1\)H NMR and CD data shown to be sarcinaxanthin mono-\(\beta\)-D-glucoside (9).

A dihexoside, presumably sarcinaxanthin diglucoside (10), was isolated for the first time.

Previously characterized less polar carotenoids were not present in three batches.

CD data for sarcinaxanthin (4) and decaprenoxanthin (1) are discussed in relation to preferred conformations.

Previous work \(^{1,2}\) on the configuration of sarcinaxanthin from *Sarcina lutea* established its isomeric relationship to the C_{50}-diol decaprenoxanthin (1).\(^{3-6}\) The structures 2 and 3 (Scheme 1) with substituted \(\varepsilon\) end groups were compatible with available \(^1\)H NMR evidence and lacking Retro-Diels-Alder fragmentation on electron impact.\(^{*}\) For differentiation between these alternatives this project was undertaken.

**RESULTS AND DISCUSSION**

The previous time-averaged \(^1\)H NMR spectrum of sarcinaxanthin could not be reproduced

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in the δ 1.5 – 1.8 region. Due to two false singlets in this region signals at δ 4.53, 4.76 had been overlooked. The latter signals, present in the previous and present spectrum, can now be assigned to two terminal methylene groups in substituted χ end-groups, consistent with medium intensity IR-absorption at 890 cm⁻¹. The centrosymmetrical structure δ (still disregarding stereochemistry) followed from the ¹H NMR spectrum and shifts induced upon addition of Eu(dpm)₃ reagent, Fig. 1. ¹H NMR assignments are included in Scheme 1.

The hydroxylated side-chain was assigned E-configuration on the basis of the chemical shifts of the –CH₂(δ 1.67), –CH₃OH(δ 4.03) and olefinic proton (δ 5.8) as compared with those of decaprenoaxanthin (I) and an aliphatic model.

The Cotton effect (see Fig. 2) of sarcincaxanthin is opposite to that of the synthetic C₄₄ model δ, favouring opposite absolute configuration at C-6,6’ in sarcincaxanthin (δ)

![Fig. 2. CD spectrum of sarcincaxanthin (δ) and of (2R, 6S, 2’R, 6’S)-2,2’-dimethyl-γ,γ-carotene (δ) in EPA (diethyl ether, isopentane, ethanol 5:5:2).](image)

and δ; cf. previous arguments for the stereochemistry of decaprenoaxanthin (I) from CD-comparison with the C₄₄ model δ. The configuration at C-2,2’ in sarcincaxanthin may, like in the ε-series, have little influence on the CD spectrum. In the ε-series the difference in chemical shift of the gem-dimethyl signals (Δ) within the 2,6-cis series (ε and δ, ε = ca. 0.20) vs. the 2,6-trans (7, Δ = 0.02) were used for assignment of absolute configuration of C-2,6; one of the two gem-dimethyl groups being more shielded in the 2,6-cis series. In the γ-series only the 2,6-cis C₄₄ model (δ) is available. However, the chemical shift difference of the gem dimethyl signals for δ (Δ = 0.22) and sarcincaxanthin (Δ = 0.23) coincide so well that a 2,6-cis relationship in sarcincaxanthin (δ) is likely. For comparison Δ = 0.08 for the unsubstituted γ end-group in optically active β,γ-carotene (δ).

The close agreement in chemical shifts of the gem dimethyl groups in the C₄₄ models δ and ε (Scheme 1) with ε and γ end-groups, as well as for decaprenoaxanthin (I) and sarcincaxanthin (δ), together with nearly identical Cotton effects for decaprenoaxanthin (I) and sarcincaxanthin (δ, Fig. 2), suggest a similar geometry of these ε and γ end-groups, in spite of the different location of the double bond. This is confirmed by molecular models: The ε-ring of I and δ is planar in the C-3,4,5,6 region with the polyene chain quasiquatorial.

and the C-2 substituent equatorial, whereas the γ-ring of δ and δ is planar in the C-4,5,6,18 region with the polyene chain and the C-2 substituent equatorial.

Δε-values for decahexanthin (I, Δε = −5.15
−5.18 value from Ref. 12 used, −15.114) and sarcinaxanthin (4, Δε = −16) are comparable. The high Δε-value (15.5) for α-ionone has been explained by predominance of the conformation with quasioequatorial side chain in order to allow interaction between the enone and the isolated olefin chromophore. In (+)-cis-α-ionone (Δε = 12.2) a certain destabilization of this conformation has been ascribed to the axial C-2 methyl group. For (+)-γ-ionone and (+)-cis-γ-ionone very low Δε-values (ca. 0.002–
0.2) are reported. It was concluded that no significant coupling between the electric transition moments of the enone/olefin chromophores occurs. Since Δε-values of ca. 16 are not exceptionally high in the carotenoid series (cf. zeaxanthin) the similar Δε-values for decahexanthin (I) and sarcinaxanthin (4) give no reason to assume any predominance of conformations with quasioxial or axial polyene chain. Such conformations would be destabilized in the C-4α and C-6α-series.

In connection with the present assignment of absolute configuration to sarcinaxanthin (4) it should be pointed out that the chirality at C-2 is the same in Cα-carotenoids with δ, ε, γ, ψ, and γ end-groups, and that the configuration at C-6 is the same for Cα-carotenoids with ε and γ end groups in cases hitherto studied. Biosynthetic considerations have been made.

Although saponification was included in the isolation procedure the presence of small amounts of a sarcinaxanthin monoester (4a) esterified with a C4H11COOH acid could be demonstrated (Batch 1) by acetylation, saponification and LiAlH4-reduction experiments and high precision MS.

A Cα-carotenoid β-D-monoglucone, tentatively identified as sarcinaxanthin β-D-monoglucone, comprised 20–40% of Batches 1, 2, and 3. The 1H NMR and CD spectra of the pentacacetate 9a here prepared are consistent with structure 9 for the mono-β-D-glucone. Structure 9 is compatible with previously reported in-chain fragmentations of the deuteriacetate 9b on electron impact.


Sarcinaxanthin dihexoside (10) was isolated for the first time and comprised around 30% of Batches 1 and 3. It was characterized as the octaacetate 10a by chromatographic behaviour, electronic and mass spectrum. By analogy with 9a, a di-β-D-glucoside structure appears likely.

Minor, less polar C4α- and C6α-carotenoids previously isolated were not present in Batches 1, 2, and 3.

The variation in carotenoid content of Sarcina lutea, particularly in mass culture, reflects sensitivity of the carotenoid synthesis to cultivation conditions.

So far sarcinaxanthin and its derivatives represent the only Cα-carotenoids with substituted γ end-groups.

EXPERIMENTAL PART

Materials and general methods were the same as before. 1H NMR 90 MHz FT spectra were recorded on a Varian XL-100 instrument and CD spectra on a Roussel Jouan Dicrographe.

Biological material. Sarcina lutea from the Department of Biochemistry at this University was grown in mass cultures (150 l) by the previously described procedure.

Batch 1 provided ca. 300 g of wet cells (31 g dry cell residue after acetone extraction) and Batch 2 370 g of wet cells. Batch 3 constituted lyophilized cells, 600 g, of S. lutea, provided by Dr. A. G. Andrews.

Pigment extraction. Cells were lyzed by lysozyme treatment and extracted with acetone; total yield of crude carotenoids: Batch 1, 53 mg, Batch 2, ca. 5 mg and Batch 3, 24 mg.

Separation of individual carotenoids was best effected on acetylated polyamide columns or TLC (SiO2) after standard saponification with 5% KOH in methanol to remove contaminants.

Sarcinaxanthin (4). Batch 1 yielded 9 mg of 4, crystallized from acetone. 4 had adsorptive properties, electronic, IR and MS spectra as previously described. 1H NMR (CDCl3) at 60 MHz and 100 MHz differed from the published spectrum: δ 0.73 s and 0.96 s, 6 H + 6 H, gem. dimethyl, 1.07 s (6 H, side-chain CH3), 1.98 s (6 H, side-chain CH3), 2.3 (m, ca. 10 H, allylic CH2 and CH), 4.03 s (4 H, CH2OH), 4.53 and 4.76 2 H + 2 H, = CH2, ca. 5.6 (t, 2 H, isopropylidene H), and 6–7 (m, ca. 10 H, olefinic H). Induced shifts upon addition of Eu(dpm)3 are illustrated in Fig. 1. The CD spectrum is reproduced in Fig. 2.

Sarcinaxanthin monoester (4a). In spite of prior saponification a monoester 4a, 0.5 mg,
less polar than 4 and with \( R_F = 0.63 \) on Schleicher & Schüll (S&S) No. 287 paper with 10 % acetone in light petroleum (a.p.e.) was isolated from Batch 1. After acetylation a monoacetate \( db \) \( (R_F = 0.85 \) on S&S 287 10 % a.p.e.) was obtained; \( m/e \) 894 (M), 802 (M − 92), 788.5725 (C\(_{24}H\(_{22}O\)\(_{17}\)) = M − C\(_{2}H\(_{2}\)\(_{2}\)), 746.5621 (C\(_{20}H\(_{16}O\)\(_{14}\)) = M − C\(_{2}H\(_{2}\)\(_{2}\)), 714.5096 (C\(_{16}H\(_{14}O\)\(_{11}\)) = M − C\(_{2}H\(_{2}\)\(_{2}\)) (2). Treatment of \( db \) with LiAIH\(_{4}\) in dry ether gave sarcoxinanthin (4) judged by MS and adsorptive properties. 4a upon standard alkali treatment provided sarcoxinanthin (4) judged by co-chromatography.

Sarcoxinanthin mono-β-D-glucoside (9). 9, eluted from acetylated polysamide with 10 % methanol in benzene, rechromatographed on TLC (SiO\(_{2}\), eluted with 50 % a.p.e.), was acetylated by a standard procedure. The pentaacetate 9a, purified by TLC (SiO\(_{2}\), eluted with 50 % a.p.e.; \( R_F = 0.58 \) on S&S No. 287 paper 10 % a.p.e., cf. Ref. 20) had an electronic spectrum like 9; MS data as previously reported.\(^{1}\) H NMR (CDCl\(_{3}\)) \( \delta \): 0.73 (s), 0.98 (s, 6 H−6 H, gem. dimethyl), 1.60 (s, 3 H, side-chain CH\(_{3}\)), 1.87 (3 H, side-chain CH\(_{3}\)), 1.98 (ca. 12 H, in-chain CH\(_{2}\)); 2.00, 2.02 and 2.09 (ca. 15 H, in-chain CH\(_{2}\)), 4.20 (ca. 2 H, −CH\(_{2}\)O gluc (AcO)\(_{2}\)), 4.5 and 4.75 (ca. 4 H, =CH\(_{2}\)), methine H and olefinic H; CD (EPA) \( \Delta \delta \) 280 nm (−10.4 µ), 240 nm (−5 µ), 215 nm (−7.8 µ).

Sarcoxinanthin diglucoside (10). 10, eluted with benzene-methanol from acetylated polysamide had \( R_F = 0.10 \) on S&S No. 287 paper (30 % a.p.e.). After standard acetylation the octaacetate (10a, 0.5 mg from Batch 1) was obtained; \( R_F = 0.23 \) on S&S No. 287 (10 % a.p.e.), electronic spectrum as for 4; \( m/e \) 1364 (M), M − 42, M − 92, M − 106, M − 158, M − 92 − 106, M − 289, M − 346, 331, 169, 145, 109, 43.

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