Bacterial Carotenoids. LIII. C₅₀-Carotenoids. 19.* Absolute Configuration of Sarcinaxanthin and Sarcinaxanthin mono-β-D-glucoside. Isolation of Sarcinaxanthin Diglycoside

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

Evidence is presented that sarcinaxanthin occurs in *Sarcina lutea* at least partly esterified with a $C_9H_{11}COOH$ acid.

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

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

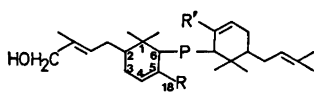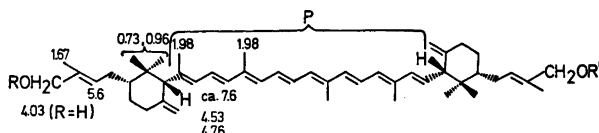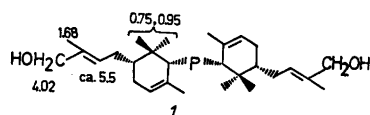Previously characterized less polar carotenoids were not present in three batches.

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 deca-prenoxanthin (1).[3-5] The structures 2 and 3 (Scheme 1) with substituted ε end groups were compatible with available ¹H NMR evidence and lacking Retro-Diels-Alder fragmentation on electron impact.[2] For differentiation between these alternatives this project was undertaken.
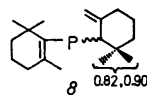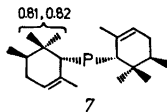
RESULTS AND DISCUSSION
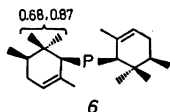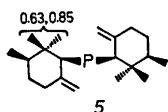
The previous time-averaged ¹H NMR spectrum of sarcinaxanthin could not be reproduced

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4   R=R′=H;   4a R=C₉H₁₁COO,R′=H;   4b R=C₉H₁₁COO,R′=Ac

9   R=β-D-gluc.,R′=H;   9a R=(Ac)₄-β-D-gluc., R′=Ac
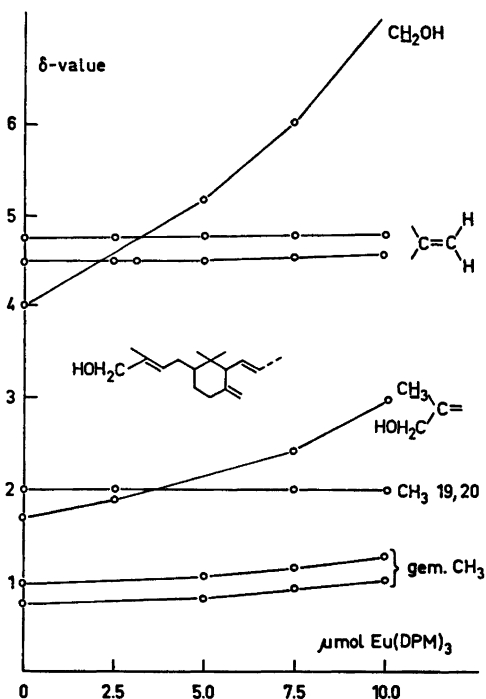
10 R=R′=gluc.,   10a R=R′=(Ac)₄-β-D-gluc.

2   R=CH₃ , R′=CH₂OH
3   R=CH₂OH, R′=CH₃

5   6   7   8

Fig. 1. Induced chemical shifts of ¹H NMR signals of sarcinaxanthin (*4*, 10 μmol) upon addition of Eu(dpm)₃ at constant volume (0.2 ml).

Fig. 2. CD spectrum of sarcinaxanthin (*4*) and of (2*R*, 6*S*, 2′*R*, 6′*S*) − 2,2′-dimethyl-γ,γ-carotene (*5*) [9] in EPA (diethyl ether, isopentane, ethanol 5:5:2).

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 γ end-groups,[6,7] consistent with medium intensity IR-absorption at 890 cm⁻¹.[6,7] The centrosymmetrical structure *4* (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 −CH₃(δ 1.67), −CH₂OH(δ 4.03) and olefinic proton (δ 5.6) as compared with those of decaprenoxanthin (*1*)[3,4] and an aliphatic model.[8]

The Cotton effect (see Fig. 2) of sarcinaxanthin is opposite to that of the synthetic C₄₂ model *5*,[9] favouring opposite absolute configuration at C-6,6′ in sarcinaxanthin (*4*)
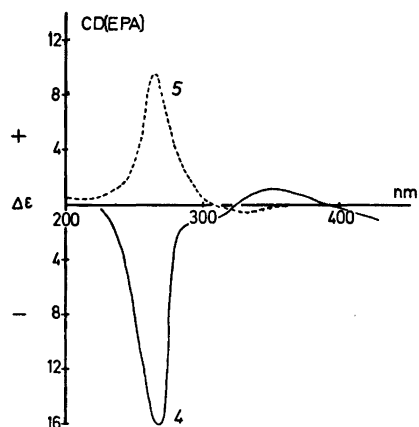
and *5*; *cf.* previous arguments for the stereochemistry of decaprenoxanthin (*1*) from CD-comparison with the C₄₂ model *6*.[5,10] The configuration at C-2,2′ in sarcinaxanthin may, like in the ε-series,[11,5] have little influence on the CD spectrum. In the ε-series the difference in chemical shift of the *gem*-dimethyl signals (*Δ*) within the 2,6-*cis* series (*4* and *6*, *Δ* = 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* C₄₂ model (*5*) is available. However, the chemical shift difference of the *gem.* dimethyl signals for *5* (*Δ* = 0.22) and sarcinaxanthin (*Δ* = 0.23) coincide so well that a 2,6-*cis* relationship in sarcinaxanthin (*4*) is likely. For comparison *Δ* = 0.08 for the unsubstituted γ end-group in optically active β,γ-carotene (*8*).[6]

The close agreement in chemical shifts of the *gem.* dimethyl groups in the C₄₂ models *5* and *6* [9] (Scheme 1) with ε and γ end-groups, as well as for decaprenoxanthin (*1*) and sarcinaxanthin (*4*), together with nearly identical Cotton effects for decaprenoxanthin (*1*)[5] and sarcinaxanthin (*4*, 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 *1* and *6* is planar in the C-3,4,5,6 region with the polyene chain quasiequatorial

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

$\Delta\varepsilon$-Values for decaprenoxanthin (1, $\Delta\varepsilon = -5$,[12] -5[13] value from Ref. 12 used, -15.1[14]) and sarcinaxanthin (4, $\Delta\varepsilon = -16$) are comparable. The high $\Delta\varepsilon$-value (15.5) for α-ionone has been explained[15] by predominance of the conformation with quasiequatorial side chain in order to allow interaction between the enone and the isolated olefin chromophore. In (+)-cis-α-irone ($\Delta\varepsilon = 12.2$) a certain destabilization of this conformation has been ascribed to the axial C-2 methyl group. For (+)-γ-ionone and (+)-cis-γ-irone very low $\Delta\varepsilon$-values (ca. 0.002 – 0.2) are reported. It was concluded[15] that no significant coupling between the electric transition moments of the enone/olefin chromophores occurs. Since $\Delta\varepsilon$-values of ca. 16 are not exceptionally high in the carotenoid series (cf. zeaxanthin[16]) the similar $\Delta\varepsilon$-values for decaprenoxanthin (1)[14] and sarcinaxanthin (4) give no reason to assume any predominance of conformations with quasiaxial or axial polyene chain. Such conformations would be destabilized in the C_{42}- and C_{50}-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 C_{50}-carotenoids with β[17], ε,[5] ψ,[18] and γ end-groups, and that the configuration at C-6 is the same for C_{50}-carotenoids with ε[5] and γ end groups in cases hitherto studied. Biosynthetic considerations have been made.[5,18,19]

Although saponification was included in the isolation procedure the presence of small amounts of a sarcinaxanthin monoester (4a) esterified with a $C_9H_{11}COOH$ acid could be demonstrated (Batch 1) by acetylation, saponification and $LiAlH_4$-reduction experiments and high precision MS.

A C_{50}-carotenoid β-D-monoglucoside, tentatively identified as sarcinaxanthin β-D-monoglucoside,[20] comprised 20 – 40 % of Batches 1, 2, and 3. The ^{1}H NMR and CD spectra of the pentaacetate 9a here prepared are consistent with structure 9 for the mono-β-D-glucoside. Structure 9 is compatible with previously reported in-chain fragmentations of the deuterioacetate 9b on electron impact.[20]

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 C_{40}- and C_{50}-carotenoids previously isolated[2] were not present in Batches 1, 2, and 3.

The variation in carotenoid content of Sarcina lutea,[1,2,21–23] particularly in mass culture, reflects sensitivity of the carotenoid synthesis to cultivation conditions.

So far sarcinaxanthin and its derivatives represent the only C_{50}-carotenoids with substituted γ end-groups.

EXPERIMENTAL PART

Materials and general methods were the same as before.[2] ^{1}H NMR 100 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[2] was grown in mass cultures (150 l) by the previously described procedure;[2] Batch 1 provided ca. 300 g of wet cells (51 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. Andrewes.

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

Separation of individual carotenoids were best effected on acetylated polyamide columns[2] or TLC (SiO_2) after standard saponification with 5 % KOH in methanol[24] 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.[2] ^{1}H NMR ($CDCl_3$) at 60 MHz and 100 MHz differed from the published spectrum:[2] δ 0.73 s and 0.96 (s, 6 H + 6 H, gem. dimethyl), 1.67 (s, 6 H, side-chain CH_3), 1.98 (s, 12 H, in-chain CH_3), 2.3 (m, ca. 10 H, allylic CH_2 and CH), 4.03 (s, 4 H, CH_2OH), 4.53 and 4.76 2 H + 2 H, $=CH_2$), 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.pe.) was isolated from Batch 1. After acetylation a monoacetate 4b ($R_F = 0.85$ on S&S 287 10 % a.pe.) was obtained; m/e 894 (M), 802 (M − 92), 788.5725 ($C_{54}H_{76}O_4 = M − C_8H_{10}$): $M = C_{62}H_{86}O_4$), 746.5621 ($C_{52}H_{74}O_3 = M − C_8H_{10} = C_2H_2O$). Treatment of 4b with $LiAlH_4$ in dry ether gave sarcinaxanthin (4) judged by MS and adsorptive properties. 4a upon standard alkali treatment provided sarcinaxanthin (4) judged by co-chromatography.

Sarcinaxanthin mono-β-D-glucoside (9). 9, eluted from acetylated polyamide with 10 % methanol in benzene, rechromatographed on TLC (SiO_2, eluted with 50 % a.pe.), was acetylated by a standard procedure.[24] The pentaacetate 9a, purified by TLC (SiO_2, eluted with 30 % a.pe.; $R_F = 0.58$ on S&S No. 287 paper 10 % a.pe., *cf.* Ref. 20) had electronic spectrum like 9; MS data as previously reported;[20] ^1H NMR ($CDCl_3$): δ 0.73 (s), 0.98 (s, 6 H + 6 H, gem. dimethyl), 1.60 (s, 3 H, side-chain CH_3), 1.67 (3 H, side-chain CH_3), 1.98 (ca. 12 H, in-chain CH_3); 2.00, 2.02 and 2.09 (ca. 15 H, acetate CH_3), 4.20 [ca. 2 H, −CH_2O gluc $(Ac)_4$], 4.5 and 4.75 (ca. 4 H, =CH_2), methine H and olefinic H; CD (EPA) $\Delta\varepsilon$ 260 nm (− 10.4 pk.), 240 nm (− 5 tr.), 215 nm (− 7.8 pk.).

Sarcinaxanthin diglycoside (10). 10, eluted with benzene-methanol from acetylated polyamide had $R_F = 0.10$ on S&S No. 287 paper (30 % a.pe.). 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.pe.), 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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