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# Bacterial Carotenoids

## XLI.* $C_{50}$-Carotenoids. 11.** $C_{45}$- and $C_{50}$-Carotenoids from *Sarcina lutea* — Sarcinaxanthin

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Previous work on the carotenoids of the common air contaminant *Sarcina lutea* is reviewed.

The carotenoids of *S. lutea* have been re-examined by modern chemical and spectroscopic methods including PMR and mass spectroscopy.

Major carotenoids were a mono-D-glucoside (*2*) of a $C_{50}$-diol, probably sarcinaxanthin, and the $C_{50}$-diol sarcinaxanthin (*3*). Decaprenoxanthin (*1*) and sarcinaxanthin (*3*) are structural isomers ($C_{50}H_{72}O_2$), probably differing only in the location of one of two primary, allylic hydroxy groups.

In one of two pilot-plant batches a carotenoid considered to be a 7,8- or 7′,8′-dihydrosarcinaxanthin (*5*) was one of three major carotenoids.

Minor carotenoids were the $C_{40}$-carotene lycopene (*4*) and three $C_{45}$-mono-ols (tentative assignments *6*,*7* and *8*).

The biosynthesis of the isolated carotenoids is considered.

The carotenoids of the common air contaminant, the yellow gram-positive bacterium *Sarcina lutea*, have been studied by several investigators. Chargaff and Dieryck [1] and Chargaff [2] described the presence of the epiphasic sarcinene, presumably a hydrocarbon, as well as a xanthophyll with the same absorption spectrum in visible light (corresponding to an aliphatic nonaene chromophore). Later Nakamura [3] isolated a yellow pigment with absorption maxima at slightly shorter wavelengths than sarcinene. Nakamura considered his pigment to be a xanthophyll ester. Still later Takeda and Ohta [4] isolated

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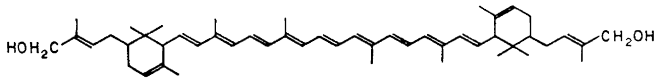
in the crystalline state a xanthophyll, sarcinaxanthin, m.p. 149 – 150°C, presumably identical with the xanthophyll described by Chargaff.[1,2,5] The only carotene isolated was lycopene.[4] Sobin and Stahly [6] found no ester or carotenoic acid, but two yellow xanthophylls. Mathews and Sistrom [7] reported the presence of sarcinene and sarcinaxanthin. In recent years Thirkell and Strang [8] have claimed the presence in both *S. lutea* and *S. flava* of a carotene, a mono-ol, a diol, and a polyol, all with nonaene-type absorption spectra in visible light. In an independant investigation [9] sarcinaxanthin from *S. flava* and sarcinaxanthin from *S. lutea* which were considered identical [8] were compared with *dehydrogenans*-P439, to which structure, *1*, $C_{50}H_{72}O_2$, had been assigned.[10,11]

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The comparative study, performed by means of micro methods involving electronic and mass spectrometry and co-chromatography of the parent compounds and various derivatives, was taken to support identity of sarcinaxanthin and *dehydrogenans*-P439 (*1*).[9] From further mass-spectrometric studies Thirkell, Strang and Chapman [12] later suggested that their sarcinaxanthin might be a mixture of the three compounds $C_{50}H_{72}O_2$, $C_{50}H_{70}O_2$, and $C_{50}H_{68}O_2$. Although M – 2 and M – 2 – 2 peaks, as well as combination peaks therewith, have been observed for carotenoids on electron impact,[13,14] it was felt that a further examination of the structure of crystalline sarcinaxanthin ought to be carried out.

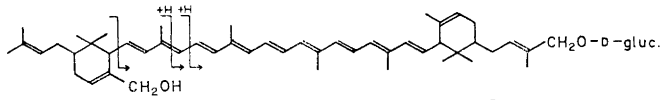
The present paper describes further studies on the carotenoids of *S. lutea*. Some preliminary data [15,16] and parts of this project [17,18] have been reported elsewhere.

## RESULTS AND DISCUSSION

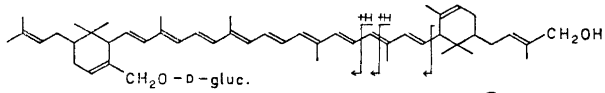
Whereas all previous studies were made on cells cultivated on agar plates, large scale submerged culture grown with high aeration was employed in the present work. Cell yields from 1.4 – 3.0 g dry cells per liter culture were obtained. In previous work, *e.g.* Ref. 8, extraction difficulties had been considerable. This was overcome by lysing the cells by means of lysozyme prior to solvent extraction, thereby allowing facile and complete pigment extraction. Even so the pigment content was low, constituting 0.008 – 0.011 % of the dry weight. In total 24 mg (spectrophotometrically determined) carotenoids were obtained from a 150 l culture (Batch 1, 215 g dried cells) and 41 mg from a 170 l culture (Batch 2, 519 g dried cells). For comparison Takeda and Ohta [4] obtained 3.4 mg crystalline sarcinaxanthin from 385 g dried cells.

Batch 1 provided pure crystalline sarcinaxanthin, m.p. 160 – 161°C, for structural studies. The characterization of the polar carotenoid and the minor carotenoids was mainly effected with pigments from Batch 2.

Experimental details on the polar carotenoid are included in the Experimental Part. Results and arguments leading to the mono-D-glucoside structure *2a* (or *2b*) have been presented separately [17] and are not repeated here. In the absence of PMR data it is emphasized that since no *retro*-Diels Alder fragmentations were observed on electron impact the attachment of the two extra $C_5$-units of the $C_{50}$-aglucone to 2,2′-position is not proved. However, the location of the two oxygen atoms of the aglucone at two different ends of the main chromophore is considered demonstrated by the observed in-chain cleavages on electron impact (indicated on structures *2a* and *2b*) of the penta-acetate and the corresponding penta(trideutero)acetate. That the $C_{50}$-diol aglucone is indeed identical with sarcinaxanthin, the main xanthophyll of *S. lutea* remains an assumption.




(2a)




(2b)

Crystalline sarcinaxanthin (2 mg) was obtained from the present isolation. *Dehydrogenans*-P439, later renamed decaprenoxanthin,[19] was available for further direct comparison.

Judged from the crystalline shapes of sarcinaxanthin (small hexagonal plates) and of decaprenoxanthin (large needles) when obtained from the same solvent system, melting points and mixed melting point determination, and finally co-chromatography tests of the pure *trans* isomers, of their mono- and diacetates as well as their dialdehydes here prepared (Table 1), the two compounds are not identical, contrary to our previous assumption.[9] This conclusion is substantiated by further spectral data given below. However, the physical and chemical data obtained do indicate a close structural relationship.

Both compounds possess the same aliphatic nonaene chromophore *3* as evidenced by their electronic spectra (see Ref. 10) and PMR spectra (Fig. 1). Also sarcinaxanthin (*3*) contains four in-chain methyl groups ($\tau$ 8.03, *ca.* 12 H) and exhibits no end-of-chain methyl signals at $\tau$ 8.19. Instrumental factors may explain the fact that the two methyl groups closest to the end of the polyene chain in sarcinaxanthin (*3*) do not produce separate signals at $\tau$ 8.08 as for decaprenoxanthin (*1*). Furthermore the $\tau$ 3 – 4 complex defining the olefinic protons of the C-8, C-8′ polyene system appears identical in the two compounds (Fig. 1). The intensity ratio (1.64) of the M – 92 and M – 106 peaks in the mass spectrum of sarcinaxanthin (*3*) is also as expected for an aliphatic nonaene chromophore.[20]

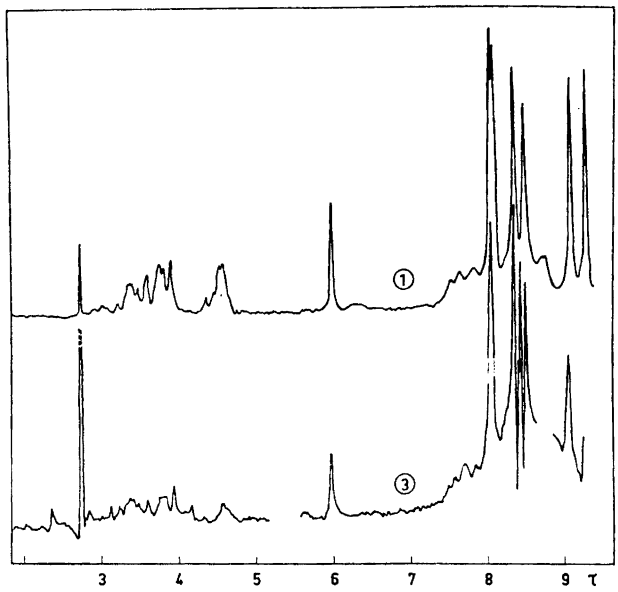



*Fig. 1.* Proton magnetic resonance spectrum ($CDCl_3$) of sarcinaxanthin (*3*) and decaprenoxanthin (*1*).

Mass spectrometry confirmed that sarcinaxanthin (*3*) has the same molecular weight (M = 704) as decaprenoxanthin (*1*). As reported by Thirkell *et al.*[12] dehydrogenation losses (M − 2, M − 2 − 2 and combination peaks therewith) are observed. The presence of two hydroxy groups was indicated by two consecutive losses of water from the molecular ion, and the formulation of the peracetate as a diacetate by following the course of acetylation was confirmed by the mass spectrum of the diacetate (M = 788, M − 42, M − 60). That the two hydroxy groups of sarcinaxanthin (*3*) are primary, allylic ones follows from the OH deformation/C − O stretching absorption at 1005 $cm^{-1}$ in the IR spectrum (Fig. 2) and the 5.96 $\tau$ singlet in the PMR spectrum (Fig. 1) of sarcinaxanthin. The hydroxy groups, in common with those in decaprenoxanthin (*1*), are not allylic to the polyene chain, since selective oxidation of the allylic hydroxy groups with nickel peroxide, *cf.* Ref. 10, yields products with unchanged visible light absorption spectra. Judged from the similarity in polarity of *3* with decaprenoxanthin (*1*) and a negative silylation test for its diacetate further hydroxy groups are disregarded. No other functional groups are disclosed by the IR spectra of sarcinaxanthin (*3*) and its diacetate. In the absence of high-precision measurements the molecular formula of sarcinaxanthin (*3*) is therefore formulated as $C_{50}H_{72}O_2$, *cf.* Ref. 12, as for decaprenoxanthin (*1*).[10]

Further information about the structure of sarcinaxanthin (*3*) is obtained from the PMR spectrum (Fig. 1). Methyl signals not yet discussed are observed at $\tau$ 8.32 (*ca.* 6H), 8.41 (*ca.* 3H), 8.49 (*ca.* 3H), 9.05 (*ca.* 6H), and *ca.*

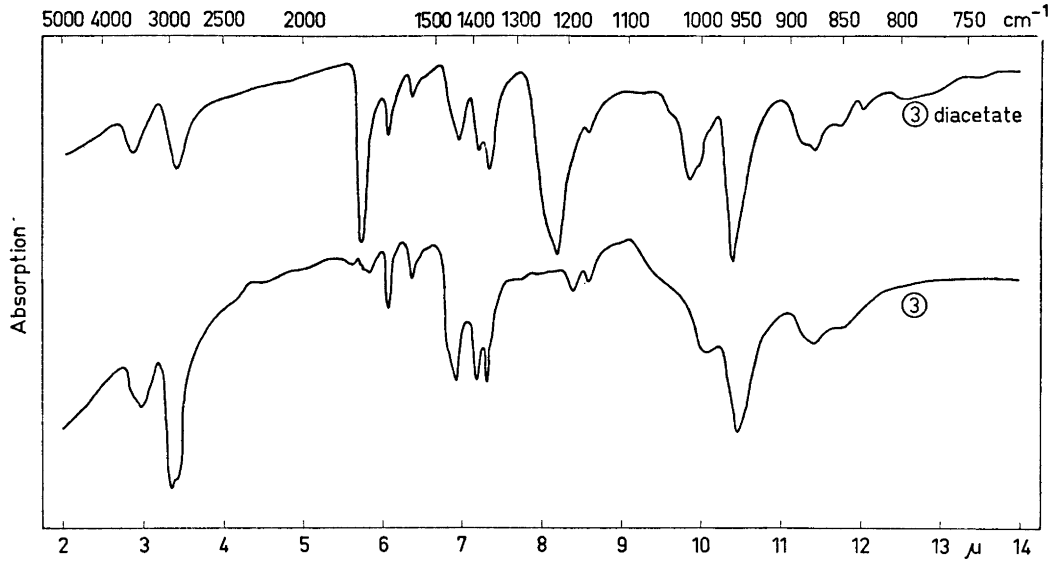
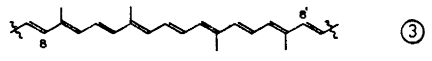
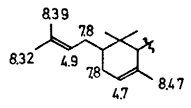
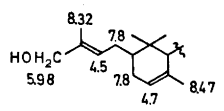


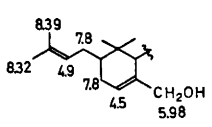

*Fig. 2.* Infrared spectrum (KBr) of sarcinaxanthin (*3*) and sarcinaxanthin diacetate.

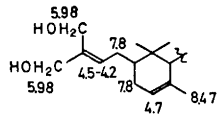
9.25 (*ca.* 6H). The latter signal, not included in the spectrum presented in Fig. 1, was present also on a time-averaged 60 Mc/sec spectrum and must originate from the end-groups. The two signals at high field ($\tau$ 9.05 and 9.25)

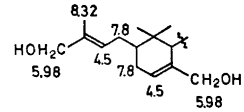


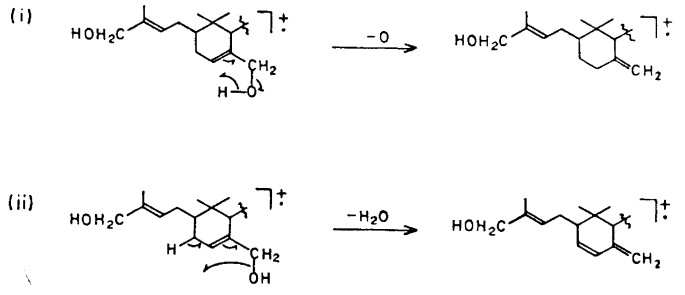
coincide with those of decaprenoxanthin (*1*) and are assigned to the *gem.* dimethyl groups of two ε-rings.

The remaining four methyl groups must be attached to isolated double bonds, and the intensity ratio of their PMR signals (2:1:1) reflects an unsymmetrical molecule.

Various combinations of the end-groups $a-e$ to be considered (predicted signals are indicated in the formula) are all compatible with the allylic $CH_2$-region in the PMR spectrum of sarcinaxanthin (analogous to that of decaprenoxanthin *1* = *3bb*). The methyl signals observed can best be accomodated with *3ea* or *3bc*, whereas the symmetrical combinations *3cc* and *3bb* (= decaprenoxanthin *1*) can be ruled out. For *3da* the expected intensity ratio is different from that observed. Moreover, failure to form an acetonide, and more particularly resistance towards formation of a condensation product with benzaldehyde [21] do not sustain a grouping of type *d*. Compound *3da* may also exhibit decreased polarity relative to, *e.g.*, *3bb*. Structures *3ea* or *3bc* consequently remain as likely possibilities.

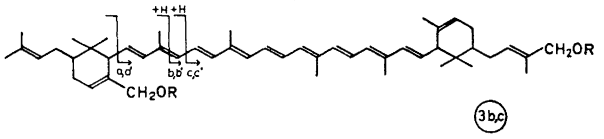
The main peaks in the upper region of the mass spectra of sarcinaxanthin (*3*) and sarcinaxanthin diacetate are compiled in the Experimental Part together with some possible assignments. Exact mass measurements were unfortunately not available. Sarcinaxanthin (*3*) shows the already mentioned dehydrogenation losses and losses due to the two primary allylic hydroxy functions (M − 16, M − 18, M − 18 − 18) in addition to the characteristic losses of toluene, xylene, and dimethylcyclodecapentaene. Other losses compatible with in-chain cleavages will be discussed below.

A major difference in the mass spectra of sarcinaxanthin (*3*) and decaprenoxanthin (*1*) (*cf.* Ref. 10) is that the characteristic loss of 140 mass units from the molecular ion of decaprenoxanthin (*1*), resulting from a RDA-rearrangement [10,11] is not observed for sarcinaxanthin (*3*). Nor does a corresponding loss of 182 mass units occur from sarcinaxanthin diacetate, *cf.* Ref. 10. This was for some time considered evidence against the presence of end-group *b* in sarcinaxanthin and hence the structure *3 ea* was preferred for sarcinaxanthin.[15,16] It seemed reasonable that the hydroxymethyl group at C-18 of end group *a* could prevent the common RDA-rearrangement provided this hydroxy group on electron impact showed a strong tendency to (i) expulsion of oxygen or (ii) elimination of water:



Moreover, previous evidence for RDA-fragmentation for hydrocarbon end-groups of type *a* is not conclusive.[22]

However, in light of the results obtained for the $C_{50}$-D-glucoside (*2a* or *2b*) discussed above, structure *3bc* must be reconsidered. Fragment ions at M − 207 (a), M − 261 (b), and M − 274 (c) of *3* compatible with the corresponding in-chain cleavages of the *2* pentaacetate are observed. Corresponding cleavages, resulting in ions of type a′,b′,c′ appear to occur in the *3* diacetate.




R=H: a,b,c
R=Ac: a',b',c'

Although the losses observed for *3* and *3* diacetate could alternatively be explained by combined losses of atomic oxygen and a hydrocarbon fragment (required by *3ea*), the analogy with the proved in-chain fragmentations of *2* pentaacetate may be taken to support structure *3bc* for sarcinaxanthin. The lacking preference for RDA-rearrangement for the molecular ions of sarcinaxanthin and its acetate may only reflect an unsymmetrical molecule with other preferred fragmentations.

The differentiation between *3ae* and *3bc* could easily be made by the PMR Europium shift technique[23,24] provided large enough samples could be obtained.

The data presented contain no support other than the appearance of the allylic methylene region in the PMR spectrum (Fig. 1) for the attachment of the $C_5$-units of *3* to the 2- rather than 3-position. 2-Substitution is preferred by analogy with decaprenoxanthin (*1*).[10,11]

The close similarity between decaprenoxanthin (*1*) and sarcinaxanthin (*3*) in chromatographic properties, necessitating the preparation of acetylated (or aldehydic) products for a clear distinction to be made on the micro scale, calls for caution in the identification of these pigments from bacteria, *cf.* Refs. 9, 25.

Sarcinene[1,2,7,8] was not encountered. The only carotene isolated was lycopene (*4*), identified from its electronic spectrum, mass spectrum (M = 536, M − 69, M − 92, M − 106, *m/e* 69) and by co-chromatography, with authentic lycopene.

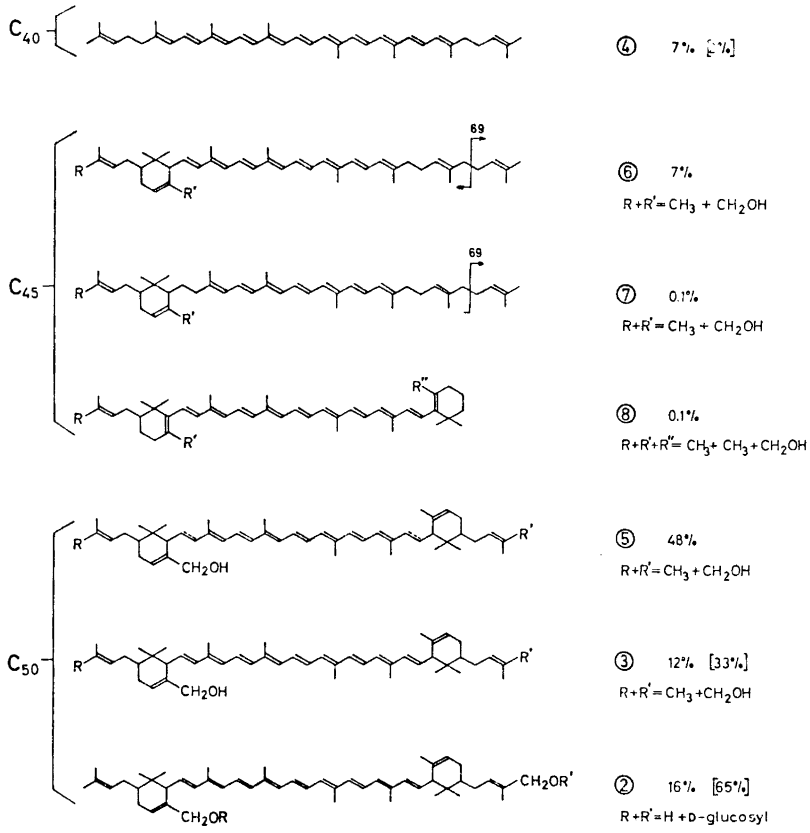
The most abundant minor carotenoid is considered to be a 7,8- or 7′,8′-dihydro-sarcinaxanthin (*5*), which exhibited aliphatic octaene chromophore, judged by its electronic spectrum. The mass spectrum exhibited the molecular ion at *m/e* 706 (compatible with $C_{50}H_{74}O_2$), M − 2, M − 18, and M − 92 ions. The PMR spectrum of a lipid-contaminated sample (for assignments see Experimental Part) supported structure *5* and ruled out the possibility of a substituted β-end group. The presence of primary allylic hydroxy group(s) was further

supported by IR absorption at 1005 $cm^{-1}$. *5* gave a diacetate on acetylation (*m/e* 790 = M, M − 2, M − 42, M − 60, and M − 92). The diacetate could not be silylated.

The mono-ol fraction comprised three minor carotenoids *6*, *7*, and *8*. Carotenoid *6* again had an aliphatic octaene chromophore and was a new $C_{45}$-carotenoid (*m/e* 622 = M, corresponding to $C_{45}H_{66}O$, M − 2, M − 18, M − 92, and base peak *m/e* 69). Acetylation provided a monoacetate (*m/e* 664 = M, corresponding to $C_{45}H_{65}OCOCH_3$, M − 92 and base peak *m/e* 43). With this limited information structure *6* seems plausible for this $C_{45}$-mono-ol.

Another minor carotenoid *7* may represent a 7′,8′-dihydro derivative of *6* or an entirely aliphatic carotenoid. *7* exhibited aliphatic heptaene chromophore with molecular ion at *m/e* 624 corresponding to $C_{45}H_{68}O$ and base peak at *m/e* 69.

The final minor carotenoid *8* exhibited an electronic spectrum characteristic of β-carotene, and gave on acetylation a monoacetate (*m/e* 662 = M, corresponding to $C_{45}H_{63}OCOCH_3$, M − 92 and M − 106, base peak *m/e* 43). The tentative structure *8* may be considered.




*Scheme 1.*

## BIOSYNTHETIC CONSIDERATIONS

For convenience the carotenoids here isolated from *S. lutea* are compiled in Scheme 1. The close biosynthetic relationship between these structures is apparent.

The occurrence of both $C_{40}$- (*4*), $C_{45}$- (*6,7,8*), and $C_{50}$-carotenoids (*5,3,2*) in the same organism may be taken to support the postulated biosynthetic pathway of $C_{45}$- and $C_{50}$-carotenoids *via* $C_{40}$-carotenoids.[10,16] The tentative structure *7* would imply cyclization at the dehydrogenation level of ζ-carotene and may be biosynthetically unsound.

The percentage figures on Scheme 1 refer to the carotenoid composition of Batch 2; those in brackets to Batch 1. Since the carotenoids of highest dehydrogenation level normally represent the ultimate products of carotenoid biosynthesis, it is reasonable to assume that sarcinaxanthin (*3*) and the D-glucoside (*2*) represent the biosynthetic end-products (*cf.* Batch 1). The growth conditions of Batch 2 therefore appear to have depressed complete dehydrogenation to the nonaene system characteristic of *3* and glucoside formation, even though the same medium and growth period were employed for each pilot-plant batch. However, the degree of aeration was probably higher for Batch 1. Considerably higher cell density was obtained in Batch 2.

The location of the carotenoids in the cell membrane of *S. lutea* and their protective effect against photodynamic destruction of the cell have been demonstrated by Mathews and Sistrom.[28,29] No specific function of glycosidic carotenoids is yet known.

## EXPERIMENTAL

*Materials and general methods.* Solvents and instruments used were as specified elsewhere [30] except that some of the mass spectra were obtained with an LKB-9000 instrument (70 eV, direct inlet system, ion source temperature 290°C) and IR-spectra of *3* and *3*-acetate were recorded on a Perkin Elmer Model 21 Instrument.

Column chromatography was carried out on Woelm neutral alumina, May and Baker magnesium oxide or Schleicher and Schüll cellulose powder No. 124. For TLC Merck Kieselgel G and for paper chromatography Schleicher and Schüll No. 287 (Kieselguhr paper), No. 288 (alumina paper) or No. 996 ($CaCO_3$ paper) were used. Chromatographic properties of the individual compounds are compiled in Table 1.

Acetylations, silylations, and saponifications were carried out by standard procedures [31] commonly used in this laboratory.

*Cultivation. Sarcina lutea*, isolated by Reistad [32] and obtained from the Department of Biochemistry, this University, was used. The medium consisted of 2 % casamino acids, 1 % glycerol, 0.5 % yeast extract, 0.5 % NaCl and tap water; pH 7.0 – 7.2. Batch 1 (150 l) was cultivated at the Karolinska Institute, Stockholm, and Batch 2 (170 l) at the Department of Biochemistry, this University. Both cultures were grown at 30°C with aeration using *ca.* 5 % inoculum. Batch 1 was harvested by centrifugation after 24 h of growth; cell yield after lyophilization 215 g. Batch 2 reached the stationary phase after 16 h of growth and was harvested by centrifugation after 26 h and frozen; yield 516 g cell residue after acetone extraction.

*Pigment extraction.* Wet cells (3.1 kg; Batch 2) suspended in phosphate buffer (1800 ml) pH 7.2 (560 ml 0.4 M $NaH_2PO_4.H_2O$ and 1440 ml 0.4 M $Na_2HPO_4.12H_2O$) were treated with lysozyme (1 g) in phosphate buffer (200 ml) for 46 h at 30°C. The lysed cells were repeatedly and completely extracted with acetone at room temperature and the pigments transferred to benzene-ether in a separatory funnel.

The pigments of the lysed cells of Batch 1 were less favourably extracted with methanol, necessitating subsequent precipitation of extracted proteins with acetone.

*Table 1.* Chromatographic properties of the carotenoids of *Sarcina lutea* and their derivatives and of decaprenoxanthin (*1*) and derivatives thereof.

| Carotenoid | Required eluent from cellulose column | Required eluent from alumina grade 3 column | $R_F$-values TLC kieselgel 20 % [c] | TLC kieselgel 40 % [c] | TLC kieselgel 1 % [a] | Kieselguhr paper 2 % [a] | Kieselguhr paper 5 % [a] | Kieselguhr paper 10 % [a] | Kieselguhr paper 50 % [b] | $Al_2O_3$ paper 30 % [a] | $CaCO_3$ paper 10 % [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $C_{50}$-Mono-D-glucoside (*2*) | 30—100 % [a] | pet.ether | | | | | | | 0.45 | | |
| *2* pentaacetate | | | | [e] | | | | | | | |
| Sarcinaxanthin (*3*) | 10—20 % [a] | 75 % [d] | | [e] | | | | 0.64 | | 0.57 | 0.75 |
| *3* monoacetate | | | | | | | 0.58 | 0.83 | | | |
| *3* diacetate | | | | | | 0.60 | 0.85 | 0.98 | | | |
| *3* monoaldehyde | | | | | | | | 0.78 | | | |
| *3* dialdehyde | | | | | | | 0.76 | | | | |
| *3* di TMS ether | | | | | 0.77 | | | | | | |
| Lycopene (*4*) | pet.ether | pet.ether | | | | 0.62 | | | | | |
| Dihydrosarcinaxanthin (*5*) | | 50 % [d] | | | | | | 0.65 | | | |
| *5* monoacetate | | | | | | | [e] | | | | |
| *5* diacetate | | | | | | | 0.95 | | | | |
| Carotenoid *6* | | 40 % [d] | | | | | 0.68 | | | | |
| *6* monoacetate | | | | | | 0.84 | | | | | |
| Carotenoid *7* | | 40 % [d] | [e] | | | | | | | | |
| Carotenoid *8* | | 40 % [d] | [e] | | | | | | | | |
| *8* monoacetate | | | | [e] | | | | | | | |
| Decaprenoxanthin (*1*) | | | | | | | | 0.66 | | 0.57 | 0.75 |
| *1* monoacetate | | | | | | | 0.64 | | | | |
| *1* diacetate | | | | | | | 0.67 | | | | |
| *1* dialdehyde | | | | | | | 0.84 | | | | |

[a] acetone-petroleum ether, [b] acetone-benzene, [c] Suitable system, [d] ether-pet.ether [e] Appropriate system; $R_F$-value not measured.

Even so transfer of the pigments to chloroform in a separatory funnel was hampered by continuous precipitation of proteins.

Spectrophotometrically determined total yield of carotenoids was: Batch 1, 24 mg (0.011 % of dried cells), and Batch 2, 41.6 mg (0.008 % of acetone-extracted cell residue).

*Separation of individual carotenoids.* The pigment extract of Batch 1 was chromatographed on alumina activity grade 2. Carotenes were eluted with 10 – 12 % ether in petroleum ether and sarcinaxanthin (*3*) with 0.5 – 2 % methanol in benzene. The polar carotenoid (*2*) could not be eluted with methanol or pyridine.

The pigments of Batch 2 were chromatographed on two cellulose columns (recovery 89 %), effecting separation of the strongly polar xanthophyll (*2*, 18 %) from the less polar carotenoids (82 %, eluted jointly with 0 – 20 % acetone in petroleum ether). The less polar carotenoids of Batch 2 (30.3 mg) were saponified with 5 % KOH in methanol-ether for 1 h in the usual manner; pigment recovery 68 %. The saponified pigment mixture was chromatographed on alumina, activity grade 3, see Scheme 1 for quantitative carotenoid composition of each batch.

*$C_{50}$-Mono-D-glucoside* (*2*). The purest fractions from the cellulose columns above (5.7 mg) were used. The paper-chromatographically homogeneous carotenoid exhibited $\lambda_{max}$ (acetone) 417, 439, and 468 nm, % III/II [33] = 85.

*2* (1.1 mg) was acetylated in standard manner for 24 h; pigment recovery 12 %. The pentaacetate, purified by TLC, exhibited the same electronic spectrum as *2*; *m/e* 1072(M), M – 2, M – 42, M – 44, M – 60, M – 79, M – 92, M – 106, M – 60 – 92, M – 60 – 106, M – 92 – 106, M – 60 – 60 – 92, M – 249, 827.4704 (calc. 827.4734 for $C_{50}H_{67}O_{10}$) = M – 275, M – 289, M – 302, M – 303, M – 316, M – 330, M – 334, M – 346, M – 347, M – 348, 331 (18 %), 169 (31 %), 109 (27 %), 43 (100 %).

*2* (1.0 mg) in pentadeuteriopyridine (2.0 ml) was acetylated with hexadeuterioacetic anhydride (0.3 ml) for 24 h; pigment recovery 17 %. The fully acetylated product exhibited the same electronic spectrum and $R_F$-value on TLC and kieselguhr paper as the undeuterated pentaacetate; *m/e* 1091(M), M – 45, M – 47, M – 61, M – 63, M – 79, M – 92, M – 106, M – 61 – 92, M – 63 – 92, M – 158, M – 92 – 106, 839.5548 (calc. 839.5487 for $C_{50}H_{55}D_{12}O_{10}$) = M – 252, M – 272, M – 286, M – 288, M – 305, M – 306, M – 319, M – 343, M – 358, M – 359, M – 360, 343 (18 %), 251, 172 (43 %), 119, 109 (43 %), 43 (100 %).

*2* (2.0 mg) was hydrolyzed [34,35] in 0.15 N HCl/methanol (5 ml) for 21 h. Methanol (5 × 10 ml) was added and evaporated. The residue was refluxed with 0.04 N polystyrenesulphonic acid (0.4 ml) for 2 h. The residue was filtered and extracted with warm methanol. The hexose liberated on hydrolysis was identified as glucose or galactose by co-chromatography with galactose, glucose, and mannose in systems 1 and 4 [35] (development with aniline-phthalic acid reagent). The hexose was further purified by chromatography in System 1 with glucose as reference. The reference was localized by spraying with the aniline-phthalic acid reagent and the unknown hexose extracted with hot water. To the hexose (*ca.* 5 μg) in water (0.02 ml) was added tris buffer (1.0 ml) and D-glucose reagent [35] (1.0 ml). After 30 min at room temperature, $H_2SO_4$ (1.0 ml of a 50 % aqueous solution) was added and the light absorption measured at 546 nm against a blank reference containing no hexose. The same test was carried out with a D-glucose standard solution (0.02 ml of a 0.025 % aqueous solution). Both the test and standard developed the bluish colour specific for D-glucose.[24]

*Sarcinaxanthin (3). trans*-Sarcinaxanthin from Batch 1 crystallized as small, regular hexagonal plates from acetone-petroleum ether, m.p. 160 – 161°C, yield *ca.* 1 mg. The absorption spectrum in visible light was identical with that of decaprenoxanthin (*1*).[10] The IR-spectrum (Fig. 1) had $\nu_{max}$ (KBr) 3330 (OH), 3000 (CH), 1640, 1580 (double bonds), 1440 ($CH_2$), 1385 and 1370 (*gem.* $CH_3CH_3$), 1000, 955 (*trans* disubst. C=C), 870 and 830 (trisubst. C=C) $cm^{-1}$ very similar to that of decaprenoxanthin (*1*).[10] The PMR-spectrum (Fig. 2) had τ ($CDCl_3$) 9.25, 9.05 (*ca.* $2CH_3$), 8.72 (lipid impurity), 8.49 (*ca.* $1CH_3$), 8.41 (*ca.* $1CH_3$), 8.32 (*ca.* $2CH_3$), 8.04 (*ca.* $4CH_3$ in-chain), 5.98 (*ca.* 4H, $CH_2OH$) and 4.8 – 3 (olefinic H). The mass spectrum (Fig. 3) exhibited *m/e* 704(M), 702(M – 2), 700(M – 2 – 2), 688(M – 16), 686(M – 18 and M – 2 – 16), 684(M – 2 – 18), 672(M – 18 – 18), 670(M – 2 – 18 – 18), 625(M – 79 [26]), 612(M – 92 [13]), 610(M – 2 – 92), 598(M – 106 [13]), 596(M – 2 – 106), 594(M – 2 – 2 – 106), 582 (M – 16 – 106), 580(M – 18 – 106), 546(M – 158 [14]), 544(M – 2 – 158), 506(M – 106 – 92), 498(M – 206,?), 497(M – 207) = a, 481(M – 16 – a), 479(M – 18 – a), 443(M – 261) = b, 430(M – 274) = c, 351(b – 92). The mass spectrum is described in Fig. 3 and Table 3.

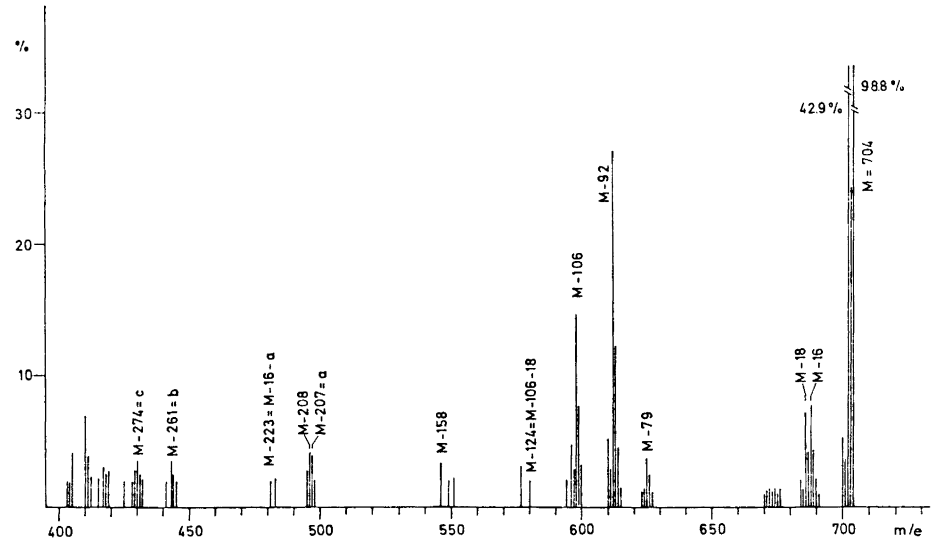



*Fig. 3.* Mass spectrum of sarcinaxanthin (*3*).

Acetylation of *3* in pyridine was effected in several experiments in the standard manner with acetic anhydride; pigment recoveries *ca.* 95 %. The formation of an intermediary monoacetate was observed when the acetylation reaction was monitored by paper chromatography (Table 1). The monoacetate and the diacetate exhibited unchanged electronic spectra. The IR-spectrum of the diacetate (Fig. 2) had $\nu_{max}$ (KBr) 3000, 1740 (acetate), 1640, 1570 (double bonds), 1440 ($CH_2$), 1385, 1365 (*gem.* $CH_3$, $CH_3$), 1220 and 1030 (acetate), 960 (*trans* disubst. C=C), 870 and 830 (trisubst. C=C) $cm^{-1}$, differing only in minor bands from that of decaprenoxanthin diacetate.[10]

The diacetate had *m/e* 788(M), 786(M−2), 746(M−42), 744(M−44), 730(M−58), 728(M−60), 709(M−79), 696(M−2−92, $m^*=616$), 682(M−106), 669(M−121,?), 661(M−129,?), 630(M−158), 622(M−106−60, $m^*=568$), 590(M−106−92), 576(M−106−106), 538(M−92−158), 512(M−60−60−158), 485(M−2−303=b′−2), 479(a′−2−60), 472(c′−2). The diacetate could not be silylated.

*3* (0.53 mg) was silylated in the usual manner; pigment recovery 94 %. The di(trimethylsilyl) ether, purified by TLC had the same electronic spectrum as *3*, *m/e* 848 (M), M−72, M−92, M−106, M−144, M−164, M−178, 73 (100 %).

Allylic oxidation of *3* (1 mg) by the procedure used for *1* for 2 h gave 70 % pigment recovery. Paper chromatography revealed the formation of a monoaldehyde (45 %) and a dialdehyde (5 %) beside unreacted *3*. The new products exhibited the same electronic spectra as *3*.

Acetonide formation [21] was attempted with *3* (0.5 mg) in dry acetone (1.8 ml) and anhydrous $CuSO_4$ (10 mg) for 44 h at room temperature. No less polar products were formed.

Condensation of *3* (1.5 mg) with benzaldehyde [38] (1 ml) at 45°C for 40 h also failed.

*Direct comparison of sarcinaxanthin* (3) *and decaprenoxanthin* (1)

Decaprenoxanthin (*1*) for comparison was obtained from *Flavobacterium dehydrogenans*.[10] On slow crystallization from acetone-petroleum ether *1* afforded long needles forming branches, m.p. 153−154°C. Mixed m.p. with sarcinaxanthin (*3*, m.p. 160−161°C) gave depression (144°C).

Separate iodine catalyzed stereomutation of *3* and *1* in benzene caused the same spectral shift and reduction in spectral finestructure, *cf.* Ref. 10. Both *trans* isomers gave rise to a neo U isomer (kieselguhr paper), *cf.* Ref. 9. Separation of *trans 3* and *trans 1* was not possible on $CaCO_3$-containing, $Al_2O_3$-containing, or kieselguhr paper.

The result of co-chromatography tests of the mono- and diacetates of *1* and *3* is given in Table 1, also including $R_F$-values for co-chromatography tests with the dialdehyde of *1* left from a previous study.[10] The PMR-spectrum of *1* is included in Fig. 1.

*Lycopene* (*4*), purified by TLC or paper chromatography had $\lambda_{max}$ 347, 363, 447, 472, and 503 nm, *m/e* 536(M), M − 69, M − 92, M − 106, 69 (100 %). Co-chromatography (kieselguhr paper) of the stereomutation mixture containing *trans*, neo A, and neo B isomers with the stereomutation mixture of synthetic lycopene gave no separation of the corresponding isomers.

*Dihydrosarcinaxanthin* (*5*). *5* (3.5 mg) was rechromatographed on magnesium oxide and crystallized from acetone-petroleum ether; yield 0.6 mg. The visible light absorption spectrum (acetone) had $\lambda_{max}$ 398, 421, and 446 nm, % III/II $^{33} = 65$, $\nu_{max}$ (KBr) 3500 (OH), 2900 (CH), 1640 (double bonds), 1440 ($CH_2$) 1385, 1370 (methyl, *gem.* dimethyl) 1005, 960 (*trans* disubst. C=C) and 890 $cm^{-1}$, $\tau$($CDCl_3$) 9.27 (*ca.* $2CH_3$), 9.03 (*ca.* $2CH_3$), 8.73 (lipid impurity), 8.40 (*ca.* $2CH_3$), 8.34 (*ca.* 2 $CH_3$), 8.17 (*ca.* 1 $CH_3$, end-of-chain), 8.03 (*ca.* 3 $CH_3$, in-chain), 5.98 (*ca.* 4H, $CH_2OH$) and 3 − 5 (olefinic protons); *m/e* 704(M), M − 2, M − 18, M − 92, 43 (100 %).

Acetylation of *5* (0.41 mg), monitored by paper chromatography revealed one intermediary acetate. Pigment recovery was 90 %. The final product, purified by TLC, exhibited the same electronic spectrum as *5* and *m/e* 790 (M), M − 2, M − 42, M − 60, M − 92, 43 (100 %). The diacetate could not be silylated. Saponification of the diacetate gave *5* (90 % recovery).

*Minor carotenoid 6*. *6* (1.6 mg), purified by TLC, had $\lambda_{max}$ (acetone) (357), 376, 397, 421 and 447 nm; *m/e* 622(M), M − 2, M − 18, M − 92, and 69 (100 %). Acetylation of *6* (0.21 mg) gave a monoacetate (no intermediary products); recovery 95 %, *6* monoacetate had the same electronic spectrum as *6*, *m/e* 664 (M), M − 92, 43 (100 %).

*Minor carotenoid 7*. *7* (0.02 mg), less polar than *6* on TLC, had $\lambda_{max}$ (acetone) 354, 373, 396 and 419 nm; *m/e* 624 (M), 69 (100 %).

*Minor carotenoid 8*. *8* was isolated after acetylation of a mixture with *6*, as a monoacetate (0.02 mg) more polar than *6* monoacetate. *8* monoacetate had $\lambda_{max}$ (acetone) 346, (426), 450, and (480) nm; *m/e* 662 (M), M − 92, 43 (100 %).

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