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Bacterial Carotenoids

XXXVI.* Remarkable C_{43}-Carotenoid Artefacts of Cross-conjugated Carotenals and New Carotenoid Glucosides from Athiorhodaceae spp.

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Further characterization and structure determination of two new carotenoid glycosides, rhodopin β-D-glucoside (*2*) and rhodopin-20-al β-D-glucoside (*7*), the first glycosidic carotenoids encountered in photosynthetic bacteria, are described.

Partial synthesis, PMR and mass spectrometry were also used in the structure elucidation of three C_{43}-carotenoid artefacts (*5*, *8*, and *10*), shown to be methyl ketones formed by smooth aldol condensation of the naturally occurring cross-conjugated carotenals *4*, *7*, and *11* with acetone during the isolation.

A new, minor cross-conjugated carotenal, considered to have structure *13*, was also isolated as the corresponding condensation product (*12*).

The chemical structures of characteristic carotenoids produced by photosynthetic purple bacteria have been dealt with in previous papers of this series.[1–4] Recently, the carotenoid composition of *Rhodospirillum tenue*, *Rhodocyclus purpureus* (preliminary name assigned by N. Pfennig), and several strains of *Rhodopseudomonas acidophila* have been analysed by Schmidt.[5] We now report on the identification of the carotenoids isolated. In particular, the structures of two new glycosidic carotenoids and a new cross-conjugated carotenal encountered in *R. acidophila* strain 7050, and of some remarkable C_{43} methyl ketone artefacts of various cross-conjugated carotenals are dealt with (Scheme 1). The C_{43} artefacts were formed in quantitative yield in the presence of small amounts of acetone during the saponification step.

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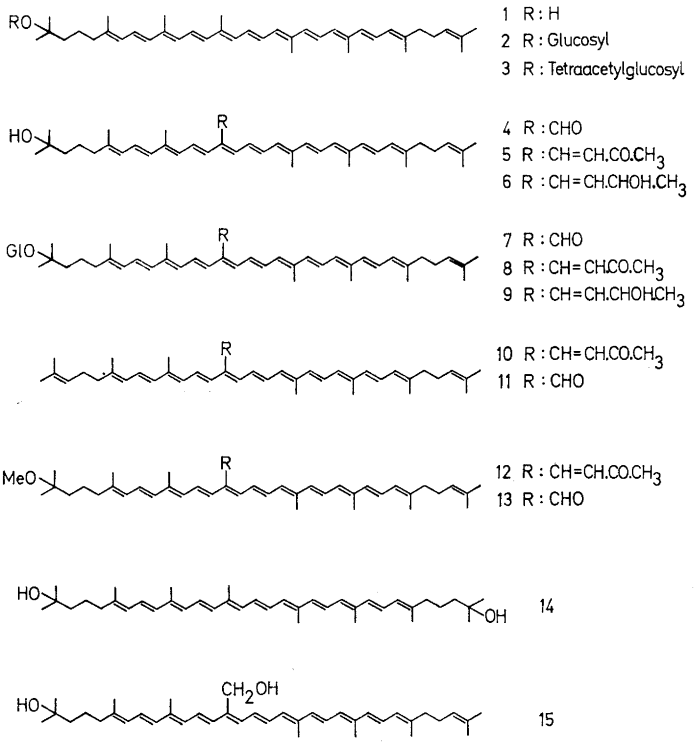
RESULTS AND DISCUSSION

Rhodopin (*1*),[6,7] one of the most widely distributed carotenoids in photosynthetic bacteria,[8] was present in all strains examined. In addition, almost all strains of *R. acidophila* contained a strongly polar derivative shown to be rhodopin β-D-glucoside (*2*). Partition data, acetylation evidence, and IR data (strong absorption at 1075 and 1010 cm^{-1}) were indicative of a glycoside, confirmed by sugar hydrolysis, followed by paper-chromatographic identification of glucose. The mass spectrum (Fig. 3) of the tetraacetate (*3*) was fully consistent with this assignment. It confirmed the molecular weight of 884 and had characteristic M − 92, M − 106, and M − 158 peaks.[9,10] A prominent ion at *m/e* 493 corresponded to cleavage of the C_{1-2} bond with transfer of two hydrogens away from the charged carotenoid fragment, and a prominent ion at *m/e* 69 (46 %) supported the presence of an isopropylidene end group. In addition, ions due to the sugar moiety[11,12] were abundant: *m/e* 331, 211, 169, 157, 127, 109, and 43. Identical PMR spectra in the region attributed to the ring protons of the sugar residue of the tetraacetates of rhodopin glucoside and the β-D-glucoside discussed below supported the β-D-glucoside formulation. In agreement with the tertiary glucoside structure, the PMR spectrum of *3* had a characteristic signal at τ 8.79 (*gem.* methyl) and *3*, provided no



Scheme 1.

silyl ether on silylation. Moreover, in-chain methyl (τ 8.03) and end-of-chain methyl (τ 8.19, 2 Me) signals confirmed the location of the aliphatic undecaene chromophore, revealed by the electronic spectrum, and the isopropylidene group gave rise to characteristic signals at τ 8.32, 8.39 (1 + 1 Me).[13] Glycosidic carotenoids have previously been isolated from blue-green algae [14–16] and non-photosynthetic bacteria,[17–19,12] but this is the first demonstration of a glycosidic carotenoid in photosynthetic bacteria.

From *R. acidophila* strain 7050 a second, dark red carotenoid glycoside was also obtained, quantitatively separated from rhodopin glucoside (*2*) only as peracetates. Again polarity properties, IR spectrum, and acetylation data were indicative of a glycoside, and in this case, D-glucose was identified after hydrolysis by means of paper chromatography and a specific colour reaction.[12] Mass spectrometry of the free glucoside (M = 770, $C_{43}H_{59}O_2.C_6H_{11}O_5$) and the peracetate (Fig. 7, M = 938, $C_{43}H_{59}O_2.C_6H_{11}O_5(OCCH_2)_4$) showed that the peracetate was a tetraacetate. In this case, also the tetraacetoxyoxonium ion (*m/e* 331), characteristic of hexosides, and ions derived therefrom [11,12] (*m/e* 211, 169, 157, 127, 115, 109, 43) were observed. From the mass-spectrometric data, it was inferred that the aglucone had the molecular composition $C_{43}H_{60}O_2$. Since the second oxygen function was shown by hydride reduction to represent a carbonyl group, the aglucone possessed a C_{43} skeleton. The round-shaped absorption spectrum in visible light (see Fig. 1) resembled that of cross-

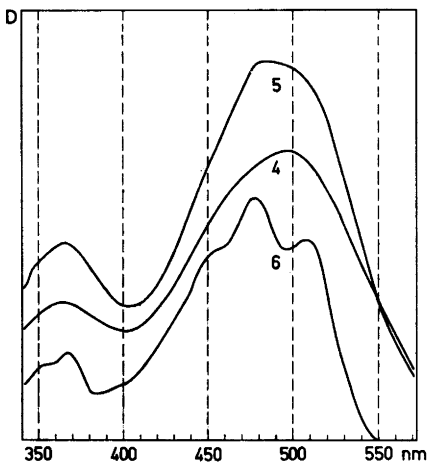


Fig. 1. Absorption spectra in visible light, measured in acetone solution of rhodopinal (*4*), 20-acetonylrhodopinal (*5*), and $LiAlH_4$-reduced 20-acetonylrhodopinal (*6*).

conjugated carotenoids of the rhodopinal (*4*) type. Mass spectrometry revealed that an extra C_3H_2O substituent was located on the polyene chain. Whereas carotenoids with the usual type of methyl substituted polyene chain give rise to characteristic M − 92 (P), M − 106 (Q), and M − 158 (T) peaks in their mass spectra ascribed to the elimination of toluene, xylene, and a dimethylcyclodecapentaene species,[9,10] P′, Q′, and T′ peaks with the appropriate mass shifts caused by extra substituents on the polyene chain [4,20,21] have been demonstrated. In the case of the red glucoside, its hydride reduction product and its tetraacetate such P′, Q′, and T′ ions were observed, demonstrating a C_4H_5O

substituent in a position usually occupied by one of the four central lateral methyl groups [4,21] (see Figs. 5, 6, 7). Hydride reduction resulted in a large hypsochromic shift, demonstrating that the carbonyl function was conjugated with the main chromophore. The electronic spectrum of the reduction product (see Fig. 1) exhibited maxima at longer wavelengths than rhodopinol (*15*), and corresponded to that of 2-rhodopinylidene ethanol [3] (Scheme 1). Structure *8* for the C_{43}-D-glucoside was considered. M − 43 and *m/e* 43 ions in the mass spectrum of the free glucoside, which could be accomodated with a methyl ketone formulation and further evidence, was sought in the PMR spectrum of the tetraacetate (Fig. 2). The PMR spectra of the tetraacetates of this

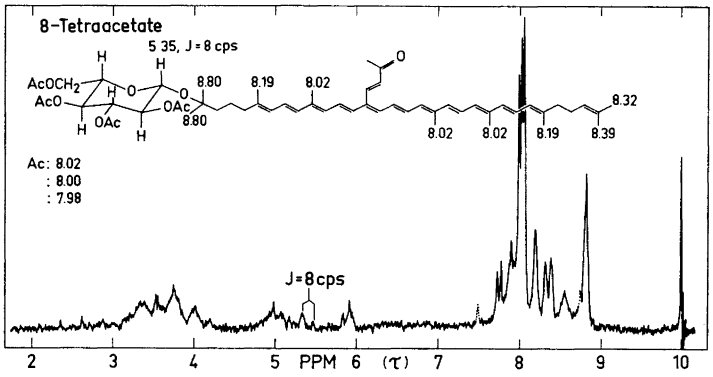


Fig. 2. Proton magnetic resonance spectrum of 20-acetonylrhodopinal β-D-glucoside (*8*) tetraacetate.

glucoside and rhodopin glucoside had many features in common. Thus the end-of-chain methyl (τ 8.19, 2 Me) and in-chain methyl (τ 8.02, theor. 3 Me) signals of *8* tetraacetate were in accordance with the location of the aliphatic undecaene chromophore. Furthermore, the presence of an isopropylidene group (τ 8.32 and 8.39, 1 + 1 Me) and a *gem.* dimethyl group (τ 8.80, 2 Me) in *8* tetraacetate were demonstrated. A signal at τ 7.82 (Fig. 2) may be ascribed to the methyl ketone, allylic methylene, or slight acetone contamination. Signals at τ 7.68 and 7.72 (*ca.* 4 H) were not identified, being downfield to allylic methylene in direct spectral comparison with rhodopin (*1*). These signals may represent the methyl keto group in different magnetic environments; the stereochemistry of this type of carotenoid is known to be complex.[3,22] A shift difference of 3 Hz makes long-distance coupling less likely.[23] Signals at τ 7.93, 7.98, and 8.02 (theor. 4 Me) were ascribed to acetate methyl. Comparison of the spectra of *8* tetraacetate and of D-glucose pentaacetate showed the anomeric proton of the former as a doublet at τ 5.35 ($J = 8$ Hz, ax. ax.)[23] in support of β-D-configuration. Observed electron impact induced in-chain fragmentations for the hydride reduced red glucoside could be accomodated with 20-substitution (*9*) (Fig. 7). The allocation of the extra substituent, however, rests on identity in electronic spectra with *5* and evidence presented for *5* below.

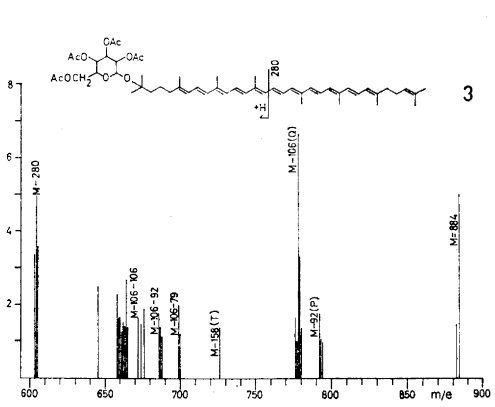


Fig. 3. Mass spectrum of rhodopin β-D-glucoside tetraacetate (*3*).

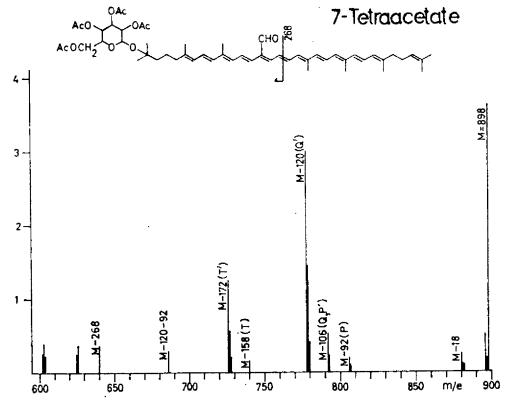


Fig. 4. Mass spectrum of rhodopinal β-D-glucoside (*7*) tetraacetate.

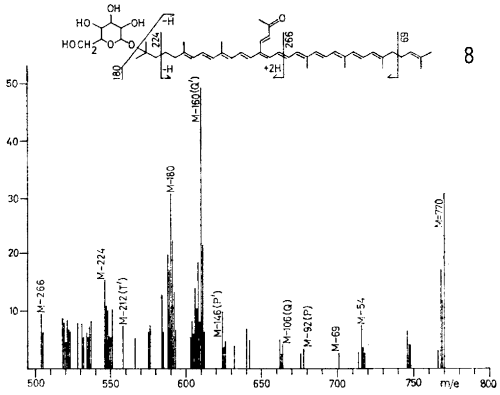


Fig. 5. Mass spectrum of 20-acetonyl-rhodopinal β-D-glucoside (*8*).

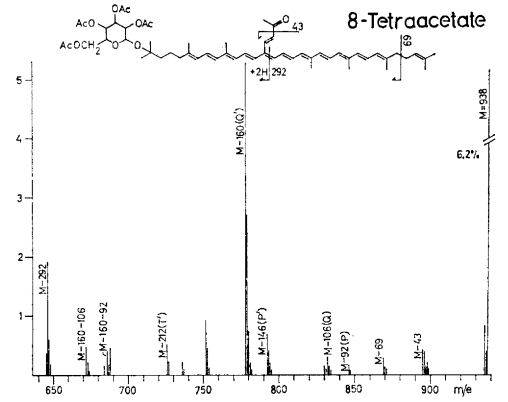


Fig. 6. Mass spectrum of 20-acetonyl-rhodopinal β-D-glucoside (*8*) tetraacetate.

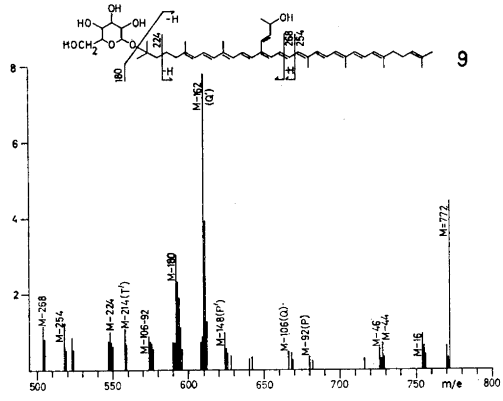


Fig. 7. Mass spectrum of *9*.

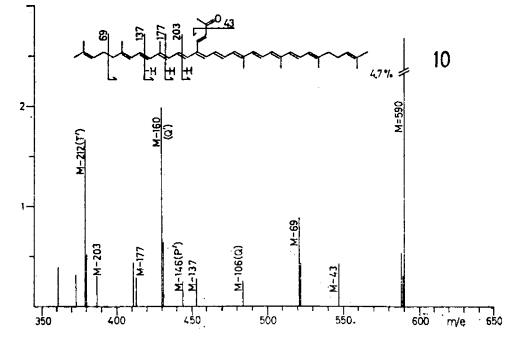


Fig. 8. Mass spectrum of 20-acetonyl-lycopenal (*10*).

The aglucone (*5*) of the red glucoside (*8*) was isolated from all strains examined. It could not be acetylated, but gave a mono(trimethylsilyl) ether on silylation, thus demonstrating the presence of a tertiary hydroxy group. Dehydration with phosphorus oxychloride provided a product identical with *10* described below. Hydride reduction caused the same spectral changes in the visible region as for the red glucoside (*8*). Smooth addition of methanol under conditions for acetal formation was not observed, and mass-spectrometric (M − 43) evidence were in accordance with a methyl ketone. The mass spectrum (M = 608) showed characteristic M − 18, Q, P′, Q′, and T′ ions (Fig. 9). Accurate mass measurements of intense ions attributed to in-chain cleavage of the C_{12-13} and C_{13-14} bonds proved the 20-substitution. The trimethylsilyl ether of *5* exhibited its molecular ion at *m/e* 680. Q, P′, Q′, and T′ ions were observed, and the *m/e* 373 ion in *5* itself was retained, confirming the assignment of this ion to cleavage of the C_{12-13} bond. *5* was not obtained free of non-carotenoid impurities. However, the PMR spectra of *5* and the hydride reduced compound (*6*) exhibited the expected signals for the in-chain, end-of-chain, isopropylidene, and *gem.* dimethyl groups. Moreover, the mass spectrum of the reduced compound (Fig. 10) was in agreement with the structure assigned (*6*).

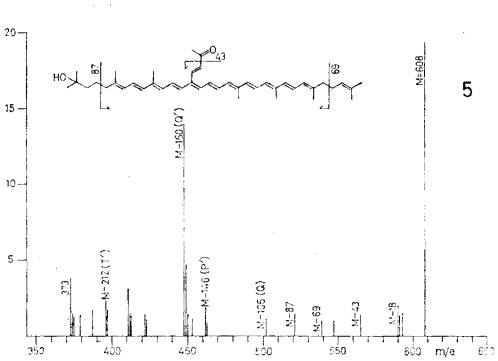


Fig. 9. Mass spectrum of 20-acetonyl-rhodopinal (*5*).

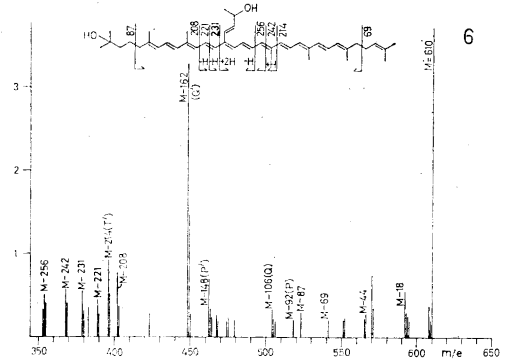


Fig. 10. Mass spectrum of *6*.

The structure of the C_{43}-aglucone (*5*) was confirmed by partial synthesis from rhodopinal (*4*) and triphenylacetylmethylenephosphorane in a Wittig reaction. Moreover, *retro*-aldol cleavage, known to operate for several keto carotenoids,[24–26] of the C_{43}-aglucone to rhodopinal (*4*) was achieved on alkali treatment.

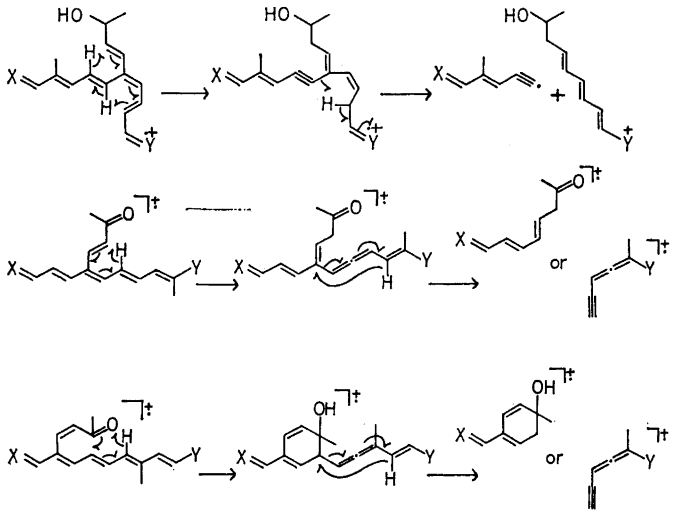
Since the C_{43}-carotenoids *5* and *8* may be conceived as aldol condensation products of acetone and rhodopinal (*4*), and rhodopinal glucoside (*7*), respectively, and such aldol condensation was subsequently demonstrated *in vitro* for rhodopinal (*4*), the natural occurrence of the C_{43}-carotenoids was checked in extracts where acetone was omitted in the isolation procedure.

In this case, no C_{43}-glucoside, but instead rhodopinal glucoside (*7*) was present (see mass spectrum of *7* tetraacetate, Fig. 4). Concentration of large acetone extracts of rhodopinal (*4*) in glassware apparatus did not lead to aldol condensation products in agreement with earlier experience,[3,4] and it is concluded that the C_{43}-carotenoids *5* and *8* are both artefacts, formed in quantitative yield from the corresponding cross-conjugated carotenals, and caused by the presence of small amounts of acetone during the saponification step.[5] The natural occurrence of several methyl ketones of apo-type structure has been reported in recent years.[27] Where acetone has been involved in the isolation procedure, the natural occurrence of such methyl ketones should be checked.

Two further C_{43}-carotenones were isolated in the present study. One (*10*) corresponded to the previously described [3,4] lycopen-20-al (*11*), and is also considered to be an artefact. The structure of *10* followed largely from spectroscopic data. The molecular formula $C_{43}H_{58}O$ was determined by mass spectrometry. The electronic spectrum corresponded to those of *5* and *8*, and the composition and attachment of the extra C_3H_2O substituent followed from P′, Q′, and T′ peaks observed in the mass spectrum (Fig. 8). Prominent ions at *m/e* 297 and 309 were attributed to in-chain cleavages at the C_{13-14} and C_{14-15} bonds. Hydroxy groups were judged absent from partition behaviour and acetylation data. The total structure of this least polar C_{43} methyl ketone (*10*) follows from dehydration of the tertiary alcohol *5* with phosphorus oxychloride, to give *10*.

R. acidophila strain 7050 in addition contained trace amounts of a slightly more strongly retained carotenoid than *10*, for which the methyl ketone structure *12* is considered. Again *12* is considered an artefact, and *13* the naturally occurring carotenal. The molecular ion of *12* corresponded to $C_{43}H_{57}O(OCH_3)$. Characteristic Q, P′, Q′, and T′ ions suggested that the extra C_3H_2O substituent was in common with *5* and *8*, and M − 32 and *m/e* 73 (base peak) together with the finding of spirilloxanthin in the same organism supported the presence of a methoxylated end group of the spirilloxanthin type. Hydride reduction gave a product with longer chromophore than *6* and *9* in agreement with a *cis* dodecaene. As discussed elsewhere,[3,22] the cross-conjugated carotenals of the rhodopinal (*4*) type are considered to possess *cis* configuration at one of the bonds in the polyene chain, adjacent to the branching point near the hetero substituent. This new carotenal (*13*), in which the methoxy group and the aldehyde function are placed by analogy at the same side of the molecule, is formulated as a 3,4-didehydro derivative of 1-methoxy-1,2-dihydro-lycopen-20-al, recently described.[4]

Some general comments on the mass spectra of the C_{43}-carotenoids examined will be made at this point. In-chain cleavages in these compounds are most intense at positions close to C − 13, and this is ascribed to the presence of the cross-conjugated substituent at this branching point. Previously, generally one hydrogen transfers have been observed for in-chain cleavages of carotenoids.[4,10] In the case of the C_{43}-carotenoids, transfer of two hydrogen atoms is involved, presumably due to the carbon-carbon double bond, present in the side chain. Possible mechanisms for some of these cleavages are given in Scheme 2, although other mechanisms may be conceived.



Scheme 2.

Regarding the excision of the various ions of the PQT and P'Q'T' series, it may be noted that P and Q ions are always weak in these compounds, and in no case is a T ion observed. The P'Q'T' ions, particularly the Q' and T' ions, are rather intense.

Turning now to the diol fraction of *R. acidophila* strain 7050, it was found to contain a mixture of the tertiary diol *14* (see Scheme 1) and rhodopinol (*15*), separable after acetylation. The identification was based on the result of acetylation in conjunction with mass spectrometry and co-chromatography tests. Both diols have previously been encountered in Thiorhodaceae sp.[3,6]

The identification of spirilloxanthin and β-carotene included mass spectrometry. β-Carotene is a rare constituent in purple bacteria.[6]

The biosynthetic and taxonomic implications of the present findings are discussed separately by Schmidt.[5]

EXPERIMENTAL

Cultures. Three strains of Athiorhodaceae, obtained from the collection of N. Pfennig, Göttingen, were used: *Rhodopseudomonas acidophila* strain 7050 (SMG 137, ATCC 25092), *Rhodospirillum tenue* strain 3661 (SMG 112), *Rhodocyclus purpureus* strain 6770 (SMG 168).

Cultivation of the biological material used has been described by Schmidt.[5]

Isolation of the carotenoids. Solvent extraction, saponification procedure, column chromatography, and quantitative composition of the carotenoid mixtures of the various strains examined are reported separately.[5] Most of the carotenoids described below were obtained from several strains and from several batches of each organism. The approximate total quantity available of each carotenoid is indicated.

Characterization of carotenoids. Eluents required from alumina or cellulose columns, R_F-values on kieselguhr paper, and absorption maxima in visible light of the individual carotenoids are reported by Schmidt; the result of co-chromatography tests with authentic carotenoids are included in the same paper.[5] Further characterization is reported below.

General methods. When not otherwise stated, these were as reported elsewhere.[28] TLC was performed on kieselgel G with mixtures of acetone and petroleum ether or chloroform and petroleum ether. Silylation was effected by the standard procedure.[29] Electronic spectra were recorded on a Coleman-Hitachi 124 instrument, IR spectra on a Perkin Elmer 254 Grating Spectrometer, and PMR spectra on a Varian A-60A spectrometer. Mass spectra were obtained on an AEI MS 902 spectrometer, using the direct insertion probe with ion source temperature 240–280°C, 70 eV bombarding electron energy, and accelerating voltage of 8 kV. For the accurate mass measurements, heptacosaperfluorotributylamine was used as standard.

β-Carotene, available *ca.* 50 μg; mass spectrum in accordance with published data.[9]

Spirilloxanthin, available *ca.* 1.5 mg; mass spectrum in accordance with published data.[10]

Rhodopin (*1*), available *ca.* 10 mg; partition ratio in petroleum ether/95 % methanol 76 : 24; mass spectrum in accordance with reported data.[10]

Rhodopin β-D-glucoside (*2*). *2* crystallized from acetone-petroleum ether, m.p. unsharp 170–175°C, total yield *ca.* 20 mg; characteristic ν_{max} (KBr) 3350, 1075, and 1010 cm^{-1} (OH), 970 (*trans* disubst. double bonds), 898 and 840 (trisubst. double bonds) cm^{-1}. Partition ratio in petroleum ether/85 % methanol was 8 : 92.

2 (3 mg) was hydrolyzed in methanol, and the resulting methyl glycoside converted to the free sugar,[14,30] subsequently identified as glucose by paper chromatography in System 5,[14] using glucose, galactose, and mannose as reference substances.

2 (14 mg) was acetylated in the usual manner. The tetraacetate (*3*) exhibited the same electronic spectrum as *2*, had $R_F = 0.50$ on aluminium oxide paper (20 % acetone in petroleum ether), and required 20–25 % ether in petroleum ether for elution from alumina activity grade 3 on quick column chromatography; τ ($CDCl_3$) 8.79 (*gem.* Me), 8.39 and 8.32 (isopropylidene Me), 8.19 (end-of-chain Me), 8.03 (in-chain Me), 8.03, 7.90 and 7.84 (acetate Me), *ca.* 5.6 (2 H, *mult.*, CH_3OAc), 5.16 (1 H, doubl., $J = 8$ cps. (ax. ax.), anomeric H), *ca.* 4.6 (*ca.* 4 H, mult., 3 ring protons + olefinic H isopropylidene), 2.9–4.0 (olefinic H); *m/e* 884 (M), M–92, M–106, (M–92/M–106 = 0.26), 493, 331 (50 %), 211 (19 %), 169 (97 %), 157 (9 %), 127 (64 %), 109 (53 %), 69 (46 %), 43 (100 %) (see Fig. 3).

20-Acetonylrhodopinal (*5*) = Product 2,[5] available *ca.* 6.5 mg. *5*, purified by chromatography on alumina activity grade 2, could not be obtained in the crystalline state, and was like other carotenoids of the rhodopinal series [3] unstable in the amorphous state. Solutions, chromatographically pure as to carotenoids, were used for experiments. In acetone ν_{max} 485 nm (see Fig. 1); characteristic ν_{max} ($CHCl_3$) 1665 (conj. ketone), 1610 (conj. double bonds) and 965 (*trans* disubst. double bonds) cm^{-1}; *m/e* 608.460 (calc. for $C_{43}H_{60}O_2$ 608.460), 565.440 (M–43, calc. for $C_{41}H_{57}O$ 565.441), M–69, M–87, 462.387 (M–106 = Q, calc. for $C_{33}H_{50}O$ 462.386), M–146 (P′), 448.371 (M–160 = Q′, calc. for $C_{32}H_{48}O$ 448.371), 396.339 (M–212 = T′, calc. for $C_{28}H_{44}O$ 396.339), 373.252 calc. for $C_{27}H_{33}O$ 373.253, 315.233 calc. for $C_{21}H_{31}O_3$ 315.232, 43 (100 %) (see Fig. 8). The partition ratio in petroleum ether/95 % methanol was 32 : 68, and in petroleum ether/85 % methanol 83 : 17.

5 (60 μg) gave no acetate on standard acetylation. *5* (0.1 mg) provided a mono(trimethylsilyl) ether in quantitative yield after 4 min at room temperature on silylation. *5* trimethylsilyl ether had $R_F = 0.50$ on kieselguhr paper (5 % acetone in petroleum ether); *m/e* 680 (M), M–90, M–92 (P), M–106 (Q), M–146 (P′), M–160 (Q′), M–212 (T′), 411, 373, 69 (100 %).

5 formed no addition products with methanol under conditions for acetal formation.[3] A presumed ketal was formed in low yield after 15 min. When isolated and kept in acidified methanol solution, it was partly reconverted to *5*.

Dehydration of *5* (0.8 mg) with phosphorus oxychloride in pyridine by standard procedure gave a single product, identical (λ_{max}, co-chromatography, and mass spectrum) with *10* described below.

Reduction of *5* (6 mg) with $LiAlH_4$ in tetrahydrofuran gave *6*, λ_{max} in acetone at (352), 365, (452), 475, and 507 nm (Fig. 1); $R_F = 0.23$ on kieselguhr paper (10 % acetone in petroleum ether); *m/e* 610.475 (M, calc. for $C_{43}H_{62}O_2$ 610.475), M–18, M–69, M–92 (P), M–106 (Q), 462.386 (M–148 = P′, calc. for $C_{33}H_{50}O$ 462.386), 448.371 (M–162 = Q′, calc for $C_{32}H_{48}O$ 448.371), 402.292 calc. for $C_{29}H_{38}O$ 402.292, M–214 (T′), 379.300 calc. for $C_{27}H_{39}O$ 379.300, 318.256 calc. for $C_{21}H_{34}O_2$ 318.256, 292.219 calc. for $C_{22}H_{28}$ 292.219, 223.149 calc. for $C_{17}H_{19}$ 223.149, 69 (100 %) (see Fig. 10).

Retro-aldol cleavage of *5* (1 mg) in methanol-water (4 ml, 4 : 1), containing 10 % KOH, for 2 h at 75°C resulted in rhodopinal (*4*), as judged by R_F-value (TLC) and mass spectrum.

Partial synthesis of *5* was effected by two methods:

(a) Rhodopinal [3] (*4*, 3 mg) in benzene (10 ml) was treated with triphenyl-acetylmethylenephosphorane [31] (230 mg) at 80°C overnight; pigment recovery was 56 %. The reaction mixture contained *5* (55 %, $R_F = 0.57$ on aluminium oxide paper, 20 % acetone in petroleum ether) and unreacted *4* ($R_F = 0.62$ on the same paper). *5* was isolated by TLC and identified by mass spectrometry. An aliquot (0.8 mg) of *5* thus prepared was reduced with $LiAlH_4$. The product was identical (λ_{max}, co-chromatography) with *6*, prepared from *5* above.

(b) Rhodopinal (*4*, 0.5 mg) in methanol-ether-acetone (8 ml, 2 : 4 : 2) was treated with 20 % methanolic KOH (2 ml), final alkali conc. 4 % and acetone conc. 20 %, for 1.5 h at room temperature. The recovered carotenoid was exclusively *5* (TLC, mass spectrometry).

Rhodopinal β-D-glucoside (*7*). When no acetone was used in the isolation procedure, *7* replaced *8* in a test extract of *R. acidophila* strain 7050. The carotenoids were extracted with methanol, purified by TLC, and the red glucoside acetylated and analyzed: λ_{max} as for *4*; *m/e* 898 (M), M − 18, M − 92 (P), M − 106 (Q), M − 120 (Q′), M − 158 (T), M − 172 (T′), 331 (9 %), 211 (7 %), 169 (44 %), 157 (17 %), 127 (9 %), 109 (37 %), 69 (71 %), 43 (100 %) (see Fig. 4).

20-Acetonylrhodopinal β-D-glucoside (*8*) = Product 3,[5] precipitated as an unstable, amorphous solid, m.p. 150 − 165°C; *m/e* 770 (M), M − 54, M − 69, M − 92 (P), M − 106 (Q), M − 146 (P′), M − 160 (Q′), M − 180, M − 212 (T′), 69 (100 %) (see Fig. 5). The partition ratio in petroleum ether/70 % methanol was 14 : 86 and remained constant when measured at pH 4 or pH 10. Solutions, chromatographically pure as to carotenoids, were used for experiments.

8 (0.1 mg) did not readily form an addition product with methanol under conditions for acetal formation [3] (*cf.* *5* above).

8 (3 mg) was hydrolyzed in the same manner as *2* above. D-Glucose was identified by paper chromatography and subsequent oxidation with D-glucose oxidase by the procedure described elsewhere.[12]

Acetylation of *8* (50 μg), followed by paper chromatographic analysis, revealed the formation of several intermediary acetates. The tetraacetate (9 mg) was prepared by standard procedure and purified by quick column chromatography on alumina activity grade 3, $R_F = 0.83$ on kieselguhr paper (20 % acetone in petroleum ether). τ ($CDCl_3$) 8.79 (6 H, *gem.* Me), 8.5 (4 H, non-allylic methylene), 8.39 and 8.32 (3 H + 3 H, isopropylidene), 8.19 (6 H, end-of-chain Me), 8.02 (in-chain Me and acetate Me), 8.00 (acetate Me), 7.98 (acetate Me), 7.82, 7.72 and 7.68 (allylic methylene and methyl ketone), *ca.* 5.85 mult. (2 H, CH_2OAc), 5.35 doubl. ($J = 8$ cps, ax. ax., anomeric H), *ca.* 4.9 (*ca.* 4 H, olefinic isopropylidene and ring protons), 3 − 4.2 (18 H, olefinic) (*cf.* Fig. 2); *m/e* 938 (M), M − 92 (P), M − 106 (Q), M − 148 (P′), M − 162 (Q′), M − 214 (T′), 493, 331 (7.4 %), 211 (5.4 %), 169 (25.6 %), 157 (14.6 %), 127 (10.8 %), 115 (21.8 %), 109 (38.5 %), 69, 43 (100 %) (see Fig. 6).

8 tetraacetate formed no silyl ether when submitted to silylation conditions. Alkaline hydrolysis of *8* tetraacetate gave *8*.

$LiAlH_4$ reduction of *8* tetraacetate (6 mg) in ether gave the allylic alcohol *9*, purified by chromatography on a cellulose column, $R_F = 0.64$ on kieselguhr paper (50 % acetone in petroleum ether), electronic spectrum as for *6* above. *m/e* 772 (M), M − 16, M − 44, M − 92 (P), M − 106 (Q), M − 148 (P′), M − 162 (Q′), M − 180, M − 106 − 92, M − 214 (T′), 268, 254, 224, 214 (see Fig. 7). The pentaacetate of *9* was prepared (5 mg); $R_F = 0.32$ on kieselguhr paper (10 % acetone in petroleum ether); τ ($CDCl_3$) 8.78 (*gem.* Me), 8.39 and 8.32 (isopropylidene Me), 8.19 (end-of-chain Me), 8.03, 8.00 and 7.97 (in-chain Me and acetate Me), and ring proton and olefinic proton signals corresponding to *8* tetraacetate. Due to lipid contaminants, the CH_3-CHOAc doublet could not be identified.

20-Acetonyllycopenal (*10*) = Product 1,[5] total yield *ca.* 0.5 mg. Partition ratio in petroleum ether/95 % methanol was 88 : 12, and in petroleum ether/85 % methanol 98 : 2. *m/e* 590.450 (M, calc. for $C_{43}H_{58}O$ 590.449), M − 43, M − 69, M − 106 (Q), M − 137, 430.360 calc. for $C_{32}H_{46}$ 430.360, 378.329 calc. for $C_{28}H_{42}$ 378.329, 309.222 calc. for $C_{22}H_{29}O$ 309.222, 297.222 calc. for $C_{21}H_{29}O$ 297.222, 223.149 calc. for $C_{17}H_{20}$ 223.149 (see Fig. 8).

1-Methoxy-1,2-dihydro-3,4-didehydro-20-acetonyllycopenal (*12*) = Product 1B,[5] total yield *ca.* 50 μg; λ_{max} 490 nm (broad) in acetone; *m/e* 620, M − 32, M − 106 (Q), M − 146 (P′), M − 160 (Q′), M − 212 (T′), 73 (100 %).

Reduction of *12* (10 μg) with $LiAlH_4$ in ether gave the corresponding allylic alcohol with λ_{max} in acetone (465), 485 and 518 nm.

1,1′-Dihydroxy-1,2,1′,2′-tetrahydrolycopene (*14*) and *rhodopinol* (*15*). The mixture of stereoisomerized *14* and *15*, eluted from the alumina column, was acetylated and separated on TLC (kieselgel G). The tertiary diol *14*, yield *ca.* 0.7 mg, could not be separated chromatographically from an authentic specimen. The mass spectra of *14* and *15* acetate were recorded, and agreed well with those published for the genuine compounds.[10,4]

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