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Bacterial Carotenoids

XLII* New Keto-carotenoids from *Rhodopseudomonas globiformis* (Rhodospirillaceae**)

KARIN SCHMIDT and SYNNØVE LIAAEN-JENSEN

Institute of Microbiology, GSF, 34 Göttingen, Germany and Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, N 7034 Trondheim, Norway

Under normal growth conditions the photosynthetic purple non-sulphur bacterium *R. globiformis* produces the four aliphatic, methoxylated keto-carotenoids *1*, *2*, *3*, and *4*; *1*, *2*, and *3* are new compounds.

From cells grown in the presence of diphenylamine the new keto-carotenoids *5*, *6*, and *7* were also isolated.

The keto-carotenoids *1* – *7* all carry the keto groups in the 4(4′)-positions. Their structures were established by means of spectroscopic methods (electronic and mass spectra; for *2*, *3*, and *4* also PMR spectra) and chemical reactions.

Biogenetic considerations suggest that the pathway of carotenoid biosynthesis in *R. globiformis* is common to that of okenone (*25*) synthesis, except that cyclization and aromatization does not occur; no cyclic carotenoids have yet been encountered in photosynthetic purple non-sulphur bacteria.

The carotenoids of the photosynthetic purple nonsulphur bacteria belonging to the family Athiorhodaceae, recently renamed Rhodospirillaceae,[1] have been extensively studied, *e.g.* Refs. 2 – 7. Aliphatic carotenoids with C_{40}-skeletons carrying tertiary methoxy or hydroxy groups in the 1,1′-positions are characteristic of this family. Conjugated keto groups in the 2,2′-positions are encountered in many carotenoids of the genus *Rhodopseudomonas*. Cross-conjugated carotenals of the rhodopinal type and glycosidic carotenoids have recently been isolated from some Rhodospirillaceae spp.[6,7]

We now report the carotenoid composition of *Rhodopseudomonas globiformis*, recently isolated by Pfennig.[8] The structures of six new keto-carotenoids, related to the carotenoids previously isolated from other Rhodospirillaceae spp., have been elucidated.

* Previous paper. *Acta Chem. Scand.* **27** (1973) 2321.

** Old nomenclature: Athiorhodaceae.

RESULTS AND DISCUSSION

Under normal, anaerobic growth conditions *R. globiformis* synthesizes four red keto-carotenoids, *1*, *2*, *3*, and *4*.

In the presence of diphenylamine (DPA), a common inhibitor of carotenoid synthesis,[9] three more saturated keto-carotenoids *5*, *6*, and *7* are also produced, see Table 1.

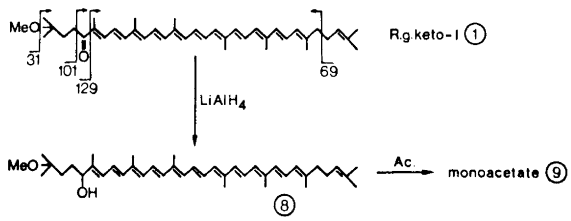
Table 1. Carotenoid composition of *Rhodopseudomonas globiformis* grown without or with 10^{-5} M DPA in the medium.

Carotenoid	% of total carotenoid −DPA	% of total carotenoid +DPA
Unsymmetrical ξ-carotene (*23*)	2.5	18
Neurosporene (*24*)		
Keto-I (*1*)	0.5	7
Keto-II (*2*)	80	18
Keto-III (*3*)	12	7
Keto-IV (*4*)	5	11
Keto-V (*5*)	0	12
Keto-VI (*6*)	0	22
Keto-VII (*7*)	0	5

Since no alkali-labile functions are present in *1* – *7* there is no need of avoiding the saponification step in future isolations.

The experimental evidence for the structures assigned to these compounds referred to as *R.g.* keto-I (*1*, 1-methoxy-1,2-dihydro-ψ,ψ-caroten-4-one [10]), *R.g.* keto-II (*2*, 1,1′-dimethoxy-1,2,1′,2′-tetrahydro-3′,4′-didehydro-ψ,ψ-caroten-4-one), *R.g.* keto-III (*3*, 1,1′-dimethoxy-1,2,1′,2′-tetrahydro-ψ,ψ-caroten-4,4′-dione), *R.g.* keto-IV (*4*, 1-methoxy-1′-hydroxy-1,2,1′,2′-tetrahydro-ψ,ψ-caroten-4-one), *R.g.* keto-V (*5*, 1-methoxy-1,2,7′,8′,11′,12′-hexahydro-ψ,ψ-caroten-4-one), *R.g.* keto-VI (*6*, 1-methoxy-1,2,7′,8′-tetrahydro-ψ,ψ-caroten-4-one), and *R.g.* keto-VII (*7*, 1-methoxy-1′-hydroxy-1,2,7′,8′-tetrahydro-ψ,ψ-caroten-4-one), will now be discussed.

R.g. keto-I (*1*) was available in small quantity. The degree of fine-structure in the electronic spectrum in hexane *contra* ethanol solution (Fig. 1) indicated the presence of a conjugated carbonyl function.[11] The mass spectrum showed diagnostically important fragment ions at M − 31, M − 101, and M − 129 characteristic of the aliphatic okenone and group end M − 69 and *m/e* 69 ions typical of the lycopene end group,[11] see Scheme 1. The common M − 92, M − 106, and M − 158 fragment ions caused by elimination from the polyene chain [11] was observed in the mass spectrum of *1* and in all other mass spectra studied here and will be omitted from further discussion. *R.g.* keto-I (*1*) could not be acetylated. Complex metal hydride reduction caused a hypsochromic shift of the electronic spectrum. The reduction product *8* exhibited visible light absorption typical of an aliphatic undecaene (Table 3, Fig. 1) and gave a mono-acetate on acetylation, confirming that *1* is a conjugated mono-ketone.



Scheme 1.

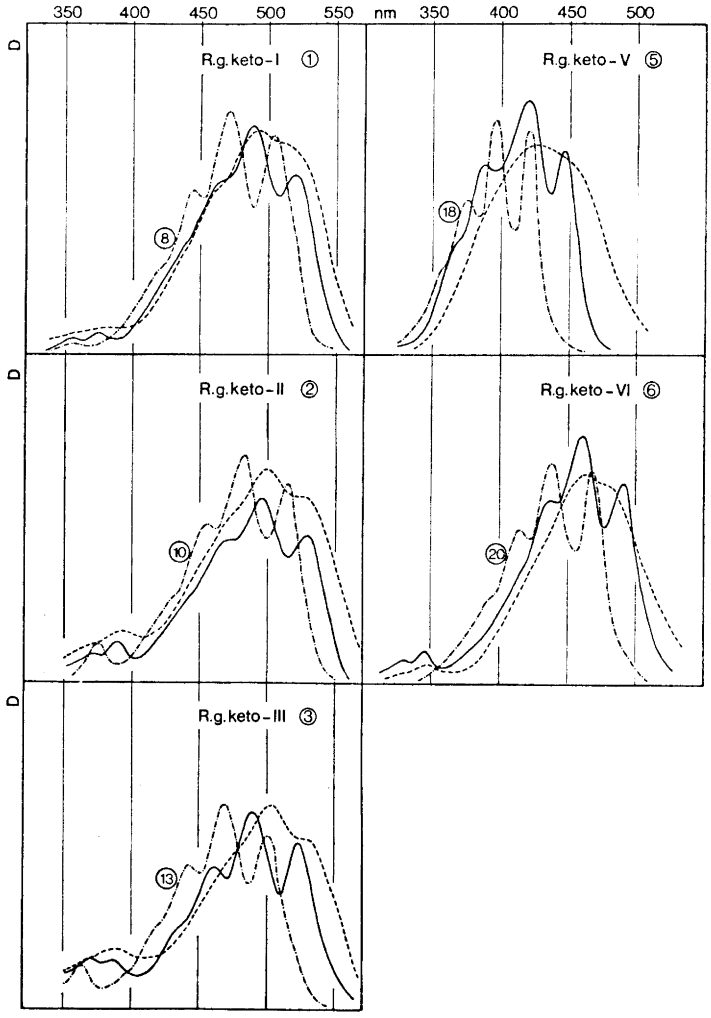


Fig. 1. Electronic spectra of *R. globiformis* keto-I (*1*), keto-II (*2*), keto-III (*3*), keto-V (*5*), and keto-VI (*6*) in petroleum ether ——— and - - - - ethanol. —.— Hydride reduced derivatives in petroleum ether.

R.g. keto-II (*2*) was the major carotenoid of *R. globiformis* grown under normal conditions. The electronic spectrum again showed the solvent effects typical of a conjugated ketone (Fig. 1).

On electron impact the molecular ion was observed at *m/e* 612 (consistent with $C_{40}H_{54}O(OCH_3)_2$) with diagnostically important fragment ions at M − 31, M − 87, and M − 101 (see Scheme 2). The PMR-spectrum (Fig. 2, including

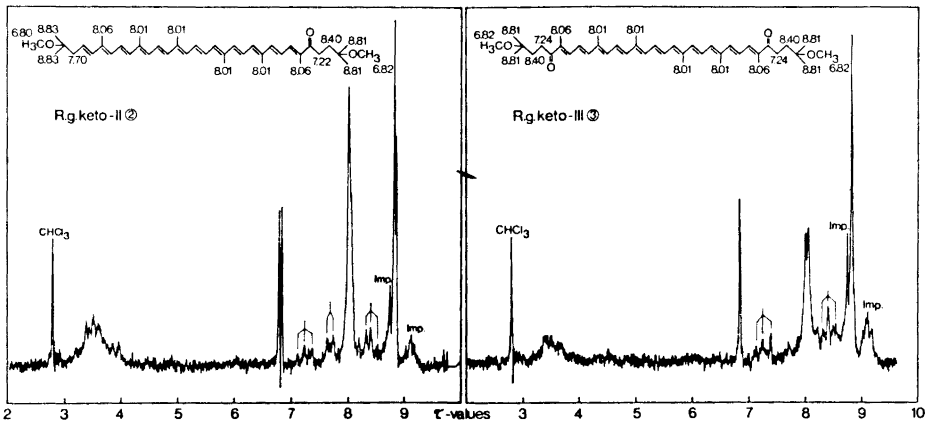
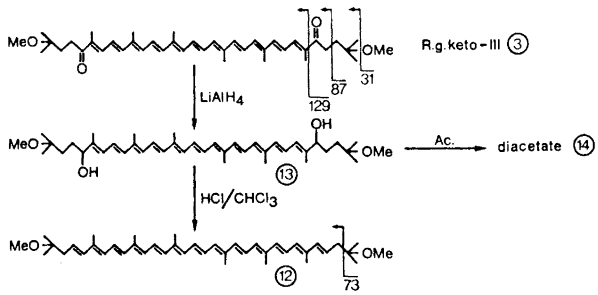


Fig. 2. PMR-spectra ($CDCl_3$) of *R. globiformis* keto-II (*2*) and *R. globiformis* keto-III (*3*).

signal assignments) was consistent with structure *2*, demonstrating the presence of two methoxy groups (τ 6.80 and τ 6.82) in magnetically non-equivalent environments. Structure *2* was confirmed by hydride reduction to *10* with an aliphatic dodecaene chromophore (Table 3, Fig. 1). Product *10* provided a monoacetate (*11*) on acetylation. On allylic dehydration with acidified chloroform [12] the reduction product *10* gave a product *12* with adsorptive properties, electronic and mass spectra indistinguishable from those of authentic spirilloxanthin (*12*).



Scheme 2.

Table 2. Adsorptive properties of the carotenoids from *Rhodopseudomonas globiformis* and their derivatives.

Carotenoid	R_F-values										Required eluent from Al_2O_3 (activity grade 2)
	Kieselguhr paper				Alumina paper					Kieselgel G	
	2 %[a]	5 %	10 %	20 %	2 %[a]	5 %	10 %	20 %	30 %	10 %[a]	
Keto-I (*1*)		0.45				0.50	0.58			0.24	2 %[a]
Keto-II (*2*)		0.19	0.50				0.50			0.17	5–8 %
Keto-III (*3*)			0.35	0.76			0.38			0.09	10 %
Keto-IV (*4*)			0.33	0.74			0.19	0.51		0.04	15 %
Keto-V (*5*)		0.88			0.53	0.74				0.32	4–5 %
Keto-VI (*6*)			0.76			0.60	0.78			0.27	5–7 %
Keto-VII (*7*)			0.40	0.76			0.38	0.72		0.06	15–20 %
8 = reduced *1*							0.25				
9 = *1* monoacetate							0.75				
10 = reduced *2*								0.52			
11 = *10* monoacetate							0.61				
12 = spirilloxanthin		0.40					0.64				
13 = reduced *3*								0.18	0.50		
14 = *13* diacetate			0.82								
15 = reduced *4*									0.41		
16 = *15* monoacetate			0.66								
17 = *4* TMS ether		0.80									
18 = reduced *5*		0.59									
19 = 11,12-dihydro-spheroidene						0.73					
20 = reduced *6*		0.39									
21 = spheroidene	0.76										
22 = reduced *7*								0.37			

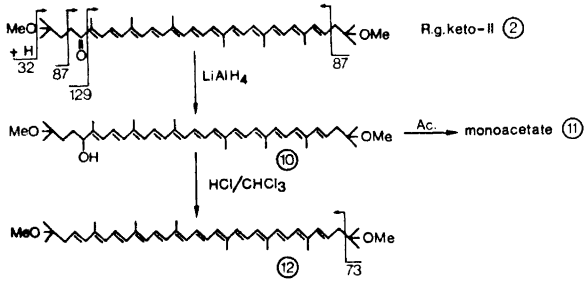
[a] Acetone in petroleum ether.

Table 3. Absorption maxima in visible light in hexane solution of the keto-carotenoids from *Rhodopseudomonas globiformis* and their reduction products with lithium aluminium hydride.

Carotenoid		λ_{max} in nm											
		Native carotenoid					After $LiAlH_4$-reduction						Polyene chain
Keto-I	(*1*)	(360)	375	(462)	488	520			448	472	503	(*8*)[a]	Undecaene
Keto-II	(*2*)	(368)	387	(470)	494.5	527	(357)	374	(456)	482.5	513.5	(*10*)	Dodecaene
Keto-III	(*3*)	(370)	387	461	489	522.5	(345)	360	444	469	500.5	(*13*)	Undecaene
Keto-IV	(*4*)	(360)	377	(463)	487	519			446	470	501	(*15*)	Undecaene
Keto-V	(*5*)			398 5	420	446			374	395	419	(*18*)	Heptaene
Keto-VI	(*6*)	(330)	345	(436.5)	460.5	490			414.5	438	467.5	(*20*)	Nonaene
Keto-VII	(*7*)			(438)	460	491			416	439	467	(*22*)	Nonaene

[a] Formula number.

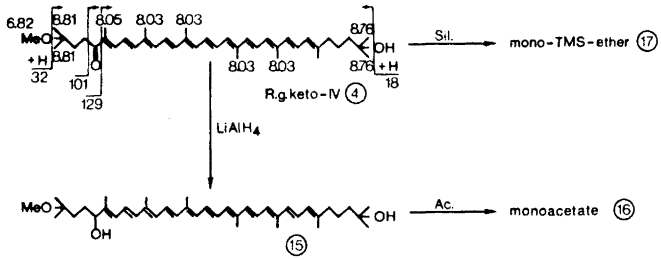
R.g. keto-III (*3*) was less abundant than *2*, but crystallized in bluish needles of m.p. 179°C, forming aggregates from petroleum ether/ether. As seen from Fig. 1 the electronic spectrum showed the typical solvent effects of conjugated carbonyl compounds. The molecular ion on electron impact occurred at *m/e* 628 (consistent with $C_{40}H_{54}O_2(OCH_3)_2$). Fragment ions at M − 31, M − 87, and M − 129 (Scheme 3) characterized the end groups. The PMR spectrum (Fig. 2, including signal assignments) revealed a symmetrical molecule with two identical tertiary methoxy groups (τ 6.80). Structure *3* was confirmed by hydride reduction to *13* with an aliphatic undecaene chromophore (Table 3, Fig. 1), and which could be converted back to keto-III (*3*) by allylic oxidation with *p*-chloranil.[13] The reduction product (*13*) had chromatographic properties (Table 2) indicative of a diol, and acetylation resulted in a diacetate (*14*). Allylic dehydration[12] of *13* gave spirilloxanthin (*12*), identified by co-chromatography tests, electronic and mass spectra.



Scheme 3.

R.g. keto-IV was a minor carotenoid of cells grown under normal conditions. The electronic spectrum (Fig. 1) exhibited the solvent effects characteristic of carotenoid ketones. Hydride reduction gave a product *15* with an aliphatic undecaene chromophore (Table 3, Fig. 1) which provided a monoacetate *16* on acetylation.

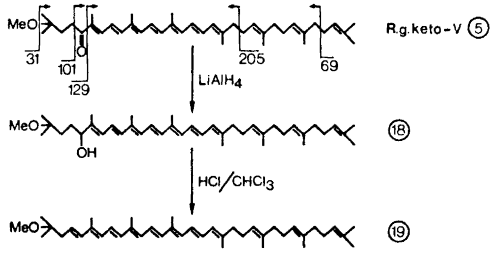
Since *R.g.* keto-IV gave a mono(trimethylsilyl)ether (*17*, judged by R_F-value and mass spectrum), it may be inferred that keto-IV is a mono-ketone with one tertiary hydroxy group. The mass spectrum of keto-IV exhibited



Scheme 4.

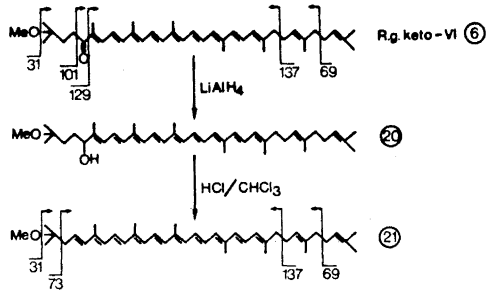
the molecular ion at *m/e* 600 (consistent with $C_{40}H_{56}O(OH)OCH_3$) and fragment ions at M − 18, M − 31, M − 101, and M − 129 defining the end groups (Scheme 4). From these data and the PMR-signals given in Scheme 4, structure *4* is inferred for *R.g.* keto-IV. The same structure has recently been suggested for a minor carotenoid isolated from *Thiothece gelatinosa*, *Thiothece*-OH-484.[14] Co-chromatography tests confirmed their identity.

R.g. keto-V (*5*), isolated from DPA-inhibited cells, is a heptaen-one judged by the electronic spectra of *5* before and after hydride reduction to *18* (Fig. 1, Table 3). The mass-spectrometric fragmentation of *R.g.* keto-V (Scheme 5) was consistent with structure *5*: *m/e* 586 = M (corresponding to $C_{40}H_{59}O(OCH_3)$), M − 69 and M − 205 fragment ions defining the hydrocarbon end group and M − 31, M − 101, and M − 129 ions defining the oxygenated end group. On allylic dehydration with acidified chloroform the hydride reduction product *18* gave a conjugated octaene product, tentatively identified as 11,12-dihydrospheroidene (*19* [5]).



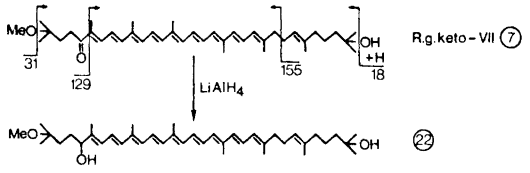
Scheme 5.

R.g. keto-VI (*6*) was the major carotenoid in DPA-grown cells. The spectral characteristics in visible light before and after hydride reduction to *20* (Fig. 1, Table 3) and allylic dehydration of *20* to spheroidene (*21*, identified by co-chromatography tests, electronic and mass spectra) together with the mass spectrum of keto-VI (*m/e* 584 = M, corresponding to $C_{40}H_{57}O(OCH_3)$; M − 31, M − 69, M − 101, M − 129, and M − 137) defined structure *6* for keto-VI, Scheme 6.



Scheme 6.

Finally *R.g.* keto-VII, isolated in minute amounts, was assigned structure *7* on the basis of its electronic spectrum which was identical with that of *R.g.* keto-VI (*6*), mass spectrum (*m/e* 602 = M, corresponding to $C_{40}H_{58}O(OH)OCH_3$; M − 18, M − 31, M − 129, and M − 137 − 18, Scheme 7) and chromatographic properties (Table 2); *R.g.* keto-VII being more polar than *R.g.* keto-VI (*6*).



Scheme 7.

Hydride reduction of *R.g.* keto-VII (*7*) gave a reduction product (*22*) with the same electronic spectrum as *20* above (Fig. 1), but more polar (Table 2).

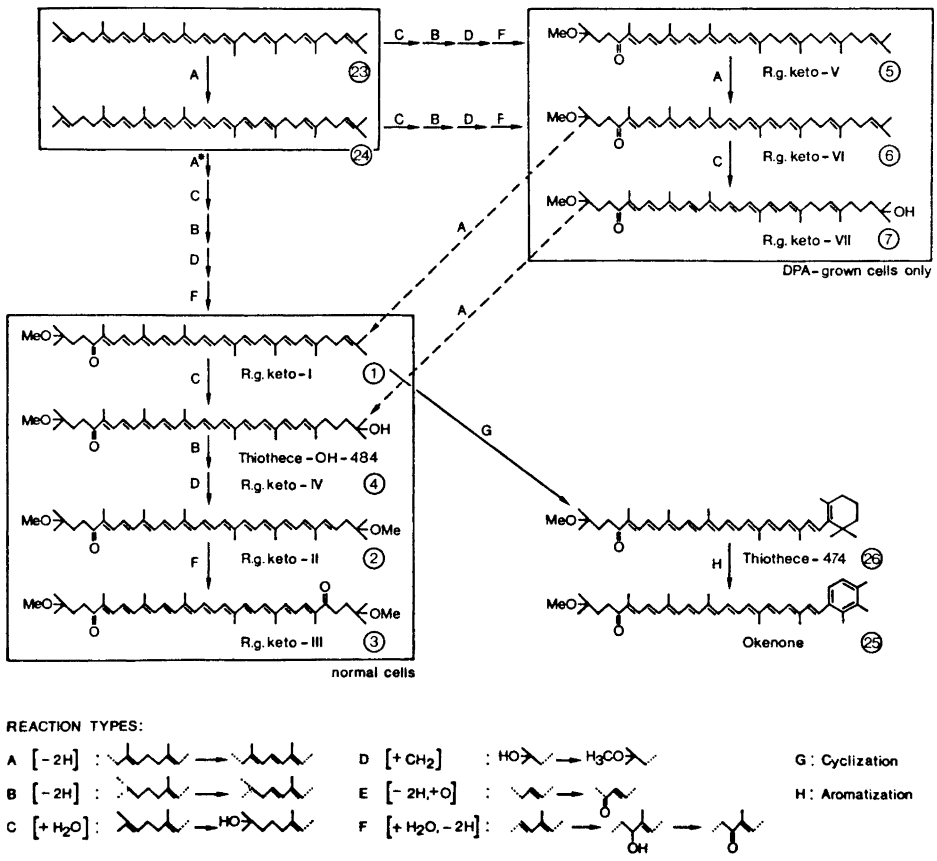
BIOSYNTHETIC CONSIDERATIONS

Since carotenoid biosynthesis normally proceeds towards products of higher dehydrogenation level and DPA is known to inhibit the dehydrogenation steps of the Porter-Lincoln series [15,16] (A, Scheme 8), consideration of the carotenoid composition of cells grown without and in the presence of DPA and the chemical structures of the carotenoids involved permits the postulation of a plausible pathway of carotenoid biosynthesis in *R. globiformis*, Scheme 8.

Starting with unsymmetrical ξ-carotene (*23*) and neurosporene (*24*), present in normal cells (Table 1) and common precursors of carotenoid biosynthesis in photosynthetic bacteria,[16b] the keto-carotenoids typical of normal cells may be formed by the reaction steps A − D (Scheme 8) discussed previously [17,5] and introduction of a carbonyl group. In spheroidenone synthesis the oxygen of the carbonyl groups is derived from molecular oxygen [18] (reaction type E); under anaerobic condition reaction type F *via* alkene, hydrated alkene (secondary alcohol) to ketone may represent a more plausible alternative.

Regarding the position of the more saturated keto-carotenoids *5*, *6*, and *7* isolated only from cells grown in the presence of DPA, these may represent normal precursors of *1*, *2*, *3*, and *4*, or alternatively, abnormal products caused by the enzymatic reactions C, D and B, F when the dehydrogenation reaction A is depressed. Previous work with *Rhodospirillum rubrum* [16] has demonstrated that DPA blocks most efficiently the dehydrogenation step (A*) leading from neurosporene (*24*) to lycopene.

The possible connection between the pathway of carotenoid synthesis in *R. globiformis* and okenone (*25*) synthesis is pointed out. The methoxylated 4-keto-carotenoid okenone (*25*) with one end group in common with *1* − *7* and one aryl end group [19] is synthesized by several purple sulphur bacteria (Thio-



Scheme 8.

rhodaceae spp., now Chromatiaceae [1])[20], possibly *via Thiothece*-474 (*26*).[14] However, *R. globiformis*, like all other Rhodospirillaceae hitherto studied, lack the ability to cyclize its carotenoids.

EXPERIMENTAL PART

Materials. Rhodopseudomonas globiformis strain 7950, SMG 161, obtained from Prof. Pfennig's collection (Göttingen institute), was grown anaerobically in the following medium:[21] KH_2PO_4 (0.5 g); $MgSO_4.7H_2O$ (0.4 g); NaCl (0.4 g); NH_4Cl (0.4 g); $CaCl_2.2H_2O$ (0.05 g); Difco yeast extract (0.5 g); mannitol (1.5 g); Na-gluconate (0.5 g); trace element solution [22] without Fe^{2+} and EDTA (10 ml); Fe-citrate solution containing 100 mg Fe-citrate/100 ml H_2O (5.0 ml); Biotine solution containing 1 mg biotine/50 ml H_2O (1.0 ml); *p*-aminobenzoic acid solution containing 5 mg *p*-aminobenzoic acid/50 ml H_2O (1.0 ml) in 1000 ml dist. H_2O (pH 4.9). After sterilization 1 ml/100 ml medium of a sterile, filtered 10 % Na-thiosulfate solution was added. For inhibition experiments DPA in a concentration of 10^{-5} M was added to the medium. Cells were grown in 500 ml screw cap bottles

at about 2000 Lux at 25 to 28°C. Cells were harvested for extraction by centrifugation after 5–8 days.

Chemicals and solvents were of analytical grade or freshly distilled.

Methods. These were as generally used in the Norwegian laboratory.[23] Hydride reduction, acetylation and silylation,[24] allylic oxidation,[25] and allylic elimination [26] were carried out by standard procedures.

Isolation of the carotenoids. The centrifuged cell pellet was extracted with acetone and the pigments transferred to ether; yield *ca.* 7 μg carotenoid/mg protein from normal cells (in total available *ca.* 45 mg carotenoids). From DPA-inhibited cells in total *ca.* 23 mg carotenoids were available.

The pigments mixture was separated by column chromatography on Woelm neutral alumina, activity grade 2. Further purification was obtained by rechromatography on alumina columns or TLC (Kieselgel G). Adsorptive properties of the carotenoids studied are compiled in Table 2. Absorption maxima in visible light are compiled in Table 3. The carotenoid composition of normal and DPA-grown cells is given in Table 1.

R.g. keto-I (*1*; 1-methoxy-1,2-dihydro-ψ,ψ-caroten-4-one). *Characterization: 1*, available *ca.* 0.2 mg, had: R_F-values Table 2; λ_{max} Table 3, Fig. 1; *m/e* 582 (M), M−31, M−69, M−92, M−101, M−106, M−129, M−158, M−172, 69. *1* could not be acetylated with acetic anhydride in pyridine. *Reduction product (8). 1*, reduced with KBH_4 in ethanol or with $LiAlH_4$ in dry ether, provided *8*: R_F-values Table 2; λ_{max} Table 3, Fig. 1. *Acetate (9). 8* on standard acetylation gave *9* with unchanged electronic spectrum; R_F-value Table 2.

R.g. keto-II (*2*, 1,1′-dimethoxy-1,2,1′,2′-tetrahydro-3′,4′-didehydro-ψ,ψ-caroten-4-one). *Characterization: 2* was precipitated together with colourless contaminants from acetone-petroleum ether, yield *ca.* 37 mg: R_F-values Table 2; λ_{max} Table 3, Fig. 1; τ ($CDCl_3$) Fig. 2 with signal assignments; *m/e* 612 (M), M−31, M−32, M−73, M−89, M−92, M−101, M−106, M−158. *Reduction product (10). 2*, reduced with KBH_4 in ethanol or with $LiAlH_4$ in dry ether, provided *10*; R_F-values Table 2; λ_{max} Table 3, Fig. 1. *Acetate (11). 10* on standard acetylation gave *11* with unchanged electronic spectrum; R_F-value Table 2. *Spirilloxanthin (12). 10* kept in 0.03 N HCl in $CHCl_3$ for 4 min gave *12*. R_F-value Table 2, no separation from authentic spirilloxanthin; λ_{max} (acetone) 460, 491, 524 nm; *m/e* 596 (M), M−73, M−87, M−92, M−106.

R.g. keto-III (*3*; 1,1′-dimethoxy-1,2,1′,2′-tetrahydro-ψ,ψ-caroten-4,4′-dione). *Characterization: 3* crystallized as bluish, shiny needles forming aggregates from acetone/petroleum ether; yield *ca.* 6 mg; m.p. 179°C; R_F-values Table 2; λ_{max} Table 3, Fig. 1 (*E* 1 %, 1 cm = 2180 in petroleum ether at 489 nm); τ ($CDCl_3$) Fig. 2 including signal assignments; *m/e* 628 (M) M−31, M−32, M−92, M−106, M−129, M−158. *Reduction product 13. 3* was reduced with KBH_4 in ethanol or with $LiAlH_4$ in dry ether to *13*; R_F-values Table 2; λ_{max} Table 3, Fig. 1. *13* was oxidized with *p*-chloranil for 3 h, resulting in a 30 % conversion to *3*. *Diacetate 14.* Standard acetylation of *13*, monitored by circular paper chromatography gave an intermediary monoacetate and a final diacetate *14* with unchanged electronic spectrum; R_F-value Table 2. *Spirilloxanthin (12). 3*, treated with HCl−$CHCl_3$ like *2* above, gave spirilloxanthin (*12*) identified by the same criteria as after dehydration of *10*.

R.g. keto-IV (*4*; 1-methoxy-1′-hydroxy-1,2,1′,2′-tetrahydro-ψ,ψ-caroten-4-one). *Characterization. 4*, available *ca.* 2 mg, had: R_F-values Table 2; λ_{max} Table 3, Fig. 1 (as for *1*); τ ($CDCl_3$) 8.82 (two *gem.* CH_3), 8.77 (two *gem.* CH_3 at *tert.* OH), 8.18 (one end-of-chain CH_3), *ca.* 8.03 (*ca.* four in-chain CH_3), 6.82 (one OCH_3), see Scheme 4; *m/e* 600 (M), M−18, M−31, M−32, M−32−18, M−92, M−101, M−106, M−129, M−129−18, M−158. *Reduction product 15. 4* was reduced in ethanol with KBH_4 or in dry ether with $LiAlH_4$ to *15*; R_F-values Table 2; λ_{max} Table 3, Fig. 1. *Acetate 16. 15* gave on acetylation, monitored by circular paper chromatography, a monoacetate *16* with unchanged electronic spectrum; R_F-value Table 2. *Trimethylsilyl ether 17. 15* gave on standard silylation at −35°C a monoether *17* with unchanged electronic spectrum; R_F-value Table 2; *m/e* 672 (M), M−15, M−32, M−72, M−90, M−92, M−101, M−106, 131.

R.g. keto-V (*5*; 1-methoxy-1,2,7′,8′,11′,12′-hexahydro-ψ,ψ-caroten-4-one). *Characterization. 5*, available *ca.* 2.5 mg, had: R_F-value Table 2; λ_{max} Table 3, Fig. 1; *m/e* 586 (M), M−31, M−69, M−92, M−101, M−106, M−129, M−106−69, M−205, 69. *Reduction product 18. 5* gave on KBH_4-reduction in ethanol or on $LiAlH_4$-reduction in dry ether *18*; R_F-value Table 2, λ_{max} Table 3, Fig. 1. *Dehydration product 19. 18*

was treated with 0.03 N HCl – $CHCl_3$ for 2 min giving *19*; R_F-value Table 2; λ_{max} 393, 416, and 442 nm in acetone.

R.g. keto-VI (*6*; 1-methoxy-1,2,7′,8′-tetrahydro-ψ,ψ-caroten-4-one). *Characterization: 6*, available *ca.* 6.5 mg, had: R_F-value Table 2; λ_{max} Table 3, Fig. 1; *m/e* 584 (M), M – 31, M – 32, M – 69, M – 92, M – 101, M – 106, M – 129, M – 137, M – 158, M – 32 – 137, 69 (base peak). *Reduction product 20. 6* was reduced with KBH_4 in ethanol or with $LiAlH_4$ in dry ether to *20*, R_F-value Table 2; λ_{max} Table 3, Fig. 1. *Spheroidene (21)*, *20* was dehydrated in 0.03 N HCl – $CHCl_3$ for 3 min providing *21*, R_F-value Table 2; λ_{max} (acetone) 432, 454 and 484 nm; *m/e* 568 (M), M – 31, M – 73, M – 92, M – 106, M – 137, M – 158. *21* could not be chromatographically separated from authentic spheroidene (*21*).

R.g. keto-VII (*7*; 1-methoxy-1′-hydroxy-1,2,1′,2′,7′,8′-hexahydro-ψ,ψ-caroten-4-one). *Characterization: 7*, available *ca.* 1 mg, had: R_F-value Table 2; λ_{max} Table 3 and Fig. 1 (as for *6*); *m/e* 602 (M), M – 18, M – 32, M – 50 (M – 32 – 18), M – 92, M – 106, M – 126, M – 129, M – 155 (M – 137 – 18), M – 137 – 18 – 32. *Reduction product 22. 7* gave on reduction with $LiAlH_4$ in dry ether *22*; R_F-value Table 2, λ_{max} Table 3 and Fig. 1 (as for *20*).

Unsymmetrical ξ-carotene (*23*; 7,8,11,12-tetrahydro-ψ,ψ-carotene). *Characterization: 23*, available *ca.* 2 mg, had: R_F-value = 0.78 on alumina paper (2 % acetone in petroleum ether); λ_{max} (petroleum ether) 374.5, 395, and 419 nm; *m/e* 540 (M), M – 69, M – 92, M – 106, M – 137, M – 205, 69 (base peak).

Neurosporene (*24*). *Characterization: 24*, available *ca.* 2 mg, had: R_F-value = 0.70 on alumina paper (2 % acetone in petroleum ether); λ_{max} (petroleum ether) 415.5, 438.5, and 468 nm.

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