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Bacterial Carotenoids

XXI.* Isolation and Synthesis of 3,4,3′,4′-Tetrahydro-spirilloxanthin

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From a photolithotropic bacterium (RG3), III (3,4,3′,4′-tetrahydro-spirilloxanthin) was isolated as the major carotenoid. Its structure was indicated by physical evidence and proved by direct comparison with a synthetic sample.

The synthesis was achieved by a somewhat modified route to that previously followed by Surmatis and Ofner,[1] by a Wittig condensation of (7-methoxy-3,7-dimethyl-2-octen-1-yl) triphenylphosphonium bromide (XII), obtained from 6-methyl-hept-5-en-2-one (IV), and crocetindial (XIV).

A photolithotropic bacterium, provisionally called RG3, was recently isolated from enrichment cultures in infrared light by Eimhjellen.[2] The organism was found by him to contain bacteriochlorophyll *b*.[3] The carotenoids of photosynthetic bacteria have been extensively studied,[4–6] and it was hoped that knowledge of the carotenoids produced by RG3 would provide information useful for the classification of this bacterium. Taxonomic aspects will be discussed separately by Eimhjellen;[2] see also Ref. 7.

RESULTS AND DISCUSSION

Isolation and identification of 3, 4, 3′, 4′-tetrahydro-spirilloxanthin (III)

The present photosynthetic bacterium produced two carotenoids, neither of which had previously been identified. Studies on the minor pigment (P500) will be reported in a separate paper.

The main carotenoid (96 % of total) was crystallized from acetone-petroleum ether; yield 19 mg, m.p. 175°C. The electronic spectrum (see Fig. 1) was indistinguishable from that of lycopene (I). However, the present pigment

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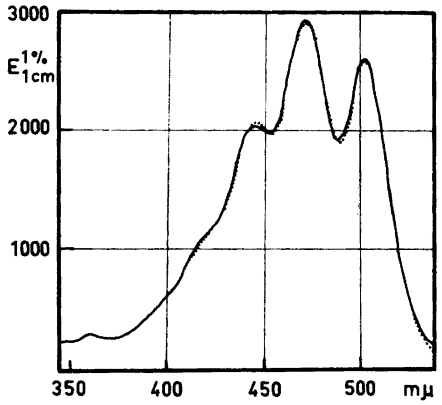


Fig. 1. Absorption spectrum in visible light of 3,4,3′,4′-tetrahydro-spirilloxanthin (III) measured in acetone solution; —— natural sample, ····· synthetic sample.

was more strongly adsorbed than lycopene (I), but less strongly than rhodopin (II). Also, the epiphasic partition behaviour (see Table 1) supported the absence of hydroxyl functions. Judging by its alkali-stability, no ester groups were

Table 1. Identity criteria for natural and synthetic 3,4,3′,4′-tetrahydro-spirilloxanthin (III).

Property	Natural sample	Synthetic sample
M.p. °C	175	174
Partition ratio petroleum ether/95 % methanol	92:8	91:9
R_F-value:* neo U	0.20 (trace) **	0.20 (trace) **
trans	0.45 (51)	0.45 (51)
neo A	0.56 (24)	0.56 (24)
neo B	0.71 (25)	0.71 (25)
Abs.max. in mμ in acetone		
trans	447 473 503	447 473 503
neo A	360 440 467 498	360 440 467 498
neo B	344 360 466 496	344 360 466 495
$E^{1\%}_{1cm}$ at 473 mμ in acetone (*trans*)	2930	2940

* Schleicher & Schüll No. 287 paper; 2 % acetone in petroleum ether.
** Per cent of total carotenoid in iodine-catalyzed equilibrium mixture.

present. The infrared spectrum (see Fig. 2) exhibited strong absorption at 1080 cm^{-1}, indicating the presence of methoxyl groups.[8] This was confirmed by the NMR-spectrum. The latter spectrum (see Fig. 3, including assignments of methyl and methylene groups) together with the other evidence indicated structure III for the main carotenoid. Structure III (1,1′-dimethoxy-1,2,1′,2′-tetrahydro-lycopene or more conveniently referred to as 3,4,3′,4′-tetrahydro-spirilloxanthin) for this carotenoid was subsequently confirmed by total synthesis.

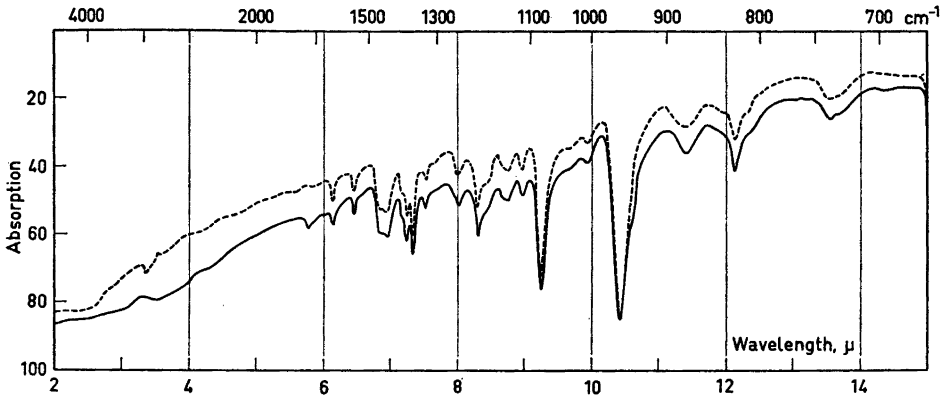


Fig. 2. Infrared spectrum in KBr pellet of 3,4,3′,4′-tetrahydro-spirilloxanthin (III); ——— natural sample, — — — synthetic sample.

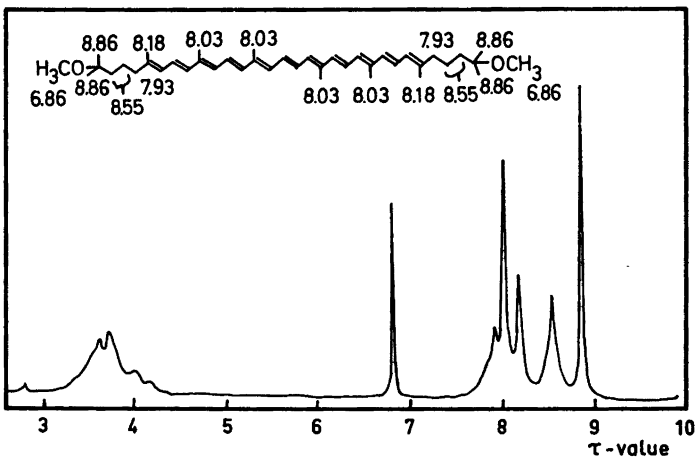
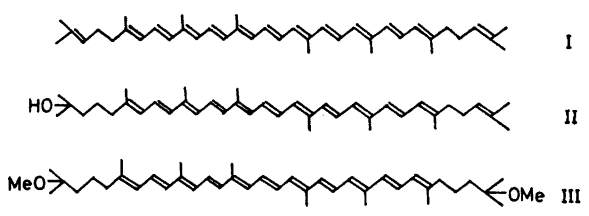


Fig. 3. NMR spectrum (60 Mc/sec) of 3,4,3′,4′-tetrahydrospirilloxanthin (III) in deuterochloroform.



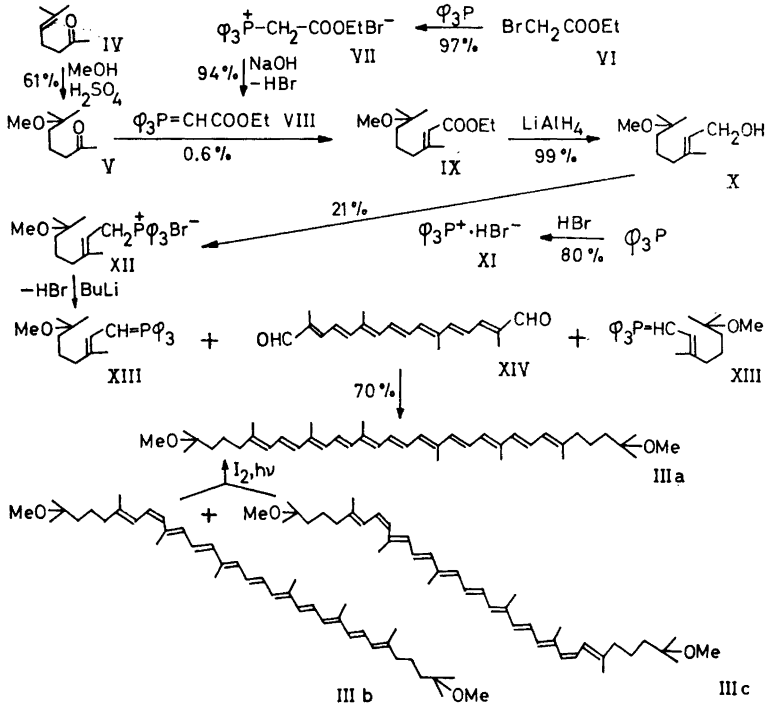
Identity criteria obtained for natural and synthetic III are listed in Table 1 (see also Figs. 1 and 2) and comprised identity in melting point, partition behaviour, adsorptive properties, stereomutation behaviour including relative abundance, R_F-values and spectral shape of the main stereoisomers after iodine-catalysis, electronic spectra including extinction coefficients, infrared spectra, and NMR-spectra.

Synthesis of 3, 4, 3′, 4′-tetrahydro-spirilloxanthin (III)

The synthesis of all-*trans* III was achieved by the route outlined below. 6-Methoxy-6-methyl-2-heptanone (V) was prepared from 6-methyl-hept-5-en-2-one (IV) by treatment with methanol and sulphuric acid by the method of Surmatis and Ofner.[1]

In a Wittig reaction, V was condensed with carbethoxymethylenetriphenyl phosphorane (VIII), prepared from carbethoxymethylenetriphenylphosphonium bromide (VII) according to Isler *et al.*[9] VII was made from ethyl bromoacetate (VI) and triphenylphosphine according to the procedure of Wittig and Haag.[10]

The resulting ethyl 7-methoxy-3,7-dimethyl-2-octenoate (IX) was obtained in 0.6 % yield only. However, the low yield obtained substantiated the observation by Pommer [11] that ketones show poor reactivity with ester phosphoranes.



A Reformatsky synthesis with V and ethyl bromoacetate to yield ethyl 3-hydroxy-7-methoxy-3,7-dimethyl-octanoate, followed by dehydration with phosphorus oxychloride to IX, according to the second spirilloxanthin synthesis of Surmatis and Ofner,[1] would have been a preferable alternative for this step.

The above product (IX) was reduced by lithium aluminium hydride in quantitative yield to the corresponding alcohol, 7-methoxy-3,7-dimethyl-2-octen-1-ol (X). In the subsequent reaction X was converted to its Wittig salt (XII) in 21 % yield by the general procedure of Surmatis and Ofner.[1] Triphenylphosphonium bromide (XI) was prepared by the general method.

In the final step crocetindial (XIV) was condensed with the ylid (XIII), formed from XII, giving 3,4,3′,4′-tetrahydro-spirilloxanthin (III)[1] in 70 % yield.

The initial product showed lower melting point and reduced fine-structure in its electronic spectrum relative to natural III. Since the purity was increased after iodine catalyzed stereomutation it appears that the final Wittig condensation yielded, in addition to the all-*trans* product (IIIa), the corresponding *cis* products (IIIb and IIIc) also. According to Zechmeister [12] IIIb and IIIc possess sterically hindered *cis* bonds. The observed similar adsorptive properties of III a, b, and c were unexpected. Isler and co-workers [13] have employed iodine-catalyzed stereoisomerization following related Wittig condensations, although this method is not commonly used.

1,1′-Dimethoxy-1,2,1′,2′-tetrahydro-lycopene (III) has previously been obtained as an intermediate in a spirilloxanthin synthesis performed by Surmatis and Ofner.[1] They prepared XII from V with higher yield, according to a different route involving ethynylation of IV to 7-methoxy-3,7-dimethyl-1-octyn-3-ol, followed by partial hydrogenation to 7-methoxy-3,7-dimethyl-1-octen-3-ol and formation of the Wittig salt (XII) with triphenylphosphonium bromide (XI) by simultaneous allylic rearrangement. Their product (III; m.p. 168°C; $E\,{}^{1\,\%}_{1\,cm} = 2990$ at 471 mμ in petroleum ether — extinction coefficients are usually *ca.* 5 % lower in acetone than in petroleum ether) was not submitted to iodine-catalyzed stereoisomerization.

EXPERIMENTAL

Materials, general methods and instruments used have been described in a previous paper.[14] For synthetic purposes all solvents were freshly distilled.

Culture. The organism used, designated RG3, was isolated in pure culture from a local habitat by K. E. Eimhjellen, Biochemistry Department, this University.

Cultural conditions. The cells were cultivated at room temperature in 5—10 l carboys in the medium described by Pfennig,[15] illuminated by tungsten lamps; yield 120 g wet cells or *ca.* 4 g per l culture.

Pigment extraction. The cells were dehydrated with acetone, and the pigments extracted with successive portions of acetone for 4 days at —12°C. The acetone extracts were pooled and concentrated, and the pigments transferred to ether on admixture with aqueous sodium chloride solution; yield 64 mg carotenoids or 0.22 % of the dry, extracted cell residue.

Saponification. Introductory experiments revealed that the carotenoids present were stable towards alkali, and saponification of the crude pigment extract in the usual manner [16]was included in the purification procedure.

Column chromatography on neutral alumina, activity grade 2,[17] revealed the presence of a major carotenoid (III, 96 % of total; required eluent 20 % ether in petroleum ether)

and a minor carotenoid (referred to as P500, 4 % of total). The latter pigment was slightly more strongly adsorbed than III on the alumina column and had an $R_F = 0.32$ on kieselguhr paper [18] (2 % acetone in petroleum ether), *cf.* Table 1.

Natural 3, 4, 3', 4'-tetrahydro-spirilloxanthin (III)

Crystallization from acetone-petroleum ether gave red needles with a metallic sheen; yield 19 mg, m.p. 175°C. Absorption properties in visible light are presented in Table 1 and Fig. 1, the IR-spectrum in Fig. 2, and the NMR-spectrum in Fig. 3. The partition ratio [19] and the properties of the iodine catalyzed equilibrium mixture [7] are given in Table 1.

Synthesis of 3, 4, 3', 4'-tetrahydro-spirilloxanthin (III)

6-Methoxy-6-methyl-2-heptanone (V). The method of Surmatis and Ofner [1] was adopted. Conc. sulphuric acid (200 ml) was added dropwise to chilled (0°C) methanol (1200 ml). 6-Methyl-5-hepten-2-one (IV; 600 g) was added, and the temperature allowed to rise to 20°C. The mixture was stirred for 24 h and poured into a separatory funnel containing water (3 l). The oil layer was collected, and the hypophase extracted with benzene (4 × 300 ml). The combined oil and benzene extracts were washed with water, dried and distilled in a Vigreux column (2 × 40 cm). The yield of V was 460 g (61 %); b.p. 78–79°C (8 mm); $n_D^{26} = 1.4247$ (Surmatis and Ofner [1] obtained 58 % yield; b.p. 94°C (14 mm); $n_D^{25} = 1.4285$). IR-properties 1715 (C=O), 1080 cm^{-1} (OCH_3); τ 8.84 (*ca.* 6 H, *gem.* CH_3), 7,84 (*ca.* 3 H, $CH_3-C=O$) and 6.87 (*ca.* 3 H, OCH_3).

Carbethoxymethyltriphenylphosphonium bromide (VII). The procedure of Wittig and Haag [10] was used. The yield of VII was 635 g (97 %); m.p. 154–155°C (Isler *et al.*[9] state m.p. 158°C).

Carbethoxymethylenetriphenyl phosphorane (VIII). The procedure of Isler *et al.*[9] was followed. The yield of VIII was 485 g (94 %); m.p. 126–127°C, λ_{max} 220 and 267 mμ ($E^{1\%}_{1\,cm} = 102$) in ethanol. Isler *et al.*[9] give m.p. 116–117°C; λ_{max} 222 mμ ($E^{1\%}_{1\,cm} = 865$) and 268 mμ ($E^{1\%}_{1\,cm} = 116$) in ethanol.

Ethyl 7-methoxy-3,7-dimethyl-2-octenoate (IX). The method of Schöllkopf [20] was used. V (220 g, 1.39 mole) and VIII (485 g, 1.39 mole) were dissolved in benzene (2500 ml). The mixture was refluxed for 6 h and kept for 14 h at room temperature. The solvent was removed *in vacuo* and the residue extracted with petroleum ether. The latter solvent was removed and the remaining oil distilled in a Vigreux column (2 × 40 cm). The yield of IX was 1.8 g (0.6 %); b.p. 93°C (1 mm); $n_D^{25} = 1.4500$ (Surmatis and Ofner [1] state b.p. 75°C (0.1 mm); $n_D^{25} = 1.4480$). IR-properties (liq.), 1715, 1217, and 1150 (α, β-unsaturated ester), 1650 (C=C), 1380 and 1360 (*gem.* CH_3, CH_3), 1080 (OCH_3) and 860 cm^{-1} (trisubst. *trans* C=C). V (97 %) was recovered unchanged.

7-Methoxy-3,7-dimethyl-2-octen-1-ol (X). IX (1.8 g) dissolved in dry ether (50 ml), was added slowly to a cold (0°C) suspension of lithium aluminium hydride (2 g) in dry ether (50 ml). The mixture was refluxed for 2 h. Moist ether and aqueous saturated ammonium chloride (20 ml) was added, and the solid matter removed by filtration. The solvent was removed *in vacuo* and the residue dried by azeotropic distillation with benzene. The yield of X was 1.45 g (99 %), $n_D^{24} = 1.4560$; IR-properties 3400 and 1020 (OH), 1665 (C=C), 1380 and 1360 (CH_3, *gem.* CH_3) and 1080 cm^{-1} (OCH_3).

Triphenylphosphonium bromide (XI). The procedure of Surmatis and Ofner [1] was followed. The yield of XI was 83 g (86 %); m.p. 189–195°C (Surmatis and Ofner [1] obtained 80 %; m.p. 202°C).

(7-Methoxy-3,7-dimethyl-2-octen-1-yl) triphenylphosphonium bromide (XII). The procedure used by Surmatis and Ofner [1] for analogous phosphonium bromides was adopted. To X (1.45 g) dissolved in methanol (17 ml) was added XI (2.8 g). The mixture was stirred for 24 h at room temperature under nitrogen. Water (200 ml) was added, and the mixture was extracted with methylene chloride. The solvent was removed *in vacuo*, and the oily residue washed with ether (100 ml). XII precipitated from a mechanically shaken, ethereal mixture and recrystallized from methylene chloride-ether. The yield of XII was 0.84 g (21 %); m.p. 159°C (Surmatis and Ofner [1] give m.p. 168–171°C).

3,4,3',4'-Tetrahydro-spirilloxanthin (III). The procedure used by Cooper, Davis and Weedon [21] for analogous Wittig condensations was followed. To a stirred suspension of finely powdered XII (500 mg, 0.98 mmole) in dry ether (10 ml) was added ethereal butyl lithium (0.4 N; 6 ml). The mixture was stirred for 30 min and crocetindial (XIV, 100 mg, 0.34 mmole) in dry methylene chloride was slowly added. The reaction was followed by periodical paper-chromatographic analysis and interrupted after 9 ½ h (when no XIV was left) by addition of water. The carotenoids were extracted with benzene and chromatographed on a column of deactivated alumina; required eluent for III was 20 % ether in petroleum ether. The yield of crude III was 142 mg (70 %); m.p. 140–160°C. Surmatis and Ofner [1] obtained a 60 % yield. The melting point could not be raised by recrystallization, rechromatography, or hydride reduction followed by rechromatography. Iodine catalyzed stereoisomerization in benzene solution in diffuse daylight for 8 h was then carried out in the usual manner.[16] The properties of *trans* III, isolated after subsequent column chromatography and crystallization from acetone-petroleum ether are listed in Table 1.

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