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Carotenoids and Related Compounds

Part XII.* Syntheses of Chlorobactene, "HO-Chlorobactene", and Rhodopin

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The structures assigned to the bacterial carotenoids mentioned in the title have been confirmed by unambiguous total syntheses. Convenient preparations have been developed for two key intermediates, apo-3-lycopenal and its 1-hydroxy-1,2-dihydro-derivative.

A reinvestigation of the carotenoids of photosynthetic green bacteria by Jensen *et al.*[1] has shown that the two principal pigments, now named chlorobactene and HO-chlorobactene, are not identical with γ-carotene (I) and rubixanthin (3′-hydroxy-γ-carotene) as reported by Goodwin and Land.[2] From the N.M.R. spectrum of chlorobactene Jensen *et al.*[1] deduced that one end of the molecule is the same as those of lycopene, and that the other is a trimethylphenyl group. The visible light absorption properties indicated that both *ortho* positions in the aryl end group are (methyl) substituted, and of the two conceivable structures Jensen *et al.* favoured that shown in (II) since the same 2,3,6-trimethylphenyl end group has previously been encountered in renieratene[3] and isorenieratene[4] (leprotene[5]). The hydroxy carotenoid was not isolated crystalline, but was characterised as a tertiary alcohol. On dehydration with phosphorus oxychloride in pyridine it was shown to give a product with visible light absorption, chromatographic, and stereomutation properties very similar to those of chlorobactene. Structure (III) was therefore proposed for HO-chlorobactene.

A minor pigment from one of the species studied (*Chloropseudomonas ethylicus*) was identified as rhodopin, a carotenoid which has been isolated from a number of photosynthetic purple bacteria.[6] Rhodopin was at one time thought[7] to be identical with lycoxanthin (VI, R = OH) but is now regarded as the tertiary alcohol (V).[8,9]

Dr. S. L. Jensen very kindly kept us informed of the progress of some of these developments, and we undertook synthetic work to provide a final

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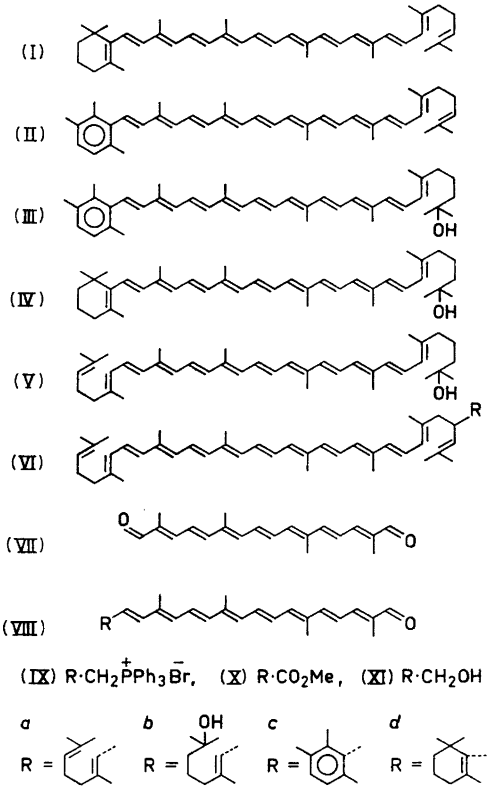
test of the structural conclusions being reached. The routes adopted are based on the $C_{10} + C_{20} + C_{10} = C_{40}$ building principle and are similar to those used recently for renieratene and some other aryl carotenoids possessing 2,3,6- or 2,3,4-trimethylphenyl end groups.[10]

A Wittig reaction of geranyl triphenylphosphonium bromide (IX*a*)[11] with crocetindial (VII)[11] gave a mixture from which apo-3-lycopenal (VIIIa) was isolated in yields up to 70 % depending on the reaction conditions. The structure of the product was confirmed by its light absorption and N.M.R. spectra, and by the formation of lycopene (VI, R = H) on further reaction with the geranyl Wittig reagent. The melting point and visible light absorption maxima of apo-3-lycopenal were in good agreement with those reported by Karrer and Jaffé [12] who obtained the aldehyde by partial oxidation of lycopene with potassium permanganate. A Wittig reaction of triphenyl(2,3,6-trimethylbenzyl) phosphonium bromide (IX*c*)[10] with apo-3-lycopenal then gave (36 %) the hydrocarbon (II) which was shown [1] to be identical with natural chlorobactene. (The introduction of the end groups in the reverse order proved less satisfactory.) The 2,3,4-trimethylphenyl isomer of chlorobactene was prepared similarly.

To synthesise HO-chlorobactene and rhodopin, the phosphonium bromide (IX*b*) was required. A Horner reaction of methyl diethylphosphonoacetate



with 6-hydroxy-6-methylheptan-2-one [13] gave the hydroxy-ester (X*b*) which was reduced with lithium aluminium hydride to the glycol (XI*b*). Partial bromination of the latter, and reaction of the product with triphenylphosphine, yielded (IX*b*). Recently Surmatis and Ofner [14] have shown that this reagent may be conveniently prepared by hydration of geranyl triphenylphosphonium bromide (IX*a*). Reaction of apo-3-lycopenal (VIII*a*) with the Wittig reagent from (IX*b*) gave (42 %) rhodopin (V), identical in all respect with the natural pigment.

Reaction of the hydroxy Wittig reagent from (IX*b*) with crocetindial (VII) gave (83 %) the hydroxy aldehyde (VIII*b*) which exhibited the expected spectral properties. It reacted with the geranyl Wittig reagent to give (45 %) rhodopin (V), and with the aryl reagent from (IX*c*) to give (47 %) the hydroxypolyene (III) which was identified [1] with natural HO-chlorobactene.

At one stage it was considered that HO-chlorobactene might be (IV), with one end group the same as those in β-carotene. This structure was therefore synthesised (40 % yield) by reaction of the Wittig reagent from (IX*b*) with β-apo-2-carotenal (VIII*d*).[15] The product readily separated from HO-chlorobactene (both natural and synthetic) in mixed chromatograms.[1]

EXPERIMENTAL

All reactions were carried out in an atmosphere of nitrogen. Melting points were determined on samples in evacuated capillary tubes, and are corrected.

Alumina (Peter Spence, Type H) for chromatography was pretreated as described by Cheeseman, Heilbron, Jones, and Weedon,[16] and graded by the method of Brockmann and Schodder.[17] Unless stated otherwise, light petroleum refers to the fraction with b.p. 40–60°.

N.M.R. spectra were run on deuterochloroform solutions on a Varian A60 spectrometer using tetramethylsilane as an internal reference; only bands due to methyl or aldehydic protons are quoted.

Apo-3-lycopenal (VIIIa). A slight excess of 0.35 M ethereal butyl lithium was added to a shaken suspension of geranyl triphenylphosphonium bromide [11] (100 mg) in ether (15 ml). Methylene chloride (0.5 ml) was added to destroy the excess of butyl lithium, and this solution was added slowly to a solution of crocetindial [11] (200 mg) in methylene chloride (10 ml) and ether (20 ml). After it had been stirred for a further 30 min, the mixture was poured into water (400 ml). The product was isolated with ether, and chromatographed on alumina (Grade IV)[17] with benzene. Three main bands were observed; the first (most readily eluted) did not separate from lycopene in mixed thin-layer chromatograms on alumina and kieselgel G, and the third consisted of crocetindial. Elution of the second band, evaporation of the solution, and crystallisation of the residue from ether gave apo-3-lycopenal (60 mg, 30 %) as purple needles, m.p. 139–140°; λ_{max} (C_6H_6) 488 mμ (inflexion at 517 mμ), $\varepsilon \times 10^{-3} = 116$; ν_{max} ($CHCl_3$) 1660 (conjug. CH=O), 1610 (conjug. C=C), and 965 cm^{-1} (*trans* CH:CH); τ 8.39, 8.32 (Me_2C:CH.), 8.19 (end-of-chain Me), 8.12 (Me on C adjacent to CHO), 8.02 (in-chain Me), 0.54 (CHO), relative intensities *ca.* 3:3:3:3:9:1. (Karrer and Jaffé [12] give m.p. 138°; λ_{max} 518 and 487 mμ). Reaction of a small amount of the aldehyde with the geranyl Wittig reagent gave a product which was identified as lycopene by its light absorption maxima and a mixed thin-layer chromatogram with an authentic specimen.

(b) A solution of sodium methoxide (from sodium 0.1 g) in methanol (20 ml) was added during 20 min to one of geranyl triphenylphosphonium bromide [11] (1.50 g) and crocetindial [11] (1.80 g) in the same solvent (150 ml). The mixture was heated under reflux for 7 h, cooled, and poured into water. Isolation of the product as above gave apo-3-lycopenal (0.90 g, 70 %), m.p. 141–142°. A mixed thin-layer chromatogram with a sample from (a) showed no separation.

Chlorobactene (II). (a) Triphenyl-(2,3,6-trimethylbenzyl) phosphonium bromide [10] (200 mg) in dry ether (20 ml) was treated with ethereal butyl lithium (0.35 M; 1.3 ml). The mixture was stirred for 15 min and then treated with dichloromethane (0.5 ml). Apo-3-lycopenal (100 mg) in dry ether (15 ml) was then added slowly, after which the reaction mixture was stirred at room temperature (1 h) and then refluxed (30 min). The solution was reduced to a small volume and applied in light petroleum to a column of alumina (Grade II). Elution with benzene, and evaporation, yielded crude chlorobactene, as a brick-red solid, m.p. 125—126° (46 mg, 36 %). Further chromatography on alumina (Grade IV), followed by crystallisations from light petroleum-methanol and light petroleum-acetone, gave chlorobactene as dark red crystals, m.p. 155—155.5° (undepressed on admixture with a specimen, m.p. 155—156°, of the natural pigment which was kindly supplied by Dr. S. L. Jensen); λ_{max} (C_6H_6) 508, 476, and 450 mμ; λ_{max} (CS_2) 530, 496, and (inflexion) 470 mμ; λ_{max} (petrol) 491, 461, and 435 mμ, $\varepsilon \times 10^{-3}$ = 143, 161, and 116 respectively; ν_{max} (CS_2) 970, and 807 cm^{-1}; τ 8.40, 8.33, 8.19, 8.04, 7.93, 7.78, and 7.74, relative intensities *ca.* 1:1:1:3:1:2:1, respectively (Found: C 90.1; H 9.95. Calc. for $C_{40}H_{52}$: C 90.15; H 9.85). The product was identified with natural chlorobactene from *Chloropseudomonas ethylicus* by mixed paper chromatograms and stereomutation studies.[1]

(b) A Wittig reaction of triphenyl(2,3,6-trimethylbenzyl)phosphonium bromide with crocetindial gave crude isorenieral in poor yield. A further Wittig reaction with geranyl triphenylphosphonium bromide yielded a product chromatographically indistinguishable from chlorobactene.

γ-Renierapurpurin (isochlorobactene). Substitution of triphenyl-(2,3,4-trimethylbenzyl) phosphonium bromide [10] for the 2,3,6-trimethylbenzyl isomer in the above preparation (a) of chlorobactene, and extension of the reaction time under reflux to 2 h, gave γ-renierapurpurin (60 mg, 83 %), m.p. 160—161°; λ_{max} (C_6H_6) 522, 485, and (inflexion) 460 mμ; λ_{max} (petrol, b.p. 60—80°) 504, 474, and (inflexion) 448 mμ, $\varepsilon_{474} \times 10^{-3}$ = 132; ν_{max} (CS_2) 760, and 700 cm^{-1}; ν_{max} ($CHCl_3$) 1600, and 970 cm^{-1}; τ 8.39, 8.33, 8.19, 8.04, 7.95, 7.80, and 7.72, relative intensities *ca.* 1:1:1:3:1:1:2 (Found: C, 90.05; H, 10.1. $C_{40}H_{52}$ requires: C 90.15; H 9.85).

Methyl 7-hydroxy-3,7-dimethyloct-2-enoate (Xb). Methanolic sodium methoxide (30 %, 9.0 g) was added slowly to a solution of 6-hydroxy-6-methylheptan-2-one (10.0 g)[13] and methyl diethylphosphonoacetate (27.0 g) in dimethylformamide (50 ml) at 0°. The mixture was kept at room temperature overnight, warmed to 35—40° for 1 h, then cooled, neutralised with glacial acetic acid, and poured into water. Isolation of the product with ether gave the hydroxy-ester (10.5 g, 76 %), b.p. 126—134°/2 × 10^{-5} mm; gas-liquid chromatography, and N.M.R. (τ 8.79, 8.15 (J = 2 cps), 7.85 (J = 2 cps) and 6.34), indicated that the product was a mixture (*ca.* 1:2) of *cis* and *trans* isomers. A small portion was purified for analysis by chromatography on fluorescein alumina from light petroleum and had b.p. 134° (bath temp.)/3 × 10^{-6} mm., n_D^{20} 1.4702; ν_{max} (liquid film) 3430, 1712, 1645, and 1154 cm^{-1}. (Found: C 65.95; H 9.8 .$C_{11}H_{20}O_3$ requires: C 65.95; H 10.05).

Triphenyl-(7-hydroxy-3,7-dimethyloct-2-enyl) phosphonium bromide (IXb). The preceding ester (5.0 g) in ether (20 ml) was added during 1 h to a stirred and cooled (−30°) suspension of lithium aluminium hydride (1.0 g) in ether (50 ml). After the mixture had been stirred at −30° for 2 h, wet ether (10 ml), and then saturated aqueous ammonium chloride (5.5 ml) were added. The mixture was allowed to warm to 20°, the ethereal layer was separated, dried ($MgSO_4$), and evaporated, giving crude 3,7-dimethyloct-2-ene-1,7-diol (2.5 g, 72 %) as a viscous oil; ν_{max} (liquid film) 3350, 1392, 1155, and 1005 cm^{-1}.

Phosphorus tribromide (3.15 g) in ether (50 ml) was added slowly (2 h) to a cooled (−15°) solution of the crude diol (2.0 g) and pyridine (1 drop) in ether (50 ml). The mixture was stirred at room temperature for 30 min, then shaken thoroughly with ice-cold 0.05 N sulphuric acid, dried, and evaporated. The resulting crude bromo-alcohol (1.4 g, 52 %) in benzene (5 ml) was added to triphenylphosphine (2.0 g) in benzene (10 ml), and the mixture was kept at room temperature for 2 days. The solid which had been deposited was then collected, washed with benzene and with light petroleum, and crystallised from methanol-ethyl acetate to give the phosphonium bromide (2.62 g, 89 %), m.p. 193—194°, undepressed on admixture with a specimen prepared according to the method of Surmatis and Ofner [14] who give m.p. 194°.

1-Hydroxy-1,2-dihydro-apo-3-lycopenal (VIIIb). A solution of sodium methoxide (from sodium 0.2 g) in methanol (5 ml) was added to a solution of the above phosphonium bromide (1.0 g) and crocetindial [11] (700 mg) in methanol (100 ml). The mixture was stirred

under reflux for 6 h, and then overnight at room temperature. A solid (crocetindial and 1,1′-dihydroxy-1,2,1′,2′-tetrahydrolycopene) which had separated was then filtered off, and the filtrate was poured into water (1 l). Isolation of the product with ether, chromatography on alumina (Grade IV) using 0.1 % methanol in benzene as the eluant, collection of the main band, evaporation, and crystallisation of the residue from light petroleum-ether, gave brick red plates, m.p. 156–157°. Repeated crystallisation from the same solvent, and finally from benzene-light petroleum, gave the hydroxy-aldehyde (0.75 g, 83 %), m.p. 167–168°; λ_{max} (C_6H_6) 489 mμ (inflexion at 516 mμ), $\varepsilon \times 10^{-3} = 144$; ν_{max} (Nujol) 3360, 1668, 1602, 1150, and 970 cm^{-1}; τ 8.79 ($Me_2C{<}$), 8.19, 8.15, 8.02, and 0.53, relative intensities *ca.* 6:3:3:9:1, respectively. (Found: C 83.05; H 9.6. $C_{30}H_{42}O_2$ requires: C 82.9; H 9.75).

Samples of the phosphonium bromide prepared by the method described above, and by that of Surmatis and Ofner,[14] yielded the same product.

Rhodopin (V). (a) From apo-3-lycopenal. A solution of sodium methoxide (from sodium (20 mg) in methanol (3.5 ml)) was added to one of apo-3-lycopenal (100 mg) and triphenyl-(7-hydroxy-3,7-dimethyloct-2-enyl)-phosphonium bromide (200 mg) in methanol (50 ml). The mixture was stirred under reflux for 12 h, cooled, and poured into water (400 ml). Isolation of the product with ether, and chromatography on alumina (Grade IV) using benzene as eluant, gave lycopene (*ca.* 5 %), apo-3-lycopenal, and rhodopin (55 mg, 42 %) which crystallised from benzene-light petroleum as purple-red needles, m.p. 182–183°; λ_{max} (petrol) 503, 473, and 445 mμ, $\varepsilon_{473} \times 10^{-3} = 164.5$; ν_{max} ($CHCl_3$) 3600, 1627, and 970 cm^{-1}; τ 8.79, 8.39, 8.33, 8.19, and 8.03, relative intensities *ca.* 2:1:1:2:4, respectively.

The synthetic product was identified with natural rhodopin from *Rhodospirillum rubrum* and *Rhodomicrobium vannielii* by direct comparison (visible light absorption data in light petroleum; infra-red light absorption data in KBr disc; co-chromatography on circular paper, Schleicher and Schüll No. 287 (kieselguhr) paper and No. 288 (Al_2O_3) paper; and mixed m.p.).

(b) From 1-hydroxy-1,2-dihydro-apo-3-lycopenal. A slight excess of ethereal butyl lithium was added to a suspension of geranyl triphenylphosphonium bromide (200 mg) in ether (10 ml). Methylene chloride (0.5 ml) was added, followed by the hydroxy-aldehyde (100 mg) in methylene chloride (5 ml). The mixture was stirred for 15 min and then poured into water (300 ml). Isolation of the product with ether, chromatography from benzene on alumina (Grade IV), and crystallisation from benzene-light petroleum gave rhodopin (61 mg, 45 %), m.p. 180–181°, undepressed on admixture with a sample from (a). Mixed thin-layer chromatograms of samples from (a) and (b) revealed no separation.

HO-Chlorobactene (III). A slight excess of ethereal butyl lithium was added to a suspension of triphenyl-(2,5,6-trimethylbenzyl) phosphonium bromide[10] (0.5 g) in ether (100 ml). The mixture was stirred for 20 min and then methylene chloride (0.5 ml) was added, followed by 1-hydroxy-1,2-dihydro-apo-3-lycopenal (0.4 g) in ether (20 ml) over a period of 30 min. The mixture was stirred for 30 min, and then poured into water (3 l). Isolation of the product with ether, chromatography on alumina (Grade IV) from benzene, collection of the main band and evaporation, yielded a red solid (*ca.* 45 %) which gave only one spot on thin-layer chromatography (Merck aluminium oxide G. acc. Stahl; 15 % acetone in light petroleum); λ_{max} (petrol) 491, 461, and (inflexion) 435 mμ; τ 8.79, 8.19, 8.03, 7.93, 7.76, and 7.74, relative intensities *ca.* 2:1:3:1:1:2, respectively. Crystallisation from aqueous acetone, then from acetone-light petroleum (b.p. 30–40°) at −70°, and finally from acetone at −30°, gave HO-chlorobactene, m.p. 131–132°; λ_{max} (petrol) 491, 461, and (inflexion) 435 mμ, $\varepsilon \times 10^{-3} = 115$, 135, and 100, respectively; λ_{max} (benzene) 495, 467, and (inflexion) 446 mμ; ν_{max} ($CHCl_3$) 3610, 1635 and 980 cm^{-1}; ν_{max} (CS_2) 3630, 970, and 810 cm^{-1}.

The product readily underwent partial dehydration to chlorobactene (identified by mixed thin-layer chromatography with an authentic specimen) and partial oxidation to isorenieral. Elementary analyses gave erratic results.

Circular paper chromatography of the synthetic material (amorphous) and natural HO-chlorobactene from *Chlorobium limicola* established their identity.[1]

1-Hydroxy-1,2-dihydro-γ-carotene (IV). A solution of β-apo-2-carotenal[15] (0.5 g), triphenyl-(7-hydroxy-3,7-dimethyloct-2-enyl)-phosphonium bromide (1.0 g), and excess

sodium methoxide in methanol (30 ml) was boiled under reflux for 15 h, then cooled and poured into water (2 l). Isolation of the product with ether, and chromatography on alumina (Grade IV) from benzene gave γ-carotene (trace) and the hydroxy-carotene which crystallised from ether-light petroleum and acetone-light petroleum and had m.p. 178–178.5°; λ_{max} (petrol) 494, 462, and 444 mμ, $\varepsilon \times 10^{-3}$ = 143, 156, and 128, respectively; ν_{max} (CS_2) 3600, 970 cm^{-1}; τ 8.96, 8.79, 8.29, 8.20, and 8.03, relative intensities 2:2:1:1:4, respectively.

The product separated readily from HO-chlorobactene in mixed chromatograms.

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