Bacterial Carotenoids. XLIII. * C₅₀-Carotenoids. 13.**

Synthesis of (2R,2'R)-2,2'-Dimethyl-β,β-carotene and Absolute Configuration of (2R,2'R)-2-(4-Hydroxy-3-hydroxymethyl-2-butenyl)-2'-(3-methyl-2-butenyl)-β,β-carotene

ARTHUR G. ANDREWES,* SYNNØVE LIAAEN-JENSEN* and GUNNER BORCHb

* Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway and b Chemistry Department A, The Technical University of Denmark, DK-2800 Lyngby, Denmark

This first assignment of absolute stereochemistry to a C₅₀-carotenoid, C.p. 450 = (2R,2'R)-2-(4-hydroxy-3-hydroxymethyl-2-butenyl)-2'-(3-methyl-2-butenyl)-β,β-carotene (5), followed from CD-correlation with natural (2R,2'R)-β,β-carotene-2,2'-diol (4) and (2R,2'R)-2,2'-dimethyl-β,β-carotene (12) synthesized here.

All fourteen ¹-⁴ known naturally occurring C₄₀- and C₅₀-carotenoids are distinguished by having additional C₄-isopentenyl units formally added to the 2- or 2,2'-positions on a C₄₀-skeleton; exemplified by bacterioruberin (I), bisanhydrobacterioruberin (2), and decaprenoaxanthin (3), Scheme 1. Provided enzymic control, this addition induces optical activity at C-2 (2'), indicated by asterisks in Scheme 1. From CD correlation bacterioruberin (I) and bisanhydrobacterioruberin (2) are known to possess the same absolute configurations.² Chiroptical properties of decaprenoaxanthin (3) have also been reported.⁴ However, despite rapid advances in the stereochemistry of C₄₀-carotenoids, no absolute configurations of C₄₀- or C₅₀-carotenoids have hitherto been reported.

Recent reports on the absolute stereochemistry of (+)-(R)-β-iron (7, Scheme 2)⁷ and tentative stereochemical assignment to (2R,2'R)-β,β-carotene-2,2'-diol (4)⁸ prompted us to investigate the stereochemistry of some C₅₀-caroteno- noids; in particular that of 2-(4-hydroxy-3-hydroxymethyl-2-butenyl)-2'-(3-methyl-2-butenyl)-β,β-carotene (5; C.p. 450 ⁹), Scheme 1. (2R,2'R)-β,β-Carotene-2,2'-diol (4) and C.p. 450 (5, stereochemistry hitherto unknown) have common chromophores and differ only in the nature of the 2-substituent. Each have termini which may be regarded as 4-substituted cyclohexenes (see structure 6, Scheme 1). From Mills' rule,¹⁰ the optical rotation of cyclohexene systems with an asymmetric carbon at position 4 is caused by a preferred conformation of the cyclohexene ring itself and the nature of the substituent is not relevant. Application of Mills' rule may lead to erroneous results,¹¹ although its validity in the carotenoid series for the stereochemical correlation of (2R,2'R)-β,β-carotene-2,2'-diol (4) and zeaxanthin (3R,3'R)-β,β-carotene-3,3'-diol has recently been confirmed.¹² As a further model for CD-correlation (2R,2'R)-2,2'-dimethyl-β,β-carotene (12) was synthesized from (+)-(R)-β-iron (7). Optically inactive 12 has previously been synthesized by Eugster et al.¹³ by a different route.

RESULTS AND DISCUSSION

(+)-(6R)-β-Iron (7) was isolated by preparative GLC from a base-isomerized mixture of natural irones.¹⁷ The synthesis of (2R,2'R)-2,2'-dimethyl-β,β-carotene (12) followed the route
shown in Scheme 2. Treatment of 7 with ethyl diethylphosphonoacetate gave (R)-β-ironyldiene acetate (8). Reduction with LiAlH₄ provided the allylic alcohol 9 which was converted to the corresponding phosphonium bromide 10. Condensation of 10 with the trienedial 11 gave (2R,2'R)-2,2'-dimethyl-β,β-carotene (12).

The electronic absorption spectrum of 12 corresponded to that of β-carotene. The ¹H NMR spectrum, including signal assignments, is reproduced in Fig. 1. The upper mass region of the mass spectrum is shown in Fig. 2.

The CD spectra of trans (2R,2'R)-2,2'-dimethyl-β,β-carotene (12), (2R,2'R)-β,β-carotene-2,2'-dil (4) and C.p. 450 (δ, available from a previous investigation ⁴) are shown in Fig. 3. Immediately apparent is the similarity between the curves of 4 and 12, possessing the same absolute stereochemistry, and differing only in the nature of the 2-substituent, hydroxyl versus methyl. This compatibility further confirms that the principle of Mills’ rule is applicable to these carotenoid systems.

The CD spectrum of C.p. 450 (5) is virtually exactly opposite to those of 4 and 12, revealing opposite absolute configuration in 2,2’-positions. C.p. 450 has therefore configuration δ. Formally, C.p. 450 is (2R,2'R)-2-(4-hydroxy-3-hydroxymethyl-2-butenyl)-2’-(3-methyl-2-butenyl)-β,β-carotene.¹³
Scheme 2.

Fig. 1. $^1$H NMR spectrum of (2R,2'R)-2,2'-dimethyl-β,β-carotene (12).
**Fig. 2.** Mass spectrum of \((2R,2'R)-2,2'-\text{dimethyl}\-\beta,\beta\text{-carotene} \,(12)\).

**Fig. 3.** CD-spectra of \((2R,2'R)-2,2'-\text{dimethyl}\-\beta,\beta\text{-carotene} \,(12, \cdots \cdots)\), \((2R,2'R)-\beta,\beta\text{-carotene}-2,2'-\text{diol} \,(4, - - -)\) and \(C.p. \, 450 \,(5, - - -)\) in EPA.

Further studies on the absolute configurations of other \(C_{20}\)-carotenoids are in progress.

**Experimental.**

Material and methods. All solvents were of analytical grade or distilled before use. Comparative TLC of carotenoid intermediates was on silica gel HF\(_{254}\) plates developed with petroleum ether-acetone solutions. Separation and purification of synthetic carotenoids were accomplished with alumina plates (1 mm) developed with petroleum ether-ethyl ether (97:3). Comparative chromatography of carotenoids was on Merck alumina HF\(_{254}\) Type E pre-coated plates (0.2 mm).

Instruments used were as specified elsewhere.\(^{14}\) Specific rotations of carotenoid intermediates were obtained on a Perkin-Elmer 141 polarimeter and CD spectra recorded in EPA (ether-isopentane-ethanol, 5:5:2) on a Roussel-Jouane Dicrographe.

\((+)-(R)-\beta\text{-Irone} \,(7)\) was isolated with an Aerograph Autoprep A-700 chromatograph using a 4.5 mm x 12 mm metal column packed with Carbowax 20M (15%) on Chromosorb W (60 - 80 mesh) at 190°C and 180 ml/min (helium). Carotenoid intermediates \((7, 8, 9)\) were examined for purity with a Perkin-Elmer F-11 gas chromatograph fitted with a 1.8 m glass column packed with OV-17 (5%) on Chromosorb W;

temperature programmed from 110 °C at 2°/min. C.p. 450 (5). It was available from an earlier reported study. The CD spectrum is given in Fig. 3.

Composition of natural Iris oil. GLC analysis of natural Iris oil (Absolute Iris Essences) showed the following iron composition: (--)-(2R,6R)-trans-a-iron (numbering and nomenclature of irones follow that used by Rautenstrauch and Ohloff, cf. 7, Scheme 2), 20.7% °f (total irones); (+)-(2S,6R)-cis-a-iron (cis refers to the 2,6-substituents), 41.8%; (++)-(2S,6R)-cis-g-iron, 37.4%.

Alkali-catalyzed isomerization of Iris oil. Iris oil (3 g) in a MeOH-H₂O (20 ml, 85:15) solution containing 10% KOH was heated at 40 °C under N₂ for 24 h. The ether extractable fraction was concentrated under vacuum and distilled at 0.3 Torr. A yellow oil (2 g) which distilled at 110-130 °C was collected and (+)-(6R)-β-iron (7, 0.55 g) isolated by preparative GLC. Iron composition of oil after isomerization: (++)-(6R)-β-iron (7), 61.6%; (++)-(2S,6R)-cis-a-iron, 8.2%; (--)-(2R,6R)-trans-a-iron, 32.2%. Analytical GLC showed the β-iron (7) collected exceeded 98% purity. The following physical properties for 7 were recorded: [α]²⁰ (EtOH) 589 nm = 16°, 578 nm = 17°, 546 nm = 20° and 436 nm = 36°; λmax 296 nm in MeOH; IR (liq.) 2980, 2920, 2880 (s, C-H), 1600, 1670 (s, C=O), 1605 (s, C=C), 1360 (s), 1250 (s, C=O), 980 cm⁻¹ (m, trans - HC = CH -); τ(CDC₁₈): singl assignments with reference to 7, Scheme 2) 0.958 3d(3 H, J = 5 Hz, CH₂-C₆H₅), 1.970 (8.95 (6 H, gern. dimethyl), 8.8-8.4 (3 H, H-a,b), 8.27 (3 H, =C-CH₃), 8.1-7.8 (2 H, H-c), 7.75 (3 H, O=C-CH₃), 3.94 d(1 H, J = 16 Hz, H-e), 2.77 d(1 H, J = 16 Hz, H-d); m/e 206 (M), 191 (M - 15), 149 (M - 71), 135 (M - 71) and 122 (M - 84).

Ethyl (+)-(6R)-β-ironylidenecacetate (8). Ethyl diethylphosphate (1.7 g) was added to a suspension of NaH (200 mg) in tetrahydrofuran (THF, 6 ml) and stirred at 20 °C for 1 h. The mixture was cooled to 0 °C and 7 (0.85 g) in THF (1 ml) added. The reaction was stirred for 3 h at 0-5 °C, then at 20 °C for an additional 15 h. Water was added and the mixture extracted with ether. The organic layer was dried with Na₂SO₄, concentrated under vacuum and chromatographed on a kieselgel column developed with benzene from which 8 was isolated (1.018 g, 89%). GLC and ¹H NMR analysis showed 8 to consist of 85% trans- and 15% cis-isomers (around the new double bond). The product 8 had: [α]²⁰ (EPA) 589 nm = 33°, 578 nm = 35°, 546 nm = 41°, and 436 nm = 81°; λmax (MeOH) 260, 295 nm; τmax (liq.) 2980, 2920, 2840 (s, C-H), 1710 (s, C=O), 1610 (s, C=C), 1450 (m), 1325 (s, C-O), 1150 (s, C=O), 1050 (s), 870 cm⁻¹ (m, trans - CH = CH -); τ(CDC₁₈): single assignment with reference to 8, Scheme 2) 9.13, 8.97 (2 H, gern. dimethyl), 9.05 (2 H, 2H, J = 5 Hz, -CH₂-C₆H₅), 8.72 t(3 H, J = 7 Hz, CH₂-C₆H₅), 8.7-8.3 (3 H, H-a,b), 8.32 (3 H, H-b) 8.1-7.8 (2 H, CH₂-c), 7.66 (3 H, CH₃-g), 5.77 q (2 H, J = 7 Hz, CH₂-CH₃), 4.35 (1 H, cis-H-f, cf. Ref. 15b), 4.25 (1 H, trans-H-f), 3.97 d(1 H, J = 16 Hz, H-e), 3.40 d(1 H, J = 16 Hz, H-d); m/e 276 (M) 263 (M - 15), 231 (M - 45) and 208 (M - 73).

(+)-(6R)-β-ironylidenec ethanol (9). 8 (0.94 g) in THF (10 ml) was added to a stirred solution of LiAlH₄ (0.2 g) in THF (20 ml) at 0 °C during 30 min. Stirring was continued for 1 h and the reaction monitored by TLC (disappearance of starting material). The complex was decomposed by cautious addition of MeOH followed by a saturated solution of NH₄Cl. After ether extraction 9 (0.84 g, quantitative yield) was isolated: [α]²⁰ (EPA) 589 nm = 30°, 578 nm = 31°, 546 nm = 37°, 436 nm = 69° and 365 nm = 327°; λmax (MeOH) 237, 265 nm; τmax (liq.) 3320 (s, OH), 2960, 2920, 2860 (s, C-H), 1000 (m, OH), 990 cm⁻¹ (s, trans - CH = CH - τ(CDC₁₈): signals assignments with reference to 9, Scheme 2) 9.13, 8.99 (6 H, gern. dimethyl), 9.09 d(3 H, J = ca. 5 Hz, -CH = CH₂), 8.7-8.3 (3 H, H-a,b), 8.32 (2 H, CH₂-C₆H₅), 1.14 (3 H, CH₃-g), 8.25-7.85 (2 H, H-e), 5.17 d(1 H, J = 7 Hz, CH₂-C₆H₅), 4.38 broad t(1 H, J = 6.5 Hz, H-f), 3.95 (2 H, H-d-c); m/e 234 (M) and 219 (M - 15).

(6R)-β-ironylidenec-ethyltriphenyolphosphine bromide (10). A solution of 9 (0.8 g) and triphenyolphosphine bromide (1.5 g) in CHCl₃ (5 ml) was stirred at 20 °C for 48 h under N₂. The solvent was removed under vacuum and the oily residue dissolved in a minimum amount of acetone (5 ml) and chromatographed on a kieselgel column. Elution with acetone removed unreacted 9 and triphenyolphosphate. 10 was eluted with MeOH and obtained as an amorphous powder after removal of solvent. Yield of 10 was 1.74 g (84%).

(2R,3R)-2,3-Dimethyl-β-β-carotene (12). NaOMe (0.6 M) was slowly added to a stirred solution of 10 (200 mg) in dry MeOH (5 ml) until the phosphonate salt was completely converted to the deep red phosphorane. A solution of 2,7-dimethylOcta-2,4,6-trienedial (11, 200 mg, prepared from the acetylenic analogue 16 in CH₂Cl₂ (0.5 ml) was added and the mixture stirred. Progress of the reaction was monitored by TLC and additional phosphorane, generated externally, was added until 11 was completely converted to 12. Water was added, the crude product extracted into ether, concentrated under vacuum and chromatographed on a kieselgel column developed with benzene. Yield of 12 (cis-trans mixture) was 42 mg (34% based on dial 11). 12 was purified on alumina plates from which the all trans-isomer was collected. Crystallized from MeOH-acetone solution 12 melted at 179 °C. Comparative TLC on pre-coated alumina plates developed with petroleum ether-ethyl ether (97:3) showed 12 to have Rᵣₑₐₙ = 0.42; β,β-carotene in this system had Rᵣₑₐₙ = 0.38.

On Schleicher  & Schuell paper No. 286 (Al₂O₃) developed with petroleum ether+ethyl ether (99+1) 12 had Rᵣₑₐₙ = 0.33; β,β-carotene in the
same system had $R_F = 0.20$. I2 had $\lambda_{max}$ (hexane) 265, 423, 449 (E1% | cm) = 2428; 
$\varepsilon = 137,000$ compared with $\varepsilon = 134,000$ for $\beta, \beta$-carotene $^{21}$, 476 nm (E1% | cm) = 2072; $\varepsilon = 117,000$, (acetone) (427), 451, 479 nm; $\tau$(CDCl3) see Fig. 1 with signal assignments; CD (EPA), see Fig. 3: m-d 554 (M), 549 (M - 15), 472 (M - 92), 458 (M - 106), 415 (M - 149), 406 (M - 158), 420 (M - 162), 373 (M - 191), 360 (M - 204), 347 (M - 217) and 282, see Fig. 2.

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