Use of Deuterium Labelling to Elucidate the Stereochemistry of the Initial Step of the Cyclization Reaction in Zeaxanthin Biosynthesis in a *Flavobacterium*

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Summary Cyclization of lycopene *in vivo* in a *Flavobacterium* species, R1519, suspended in deuterium oxide medium gives (2*S*,2′*S*)-[2,2′-$^2H_2$]zeaxanthin, thus demonstrating that the initial proton (deuteron) attack in the cyclization reaction is on the *re*,*re* face of the C-1,2 double bond.

CYCLIZATION is one of the fundamental reactions of carotenoid biosynthesis, and is generally believed to involve proton attack at C-2 of an acyclic precursor, probably lycopene. We now report the use of deuterium labelling to elucidate the stereochemistry of this process.

A *Flavobacterium* species, R1519, when cultured in the presence of nicotine (7·5mM) accumulates lycopene (ψ,ψ-carotene) in place of its normal main pigment (3*R*,3′*R*)-zeaxanthin (β,β-carotene-3,3′-diol). On removal of the inhibitor, the accumulated lycopene is converted into zeaxanthin.[1] In the present experiment *Flavobacterium* R1519 cells were grown in a medium containing nicotine so that they accumulated lycopene, and were then washed to remove the inhibitor and resuspended and incubated in medium prepared with D_2O in place of H_2O. According to the accepted mechanism[2] cyclization of the accumulated lycopene under these conditions should be initiated by D^+ instead of H^+ to give [2,2′-$^2H_2$]zeaxanthin. Mass spectrometric analysis confirmed that the zeaxanthin produced was largely the expected dideuterio species. The location of the deuterium and the absolute configuration at C-2 and C-2′ were established by ^1H n.m.r. spectroscopy.

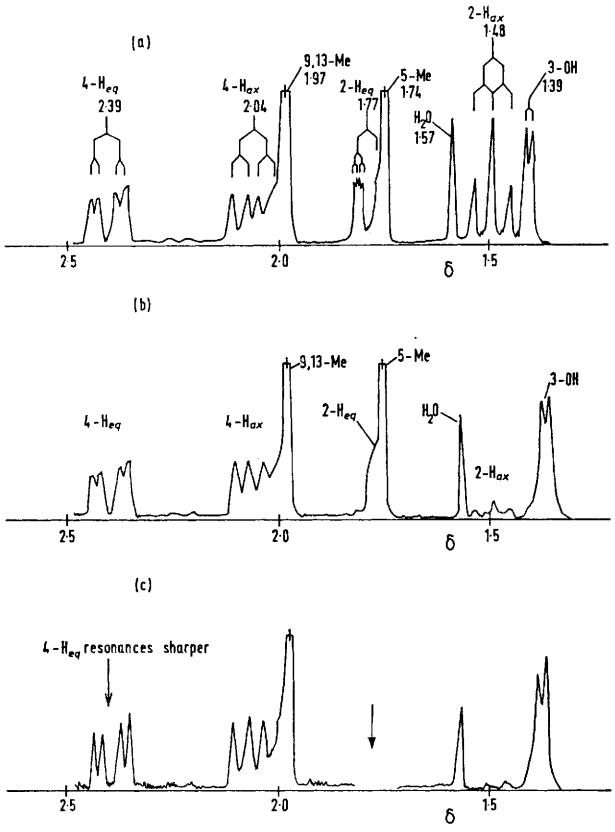
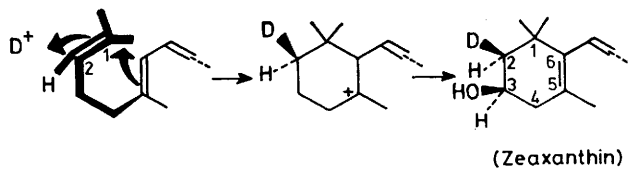


FIGURE. Features of the 270 MHz ^1H n.m.r. spectra of zeaxanthin; (a) synthetic (3*R*,3′*R*)-zeaxanthin; (b) biosynthetic (3*R*,3′*R*)-[2,2′-$^2H_2$]zeaxanthin; (c), as (b) but with double resonance irradiation at δ 1·76 (↓).

^{13}C N.m.r. studies[3] have shown that in (3*R*,3′*R*)-zeaxanthin the 3β- and 3′β-hydroxy-groups occupy the equatorial position, so the 3α- and 3′α-protons are axial. The 2β-proton is therefore quasi-axial and the 2α-proton quasi-equatorial. In the 270 MHz ^1H n.m.r. spectrum of unlabelled, synthetic (3*R*,3′*R*)-zeaxanthin (Figure, a) the quasi-equatorial α-protons at C-2 and C-2′ give a complex absorption at δ 1·77 (d,d,d; *J* 2, 3·5, 12 Hz) whereas the resonance of the quasi-axial β-protons at C-2 and C-2′ appears at δ 1·48 (d,d; *J* 11·5, 12·0 Hz). This assignment is based on the following evidence:[4] (i) in cyclohexane derivatives, axial protons resonate approximately 0·5—1·0 p.p.m. towards higher field than the corresponding equatorial protons, and (ii) coupling between the 2-axial and the 3-axial protons gives a much larger coupling constant (11·5—12·0 Hz) than coupling between the 2-equatorial and 3-axial protons (3·0 Hz).

The spectrum of our deuteriozeaxanthin sample (Figure, b) showed clearly that the signal at δ 1·48 due to the 2-axial protons was almost completely absent, whereas the signal due to the 2-equatorial protons was present as a broad singlet at δ 1·76, largely overlapping with the 6 proton signal of the C-5 and C-5′ methyl groups at δ 1·74. Integration confirmed that this combined signal (δ 1·74—1·76) was due to 8 protons. Further proof that the 2- and 2′-equatorial protons were still present was obtained by irradiation at δ 1·76. Examination of the 4-equatorial proton resonance at δ 2·39 revealed that this irradiation had removed the H-4 equatorial–H-2 equatorial long range W coupling (Figure, c).



SCHEME. Stereochemistry of the cyclization reaction in the biosynthesis of zeaxanthin from lycopene in *Flavobacterium* R1519 resuspended in deuterium oxide medium.

In the deuteriozeaxanthin sample therefore it is clearly the 2β- and 2'β-axial protons that have been replaced by deuterium, and consequently the zeaxanthin sample has the (2*S*,2'*S*) configuration. The stereochemistry of the cyclization reaction is thus as shown in the Scheme: *i.e.* the initial proton (or deuteron) attack must be on the *re,re* face[5] of the C-1,2 double bond of the acyclic precursor, in this case lycopene. It is interesting that the stereochemistry of substitution at C-2 is opposite to that in the C_{50} carotenoids 'C.p. 450' [2-(4-hydroxy-3-hydroxymethylbut-2-enyl)-2'-(3-methylbut-2-enyl)-β,β-carotene][6] and decaprenoxanthin [2,2'-bis(4-hydroxy-3-methylbut-2-enyl)-ϵ,ϵ-carotene][7] but the same as that established[8] for β,β-caroten-2-ol and β,β-carotene-2,2'-diol. There may thus be differences in the stereochemistry of cyclization in the biosynthesis of different types of cyclic carotenoids in different organisms.

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