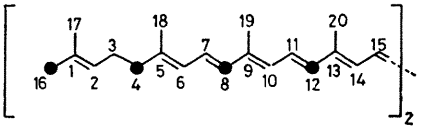
Stereochemistry of Cyclization in Carotenoid Biosynthesis; Use of ^{13}C-Labelling to Elucidate the Stereochemical Behaviour of the C-1 Methyl Substituents during Zeaxanthin Biosynthesis in a *Flavobacterium*

By GEORGE BRITTON,* TREVOR W. GOODWIN, WILLIAM J. S. LOCKLEY, ANN P. MUNDY, and NARENDRA J. PATEL
(*Department of Biochemistry, University of Liverpool, P.O. Box* 147, *Liverpool* L69 3BX)
and GERHARD ENGLERT
(*F. Hoffman–La Roche and Co. Ltd.*, CH-4002, *Basel, Switzerland*)

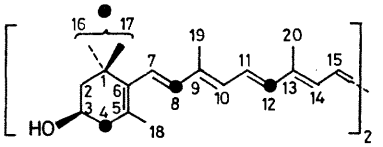
Summary [2-^{13}C]Mevalonate has been incorporated into the carotenoid zeaxanthin and its acyclic precursor, lycopene, in a *Flavobacterium* species; n.m.r. analysis showed ^{13}C enrichment in both compounds at C-4, C-8, C-12, C-16, C-4′, C-8′, C-12′, and C-16′ so that in lycopene the C-1 methyl substituent *trans* to the main carbon chain was enriched and in zeaxanthin the 1α (axial) methyl substituent was labelled, enabling us to define the stereochemistry of the behaviour of the C-1 methyl groups during cyclization.

IN order to define the stereochemistry of the cyclization reaction which gives the β-ring in carotenoid biosynthesis, two features have to be elucidated.[1] The first of these, the stereochemistry of hydrogen attack at C-2 of the acyclic precursor, was recently determined for zeaxanthin (3*R*,3′*R*-β,β-carotene-3,3′-diol) in a *Flavobacterium* species by use of deuterium labelling.[2] We now report the use of ^{13}C-labelling, for the first time in studies of carotenoid biosynthesis, to determine the behaviour of the C-1 methyl substituents during the formation of zeaxanthin in *Flavobacterium*.

In the first of two experiments a *Flavobacterium* preparation was incubated with [2-^{13}C]mevalonate in the presence of nicotine (10 mM). Under these conditions cyclization is inhibited and the acyclic precursor lycopene (ψ,ψ-carotene) accumulates as the main carotenoid in place of the normal zeaxanthin. Examination of the lycopene (**1**) sample by ^{13}C n.m.r. spectroscopy (67·89 MHz) showed that the ^{13}C label (1—2% enrichment at each position) was localized at four positions, C-4 (δ 40·30 p.p.m.), C-8 (135·50), C-12 (137·43), and C-16 (25·67).[3] These are exactly the positions predicted from a consideration of the general biosynthetic pathway. Of particular importance is the fact that the C-16 methyl group, *i.e.* that methyl substituent at C-1 which is *trans* to the main carbon chain of the lycopene molecule, was enriched with ^{13}C, whereas the C-17 methyl group (δ 17·70 p.p.m.), which is *cis* to the main carbon



(1)



(2)

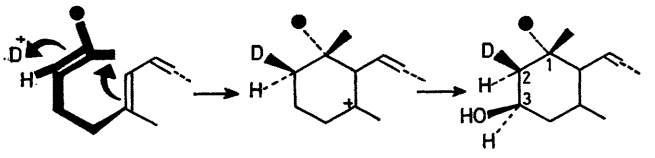
● = ^{13}C

chain was not enriched. This result is significant in that (a) it confirms the isoprenoid labelling pattern in lycopene, (b) it shows that the two C-1 methyl substituents retain their individuality up to the lycopene stage of biosynthesis, and (c) it defines the positions of ^{13}C-labelling in the acyclic precursor which normally undergoes cyclization to give β-carotene and thence zeaxanthin.

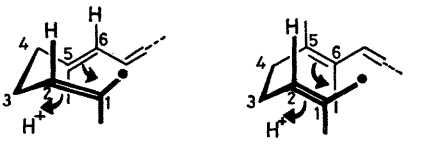
In the second experiment a *Flavobacterium* preparation was incubated with [2-^{13}C]mevalonate with no nicotine present. A ^{13}C enrichment of 3—4% was achieved in the appropriate positions of the zeaxanthin (**2**) isolated. The ^{13}C n.m.r. spectrum showed the ^{13}C label again to be located in the C-4 (δ 42·76 p.p.m.), C-8 (138·56), and C-12 (137·66) positions, and in one of the methyl substituents at C-1 (δ 28·84 p.p.m.). ^{13}C N.m.r.[4] and ^{1}H n.m.r.[2] studies have shown that in (3*R*,3′*R*)-zeaxanthin the 3β-hydroxy-group occupies the equatorial position so that the 3α-proton is axial, the 2β-proton is quasi-axial, and the 2α-proton quasi-equatorial. The β-methyl substituent at C-1 will therefore occupy the equatorial position and the α-methyl substituent will be axial. In the ^{13}C n.m.r. spectrum of

zeaxanthin separate signals are given by the two C-1 methyl substituents, at δ 28·84 and 30·35 p.p.m. In cyclohexane derivatives axial substituents resonate at higher field than the corresponding equatorial substituent.[5] The signal at δ30·35 p.p.m. in the zeaxanthin spectrum can therefore be assigned to the equatorial (1β) substituent and that at δ 28·84 p.p.m. to the axial (1α) methyl group. Studies with lanthanide shift reagents are in full agreement with this conclusion.

The ^{13}C label in the *Flavobacterium* sample is thus located in the axial 1α-methyl substituent. The behaviour of the C-1 methyl substituents during cyclization is therefore as illustrated in the Scheme. The stereochemistry of hydrogen introduction at C-2, previously deduced from deuterium-labelling experiments,[2] is also included. These two results together define the stereochemistry of the reaction at the C-1–C-2 double bond (equivalent to an overall *trans* addition). It is still not possible to distinguish between the alternative chair (**3**) and boat (**4**) foldings, both of which would lead to the same labelling pattern.



SCHEME. Stereochemical course of the cyclization of lycopene to give zeaxanthin in *Flavobacterium*. ● = ^{13}C.



(3) (4)

● = ^{13}C

It is interesting that the labelling pattern in the C-1 methyl groups of zeaxanthin in *Flavobacterium* is opposite to that deduced for trisporic acid and β-carotene in *Blakeslea trispora*.[6]

We thank F. Hoffmann-La Roche and Co. Ltd., for cultures of *Flavobacterium* and for facilities for determining ^{13}C n.m.r. spectra, and the S.R.C. for financial support and a research studentship (A.P.M.)

(*Received*, 13*th September* 1978; *Com.* 993.)

[1] G. Britton, *Pure Appl. Chem.*, 1976, **47**, 223.
[2] G. Britton, W. J. S. Lockley, N. J. Patel, T. W. Goodwin, and G. Englert, *J.C.S. Chem. Comm.*, 1977, 655.
[3] G. Englert, *Helv. Chim. Acta*, 1975, **58**, 2367.
[4] G. P. Moss, *Pure Appl. Chem.*, 1976, **47**, 97.
[5] W. McFarlane, in 'Elucidation of Organic Structures by Physical and Chemical Methods,' Pt. 1, 2nd Ed., eds. K. W. Bentley and G. W. Kirby, Wiley-Interscience, New York, 1972.
[6] J. D. Bu'Lock, D. J. Austin, G. Snatzke, and L. Hruban, *Chem. Comm.*, 1970, 255.