RECENT STUDIES OF CAROTENOID BIOSYNTHESIS
IN BACTERIA

J. C. B. McDermott†, A. Ben-Aziz‡, R. K. Singh, G. Britton
AND T. W. Goodwin

Department of Biochemistry, University of Liverpool, Liverpool L69 3BX

ABSTRACT

The effects of nicotine on carotenoid biosynthesis have been investigated in a non-photosynthetic Flavobacterium strain 0147, and in two photosynthetic bacteria, Rhodopseudomonas spheroides and Rhodomicrobium vannielii.

In Flavobacterium, nicotine inhibits the synthesis of the normal main carotenoid, zeaxanthin, and causes an accumulation of lycopene at high nicotine concentration, and rubixanthin at lower nicotine concentration. On removal of the inhibitor, the accumulated lycopene is converted under anaerobic conditions into β-carotene, which in turn is converted into zeaxanthin in the presence of oxygen. Similar in vivo conversions of rubixanthin into β-cryptoxanthin (anaerobic) and zeaxanthin (aerobic) have been demonstrated.

Two alternative routes from lycopene to zeaxanthin are therefore possible in Flavobacterium, via β-carotene or via rubixanthin.

Nicotine also inhibits production of the normal carotenoids in two photosynthetic bacteria.

In Rhodopseudomonas spheroides, at high nicotine concentrations, the normal main carotenoid spheroidene is replaced by neurosporene, but at low nicotine concentrations the amount of spheroidene is increased, while the hydroxyspheroidene content decreases to zero. This offers support for the postulated pathway neurosporene → spheroidene → hydroxyspheroidene.

In Rhodomicrobium vannielii, formation of the cyclic β-carotene is completely inhibited at low nicotine concentrations, and at higher nicotine concentrations the normal main carotenoid, rhodopin, is greatly decreased, while lycopene becomes the main product.

In addition to inhibiting the cyclization reaction, nicotine thus apparently inhibits the hydration of the terminal (C-1) double bond in the biosynthesis of the characteristic hydroxy- and methoxycarotenoids of photosynthetic bacteria.

In Rhodopseudomonas spheroides, neurosporene appears to be the key intermediate in the formation of other carotenoids, whereas lycopene plays the most important role in Rhodomicrobium vannielii.

† Present address: Department of Botany, University of Liverpool, Liverpool L69 3BX.
‡ Present address: Volcani Institute of Agricultural Research, National and University Institute of Agriculture, Bet Dagan, Israel
INTRODUCTION

Carotenoids of many varied structural types are synthesized by bacteria, but very little work has been done on the biosynthesis of these bacterial carotenoids. In higher plants and fungi, however, much work has been done on biosynthesis, especially of carotenes, and the overall pathway of carotene biosynthesis is reasonably well defined. Actual in vitro conversions of postulated intermediates into the expected products have been achieved with crude cell-free preparations. Most of this work has, however, been limited to studies of the biosynthesis of carotene hydrocarbons, it being assumed that oxygenated derivatives are formed from these at a late stage in the pathway.

The biosynthesis of carotenoids has been the subject of several recent reviews. In the case of bacteria, biosynthetic pathways have often been postulated from a consideration of the structures of the carotenoids present, but with little or no experimental evidence to support the proposals. Most of the work that has been undertaken on carotenoid biosynthesis in bacteria has relied on studies of the effects of the inhibitor diphenylamine on carotenoid composition, but very little work has been done involving the use of isotopic labelling. The fact that many bacteria appear to be reluctant to incorporate mevalonic acid into carotenoids has probably restricted quite considerably the study of the biosynthesis of bacterial carotenoids.

The availability of a Flavobacterium (strain 0147), which accumulates, as essentially the only carotenoid, large amounts of zeaxanthin (I), and which is capable of incorporating mevalonic acid very efficiently into zeaxanthin, has promoted a series of investigations into the biosynthesis of carotenoids, especially zeaxanthin, in this organism, and this work has led to new studies of the biosynthesis of acyclic carotenoids in photosynthetic bacteria.

BIOSYNTHESIS OF ZEAXANTHIN IN FLAVOBACTERIUM 0147

Early work, including determination of o.r.d. curves, established that the zeaxanthin from Flavobacterium strain 0147 had the same absolute configuration as that from higher plant sources, i.e. 3R, 3'R (I). The stereochemistry of hydroxylation was also found to be the same as in higher plants, i.e. the 5-pro-R hydrogen atom from mevalonic acid (MVA) was lost and the 5-pro-S hydrogen atom retained during the hydroxylation (Figure 1). This indicates that hydroxylation takes place with retention of configuration at C-3, suggesting that a mixed-function oxidase type of enzyme may be responsible.
RECENT STUDIES OF CAROTENOYD BIOSYNTHESIS IN BACTERIA

Many questions, however, are not answered by this work, in particular the stage at which hydroxylation takes place—as the final step or early in the biosynthetic sequence. This problem has now been approached by a series of inhibitor studies.

The inhibition of carotenoid biosynthesis by diphenylamine (DPA) is well known. This substance apparently inhibits the desaturation sequence, causing an accumulation of phytoene, and has been widely used in studies of carotenoid biosynthesis in several microorganisms. However, no specific inhibition of zeaxanthin biosynthesis was observed in *Flavobacterium*.

Recently two other compounds, 2-(p-chlorophenylthio)triethylammonium hydrochloride (CPTA) and nicotine have been found to inhibit production of normal carotenoids in several different biological systems. With these compounds, however, it is the formation of cyclic carotenes that is inhibited, and in the organisms studied, lycopene accumulated in place of the normal carotenoid complement. In particular, in *Mycobacterium* species, formation of the normal main pigment, β-carotene (II), was inhibited by nicotine, the β-carotene being replaced almost quantitatively by lycopene (III). Furthermore, after removal of the inhibitor, the cells were able to produce cyclic carotenes, apparently at the expense of the accumulated lycopene.

Figure 1. Stereochemistry of hydroxylation in zeaxanthin biosynthesis

31
The use of an inhibitor of cyclization would clearly be very useful for elucidating details of the biosynthesis of zeaxanthin. The effect of nicotine on zeaxanthin biosynthesis in Flavobacterium 0147 has therefore been studied.

**Effect of nicotine on zeaxanthin biosynthesis in Flavobacterium 0147**

When Flavobacterium strain 0147 was grown in the presence of nicotine (5 mM), the cells produced were red, in contrast to the yellow of normal cultures. It was found that at this nicotine concentration, zeaxanthin was almost completely replaced by lycopene, showing that in this Flavobacterium, as in Mycobacterium species\(^37\), the cyclization reaction is inhibited by nicotine, and suggesting that lycopene is the compound that cyclizes.

![Figure 2. Variation of carotenoid composition in Flavobacterium 0147 with nicotine concentration: ——, zeaxanthin; ————, rubixanthin; ————, lycopene](image)

The effect of different concentrations of nicotine on the carotenoid composition in Flavobacterium 0147 was then investigated. The results are shown in *Figure 2*. This illustrated that at nicotine concentrations above 5 mM, lycopene was the main pigment. Below 2.5 mM nicotine, however, the lycopene content was very small and the zeaxanthin content was greatly decreased. The major pigment at these lower nicotine levels, especially 1.0 mM, was rubixanthin (IV). No hydroxylycopenes were detected at any nicotine concentration.

![Rubixanthin (IV)](image)
RECENT STUDIES OF CAROTENOID BIOSYNTHESIS IN BACTERIA

If nicotine inhibits only cyclization, the finding of lycopene and not hydroxylycopenes suggests that lycopene is the compound that cyclizes in Flavobacterium 0147, and that hydroxylation does not occur before cyclization. The finding of rubixanthin as the main pigment at lower nicotine concentrations is interesting. It was perhaps expected that at low nicotine concentrations, a smaller amount of inhibition would be observed, with a small amount of zeaxanthin being replaced by lycopene. The facts that considerable inhibition occurs, and that rubixanthin replaces zeaxanthin, show that inhibition of the second cyclization is substantially complete (leading to the production of rubixanthin) before any inhibition of the first cyclization (leading to an accumulation of lycopene) occurs.

In cultures of several microorganisms grown in the presence of diphenylamine, removal of the inhibitor allows synthesis of the normal carotenoids to proceed, often at the expense of the accumulated phytoene (V)\textsuperscript{16,19-22,32,38,39}. If the lycopene that accumulates in nicotine-inhibited cultures of Flavobacterium 0147 is an intermediate in zeaxanthin biosynthesis, then removal of the nicotine might allow zeaxanthin to be produced at the expense of the lycopene.

Cultures were therefore grown in the presence of nicotine (7.5 mM). The cells were then washed to remove the inhibitor, and were reincubated in nicotine-free medium. As shown in Table 1, cells reincubated under aerobic conditions had synthesized zeaxanthin, with a concomitant decrease in the accumulated lycopene. The cells reincubated anaerobically, however, had synthesized virtually no zeaxanthin, but the lycopene had been replaced by β-carotene. This indicates that the cyclization of lycopene to β-carotene is an anaerobic process, but the hydroxylation process requires oxygen.

Table 1. Changes in carotenoid composition in resuspended nicotine-grown Flavobacterium 0147 (1). Cells grown 42 h in 7.5 mM nicotine

<table>
<thead>
<tr>
<th></th>
<th>Lycopene (µg)</th>
<th>Lycopene (%)</th>
<th>β-Carotene (µg)</th>
<th>β-Carotene (%)</th>
<th>Zeaxanthin (µg)</th>
<th>Zeaxanthin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Control</td>
<td>466.1</td>
<td>79.5</td>
<td>—</td>
<td>—</td>
<td>17.7</td>
<td>3.0</td>
</tr>
<tr>
<td>Reincubation:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anaerobic 48 h</td>
<td>56.5</td>
<td>8.1</td>
<td>528.0</td>
<td>75.9</td>
<td>22.3</td>
<td>3.2</td>
</tr>
<tr>
<td>(b) Control</td>
<td>309</td>
<td>80.9</td>
<td>—</td>
<td>—</td>
<td>3</td>
<td>0.8</td>
</tr>
<tr>
<td>Reincubation:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aerobic 24 h</td>
<td>36</td>
<td>8.5</td>
<td>18</td>
<td>4.1</td>
<td>336</td>
<td>78.8</td>
</tr>
</tbody>
</table>
To confirm that this does represent a real *in vivo* conversion of lycopene into β-carotene and zeaxanthin and not *de novo* synthesis of β-carotene and zeaxanthin while the accumulated lycopene is broken down, a similar series of experiments, but involving the use of isotopic labelling, was designed.

Table 2. Carotenoid changes in resuspended nicotine-grown cells of *Flavobacterium* 0147 (2). Cells grown 42 h in 7.5 mM nicotine + [2-14C] MVA

<table>
<thead>
<tr>
<th></th>
<th>Lycopene (µg)</th>
<th>β-Carotene (µg)</th>
<th>Zeaxanthin (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(14Cdpm)</td>
<td>(14Cdpm)</td>
<td>(14Cdpm)</td>
</tr>
<tr>
<td>(a) Control</td>
<td>383</td>
<td>25424</td>
<td>30</td>
</tr>
<tr>
<td>Reincubation:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anaerobic 46 h</td>
<td>32</td>
<td>3172</td>
<td>7</td>
</tr>
<tr>
<td>(b) Control</td>
<td>353.9</td>
<td>47438</td>
<td>11.1</td>
</tr>
<tr>
<td>Reincubation:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anaerobic 24 h</td>
<td>166.9</td>
<td>18106</td>
<td>11.4</td>
</tr>
<tr>
<td>Reincubation:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aerobic 46 h</td>
<td>38.0</td>
<td>2693</td>
<td>560.9</td>
</tr>
<tr>
<td>Reincubation:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aerobic 20 h</td>
<td>59.1</td>
<td>3370</td>
<td>34036</td>
</tr>
<tr>
<td>aerobic 7 h</td>
<td>103.2</td>
<td>9638</td>
<td></td>
</tr>
</tbody>
</table>

Cells were grown in the presence of nicotine (7.5 mM) and [2-14C] mevalonic acid (MVA). One portion of these cells was analysed (control), and the remainder were washed free from nicotine and MVA, and reincubated as described in Table 2. The control cells had lycopene as the main pigment, and the bulk of the carotenoid radioactivity was associated with lycopene. In washed cells reincubated under anaerobic conditions, β-carotene had become the main pigment, and contained the radioactivity formerly associated with lycopene. In cells reincubated aerobically, lycopene was replaced by zeaxanthin, and the zeaxanthin contained essentially all the radioactivity previously in lycopene. This clearly demonstrates the *in vivo* conversion of lycopene into β-carotene under anaerobic conditions, and of lycopene to zeaxanthin in the presence of oxygen. A further batch of washed cells was reincubated anaerobically so that the accumulated lycopene would be converted, as previously shown, into β-carotene. The incubation was then made aerobic and a substantial amount of zeaxanthin was formed at the expense of the β-carotene. The decrease in the radioactivity of the β-carotene was paralleled by the increase in radioactivity of the zeaxanthin.

This demonstrates the sequence of reactions

lycopene → β-carotene → zeaxanthin

and confirms that hydroxylation occurs after formation of the cyclic β-carotene.
RECENT STUDIES OF CAROTENOID BIOSYNTHESIS IN BACTERIA

At lower nicotine concentrations, zeaxanthin formation is inhibited, and rubixanthin becomes the main pigment. A series of experiments similar to those described above was undertaken to determine whether any in vivo conversion of rubixanthin into zeaxanthin could occur.

Table 3. Carotenoid changes in resuspended nicotine-grown cells of Flavobacterium 0147 (3).
Cells grown 36 h in 1.0 mM nicotine

<table>
<thead>
<tr>
<th></th>
<th>Rubixanthin (μg)</th>
<th>β-Cryptoxanthin (μg)</th>
<th>Zeaxanthin (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>(a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>317.2</td>
<td>83.3</td>
<td>63.6</td>
</tr>
<tr>
<td>Reincubation:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anaerobic 24 h</td>
<td>172.0</td>
<td>64.9</td>
<td>31.2</td>
</tr>
<tr>
<td>(b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>102.6</td>
<td>60.9</td>
<td>64.7</td>
</tr>
<tr>
<td>Reincubation:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aerobic 48 h</td>
<td>72.0</td>
<td>39.4</td>
<td>109.8</td>
</tr>
</tbody>
</table>

No lycopene was detected

Cells were therefore grown in the presence of nicotine (1 mM). As shown in Table 3 rubixanthin was the main pigment under these conditions, and no lycopene was detected. When the cells were washed to remove nicotine and reincubated under aerobic conditions, there was a substantial synthesis of zeaxanthin at the expense of the accumulated rubixanthin. In cells reincubated anaerobically, a significant amount of rubixanthin was replaced by β-cryptoxanthin (VI).

These findings were confirmed by an experiment in which cells were grown in nicotine (1 mM) and [2-14C] MVA (Table 4). In these cells (control) much of the radioactivity was associated with rubixanthin. When the cells were washed to remove the nicotine and MVA, and then reincubated anaerobically, the amount of β-cryptoxanthin was significantly increased, and the decrease in radioactivity in rubixanthin was accompanied by an increase in radioactivity in β-cryptoxanthin. In cells reincubated aerobically, a large production of zeaxanthin occurred, and the large decrease in radioactivity in rubixanthin was accompanied by a large increase in radioactivity in zeaxanthin.
Table 4. Carotenoid changes in resuspended nicotine-grown cells of Flavobacterium 0147 (4). Cells grown 22 h in 1.0 mM nicotine + [2-{14}C] MVA

<table>
<thead>
<tr>
<th></th>
<th>Rubixanthin (µg)</th>
<th>β-Cryptoxanthin (µg)</th>
<th>Zeaxanthin (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(¹⁴Cdpm)</td>
<td>(¹⁴Cdpm)</td>
<td>(¹⁴Cdpm)</td>
</tr>
<tr>
<td>Control</td>
<td>348.0</td>
<td>12.4</td>
<td>481.8</td>
</tr>
<tr>
<td>Reincubation:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anaerobic 44 h</td>
<td>279.4</td>
<td>67.1</td>
<td>564.9</td>
</tr>
<tr>
<td>Reincubation:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aerobic 44 h</td>
<td>194.4</td>
<td>3.2</td>
<td>813.7</td>
</tr>
</tbody>
</table>

These experiments show that in Flavobacterium strain 0147, the in vivo conversion of rubixanthin into β-cryptoxanthin and zeaxanthin can occur.

Conclusions

The above experiments indicate that in Flavobacterium 0147, zeaxanthin biosynthesis is inhibited by nicotine, with lycopene or rubixanthin replacing zeaxanthin as the main pigment. Thus nicotine appears to be acting as an inhibitor of cyclization in this system as it does in the Mycobacterium system of Howes and Batra. The experiments, in which cells grown in 7.5 mM nicotine were washed free from inhibitor and reincubated aerobically or anaerobically, showed that the accumulated lycopene could be cyclized under anaerobic conditions to β-carotene, which, in the presence of oxygen, was hydroxylated to zeaxanthin, thus establishing the pathway lycopene → β-carotene → zeaxanthin in Flavobacterium 0147. Similar experiments with cells grown in 1 mM nicotine showed that the accumulated rubixanthin was cyclized under anaerobic conditions to β-cryptoxanthin, and, in the presence of oxygen, converted into zeaxanthin. This established the operation of an alternative pathway

lycopene → rubixanthin → β-cryptoxanthin → zeaxanthin

in Flavobacterium.

It seems likely that this illustrates the fact that the biosynthetic reactions (cyclization, hydroxylation) are reactions of one suitable end-group in the molecule (in this case one-half of the lycopene molecule) rather than reactions of individual compounds. Thus low levels of nicotine inhibit cyclization in one half of the lycopene molecule, while allowing cyclization and subsequent hydroxylation to take place in the other half to form rubixanthin. When the inhibitor is removed, cyclization of the previously inhibited half of the molecule is possible, thus forming β-cryptoxanthin and zeaxanthin.

Higher nicotine concentrations inhibit the cyclization reaction at both ends of the pre-cyclization intermediate lycopene, which therefore accumulates. Removal of the inhibitor then allows cyclization to occur at both ends of the molecule, under anaerobic conditions, followed by hydroxylation to give the normal pigment, zeaxanthin.

The two alternative schemes for zeaxanthin biosynthesis illustrated in Figure 3 (via rubixanthin and β-carotene respectively) may thus merely...
Figure 3. Possible alternative pathways of zeaxanthin biosynthesis in Flavobacterium 0147
indicate the sequence of events when one or both end-sites are inhibited, and the inhibitor is then removed. The cyclizing enzyme uses as substrate any compound with a suitable end-group (as in lycopene), and the hydroxylating enzyme hydroxylates the β-ring once formed, regardless of the nature of the other half-molecule. This is also indicated by the finding of 3-hydroxy-β-zearcarotene (VII) in other zeaxanthin-producing strains of Flavobacterium. In this compound cyclization and hydroxylation have occurred at the neurosporene (VIII) stage, i.e. when only one half of the molecule has reached the required ‘half-lycopene’ level of unsaturation.

At least in the biosynthesis of zeaxanthin in Flavobacterium, pathways of carotenoid biosynthesis should perhaps be considered in terms of series of reactions of half-molecules rather than sequences of individual intermediate carotenoids.

**CAROTENOID BIOSYNTHESIS IN PHOTOSYNTHETIC BACTERIA**

Photosynthetic bacteria characteristically produce acyclic carotenoids with tertiary hydroxy and methoxy groups at C-1 and C-1', e.g. spheroidene (IX), spirilloxanthin (X). In some species, formation of the normal methoxy-carotenoids is inhibited by diphenylamine, and large amounts of phytoene accumulate1 7—22. When the inhibitor is removed, synthesis of the normal carotenoids occurs at the expense of some of the accumulated phytoene.

![Diagram of spheroidene (IX) and spirilloxanthin (X)]

Based upon the structures of minor compounds which appear in diphenylamine-treated cultures, and upon kinetic data for the synthesis of the normal pigments in cells from which diphenylamine has been removed by washing, schemes have been postulated for the biosynthesis of spheroidene and spirilloxanthin19 -24, 41, 42. These schemes include a step in which the terminal double bond is hydrated, thus introducing the tertiary hydroxyl group at C-1. This hydration reaction may be somewhat similar mechanistically to the cyclization reaction which occurs in the formation of other carotenoids14, 40, and therefore, since nicotine is known to inhibit events at the terminal double bond of the acyclic precursor during cyclization37, this compound might also inhibit the hydration reaction in carotenoid biosynthesis in photosyn-
RECENT STUDIES OF CAROTENOID BIOSYNTHESIS IN BACTERIA

thetic bacteria. Any such inhibition should cause the accumulation of the pre-hydration intermediates in the biosynthetic pathway, and thus provide a basis for experiments to give more information about the biosynthesis of the typical hydroxy- and methoxycarotenoids of photosynthetic bacteria.

Effect of nicotine on carotenoid biosynthesis in photosynthetic bacteria

The effects of nicotine at concentrations up to 10 mM were investigated in several photosynthetic bacteria of the Athiorhodaceae. In some species, although the relative amounts of individual carotenoids were altered, no specific inhibition of the main carotenoids or accumulation of possible intermediates was observed. In two species, however, *Rhodopseudomonas spheroides* and *Rhodomicrobium vannielii*, cells grown in the presence of nicotine did have carotenoid compositions very different from those of normal cells, and in each case it appeared that certain biosynthetic reactions were specifically inhibited.

*Rhodopseudomonas spheroides*

*Rhodopseudomonas spheroides* grown under anaerobic conditions synthesizes as its main carotenoids spheroidene (IX) and hydroxyspheroidene (XI), with other related compounds present in small amounts \(^{19,43,44}\). Schemes have been postulated for the biosynthesis of spheroidene and hydroxyspheroidene in *Rps. spheroides* \(^{20-22,24,41}\), but little experimental evidence is available to confirm the operation of these pathways.

![Hydroxyspheroidene](image)

The effect of nicotine at concentrations of 0–10 mM on the carotenoid composition of *Rps. spheroides* was studied. Figure 4 shows the variation of carotenoid composition with nicotine concentration, and shows that at high nicotine concentrations, the amount of spheroidene and spheroidene derivatives present was very much reduced compared with the nicotine-free control. Above 5 mM nicotine, spheroidene was replaced as the main carotenoid by neurosporene, which is present only in traces in control cells. Nicotine therefore inhibits spheroidene formation, and the results suggest that the neurosporene that accumulates is an intermediate in spheroidene biosynthesis.

At lower nicotine concentration, the spheroidene level was significantly increased over the control, and this increase paralleled a decrease in the amount of hydroxyspheroidene present. No hydroxyspheroidene was detected at nicotine concentrations above 5 mM. This confirms that it is the hydration reaction that is inhibited by nicotine, and suggests that inhibition of the second hydration, that of spheroidene to hydroxyspheroidene is essentially
complete before inhibition of the first hydration, of neurosporene, occurs to an appreciable extent.

It appears therefore that in *Rhodopseudomonas spheroides* nicotine does inhibit the hydration of the terminal double bonds in the biosynthesis of spheroidene and hydroxyspheroidene. The fact that at high nicotine concentrations this inhibition results in an accumulation of neurosporene supports the hypothesis that neurosporene is an intermediate in spheroidene formation. That no hydroxyneurosporene (XII) was detected supports the view that it is the hydration step that is inhibited, and the fact that no 3,4-didehydroneurosporene (XIII) was detected suggests that the desaturation of the 3,4-bond does not occur until after the tertiary hydroxyl group has been introduced.

These experiments therefore offer support for the operation of a pathway such as that previously proposed (Figure 5) for spheroidene and hydroxyspheroidene biosynthesis in *Rhodopseudomonas spheroides*.

It is noteworthy that 'demethylated spheroidene' (XIV), a compound postulated as an intermediate in spheroidene biosynthesis but not previously detected, is found in some of these cultures.
Figure 5. Postulated scheme for biosynthesis of hydroxy-spheroidene from neurosporene
(Rhodopseudomonas spheroides)

Rhodomicrobium vannielli

Another photosynthetic bacterium in which carotenoid biosynthesis is susceptible to nicotine inhibition is Rhodomicrobium vannielli. This is apparently unique among non-sulphur photosynthetic bacteria in that it synthesizes small amounts of the cyclic β-carotene, although the main carotenoid present is the acyclic 1-hydroxy-1,2-dihydrolycopene, rhodopin (XV)\textsuperscript{45–48}. 
At nicotine concentrations above about 2 mM, growth of *Rm. vannielii* is inhibited. At 1 mM nicotine, however, growth is not appreciably inhibited, but the carotenoid composition of the organism is very different from that of the nicotine-free control. Figure 6 shows that, as expected, β-carotene synthesis is completely inhibited at very low nicotine concentrations. At rather higher nicotine concentration (1 mM), the amount of the normal main pigment, rhodopin, is also drastically reduced, and lycopene becomes the main carotenoid.

![Carotenoid structure](image)

(XVI)

Anhydrorhodovibrin

The amounts of other carotenoids present in control cells, e.g. spirilloxanthin, anhydrorhodovibrin, (XVI), also decreases as the nicotine concentration increases. The total decrease in β-carotene, rhodopin, anhydrorhodovibrin and spirilloxanthin is almost exactly mirrored by the increase in lycopene.

![Graph](image)

*Figure 6. Variation of carotenoid composition of Rhodopseudomonas vannielii with nicotine concentration:* --- lycopene; --- rhodopin; --- spirilloxanthin; --- anhydrorhodovibrin; --- β-carotene; --- total rhodopin + spirilloxanthin + anhydrorhodovibrin + β-carotene

These results indicate that in *Rm. vannielii* as in other organisms, the cyclization reaction in the formation of β-carotene is inhibited by nicotine. The hydration reaction is apparently also inhibited in this organism, with lycopene accumulating at the expense of rhodopin (*Figure 7*). The increase in
Figure 7. Nicotine inhibition of carotenoid biosynthesis in *Rhodomicrobium vanniellii*
lycopene content at the expense of the total of other carotenoids also suggests that lycopene is the likely precursor of all the other carotenoids normally present in *Rhodopseudomonas vannielli*.

It is interesting that lycopene is apparently the key intermediate in carotenoid biosynthesis in *Rhodopseudomonas vannielli*, whereas neurosporene is the key compound in the case of *Rhodopseudomonas spheroides*.

**ACKNOWLEDGEMENT**

We are grateful to F. Hoffmann-La Roche and Co. Ltd., Basel, for kindly supplying cultures of *Flavobacterium* 0147, and to this company and the Science Research Council for financial support. A.B.-A. was in receipt of a research fellowship from the Hebrew University of Jerusalem, and a Johnston Fellowship in Biochemistry from the University of Liverpool.

**REFERENCES**

RECENT STUDIES OF CAROTENOID BIOSYNTHESIS IN BACTERIA

48  A. Ben-Aziz, G. Britton and T. W. Goodwin, unpublished results.