Biosynthesis of isoprenoids in *Escherichia coli*: The fate of the 3-H and 4-H atoms of 1-deoxy-D-xylulose

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The ubiquinone obtained from *E. coli* after feeding of 1-deoxy[3-^{2}H]xylulose shows labeling of the *E*-methyl group in the terminal unit and of all other positions derived from the terminal methylene group of isopentenyl pyrophosphate, whilst label from 1-deoxy[4-^{2}H]xylulose is retained exclusively in the double bond corresponding to the dimethylallyl pyrophosphate starter unit.

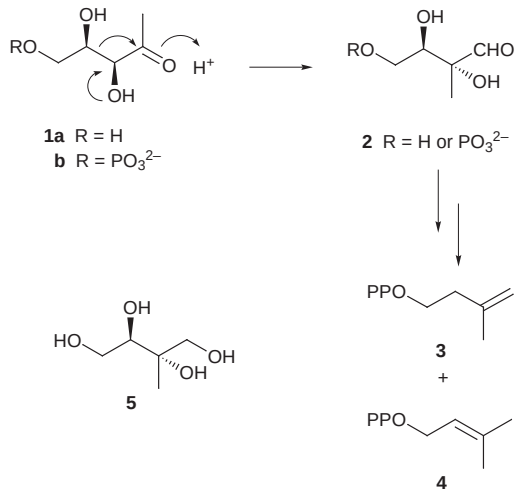
Recent studies have indicated that in *E. coli* the early steps of isoprenoid biosynthesis, *i.e.* formation of isopentenyl pyrophosphate (IPP) **3** and dimethylallyl pyrophosphate (DMAPP) **4**, occur *via* a mevalonate-independent pathway,[1,2] known to be operative also in other bacteria[1] as well as in green algae[3] and higher plants.[4] This alternative route proceeds *via* the intermediacy of 1-deoxy-D-xylulose **1a** (or the corresponding 5-phosphate **1b**) as first demonstrated for the formation of ubiquinone and menaquinone in *E. coli*,[2] and subsequently confirmed for the formation of a variety of terpenes in higher plants.[5]

The first committed step in the formation of IPP and DMAPP from **1a** or **1b** (Scheme 1) is believed to be a rearrangement to the branched aldehyde **2**, as suggested by the labeling patterns observed for the biosynthesis of the related tetrol **5** in *Corynebacterium ammoniagenes*[6] and the demonstration that **1a** indeed serves as a precursor of **5** in leaves of *Liriodendron tulipifera*.[7] Clearly, the overall conversion of **1a** to IPP and DMAPP requires *inter alia* three reductive steps, but the sequence of events remains unknown. To narrow down the number of mechanistic possibilities we have now investigated the fate of the H-atoms at positions 3 and 4 of 1-deoxyxylulose **1a** during the biosynthesis of ubiquinone in *E. coli* by using specifically deuterated forms of the precursor.

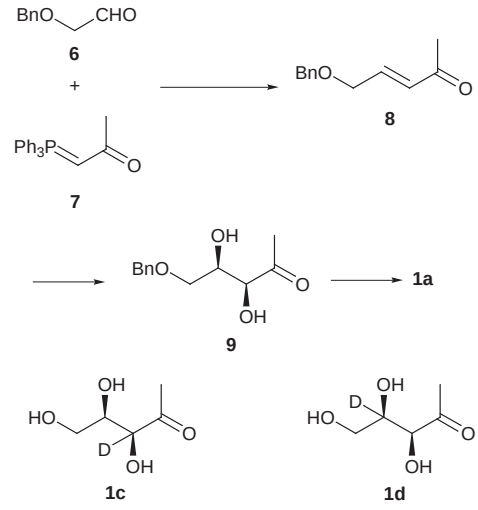
The required labeled substrates were prepared by exploitation of a recently described synthetic route centered on the Sharpless asymmetric dihydroxylation of **8** to **9** (Scheme 2).[8] For the preparation of 1-deoxy[3-^{2}H]xylulose **1c**, 1-triphenylphosphoranylidenepropan-2-one **7** was deuterated at the 1-position by deuterium-exchange in CH_2Cl_2–D_2O–CD_3OD (10:1:1) in the presence of 0.1% TFA. The resulting 1-deoxyxylulose was shown to contain 85% deuterium at C-3 and 5% at C-1 by a combination of ^{1}H and ^{2}H NMR spectroscopy of its 5-benzyl ether. The [4-^{2}H]-labeled 1-deoxyxylulose **1d** was assembled from benzyloxy[1-^{2}H]acetaldehyde, itself available from the unlabeled compound **6** through reduction with $LiAlD_4$ (> 99 atom%) and subsequent Swern oxidation of the resulting deuterated alcohol. A deuterium content of 70% was estimated for **1d** by ^{1}H NMR analysis of the corresponding 5-benzyl ether.

Samples of the labeled precursors (640 mg) were fed to 1 l cultures of *E. coli* (K-12 strain). The cells were grown for 24 h at 37 °C in a minimal medium containing 3.0 g l^{-1} glucose.[9] Ubiquinone **10** was obtained in a yield of 2.5 mg l^{-1}, which is twice as high as normally observed in the absence of the precursor. The biosynthetic samples were analyzed by mass spectrometry and NMR spectroscopy.[10]

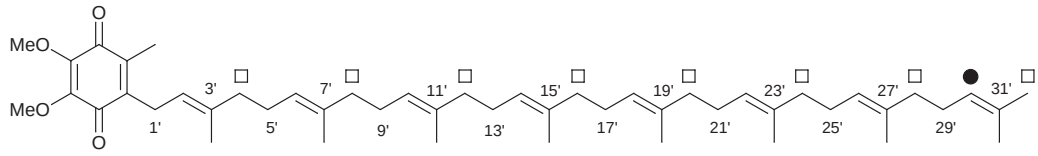
The ubiquinone produced in the presence of **1c** was shown by integration of the 500 MHz ^{1}H NMR spectrum to contain 31% CH_3 and 69% CDH_2 in the (*E*)-31'-methyl position (signals at δ 1.679 and 1.661, respectively). In addition, integration of the signal cluster at δ 1.91–2.0 also indicated 69% monodeuteration of the CH_2 groups labeled with a square in formula **10**. These data correspond to a specific incorporation rate of 81% per unit derived from added **1c**.† The location of the deuterium was confirmed by peaks at δ 1.976 and 1.689 in the ^{2}H NMR spectrum in a ratio of approximately 7:1; an additional minor peak at δ 1.608 is due to the labeling of the (*Z*)-methyl groups by the small amount of deuterium present at C-1 of the precursor. The retention of deuterium from the 3-position of 1-deoxyxylulose **1c** in the positions arising from the terminal methylene group of IPP was further verified by ^{13}C NMR



Scheme 1



Scheme 2



10 □ position of the label from **1c**
● position of the label from **1d**

analysis. The signal for the (*E*)-31'-methyl group bearing one deuterium was observed as a triplet (J_{C-D} = 19.1 Hz) shifted by −288 ppb from the signal of the unlabeled compound at δ 25.672. Monodeuteration of the labeled methylene positions was evidenced by the appearance of a broad triplet (J_{C-D} = 19.5 Hz) with an α-shift of −385 ppb from the normal signals at δ 39.735 and 39.714. The presence of deuterium at the positions indicated in **10** also resulted in β-shifts of −30 ppb for the adjacent quaternary olefinic carbons (δ 137.6–134.8) and of −72 ppb for the adjacent methylene carbons (δ 26.5–26.8). The signals due to the methyl group at C-3' (δ 16.334) and the other internal methyl groups (δ *ca*. 16.0) displayed γ-shifts of −30 ppb, while a γ-shift of −17 ppb was observed for the (*Z*)-methyl group at C-31' (δ 17.660).

Mass spectral analysis (EI) of the ubiquinone sample from the feeding experiment with **1d** disclosed the presence of an amount of d_1 molecular ions corresponding to *ca*. 45% of the total. From this value an approximate 65% specific incorporation of the label from **1d** into a single C_5 unit of **10** can be estimated. As a first indication that the label was localized at C-30' the same proportion of d_1 ions was observed for the $C_5H_9^+$ fragment expected to originate from the terminal C_5 unit of the isoprenyl chain. In keeping with this, the ^{2}H NMR spectrum displayed a single sharp signal at δ 5.138 and the ^{1}H NMR spectrum showed by integration the loss of *ca*. 0.36 H from the olefinic region, but no diminution of the resolved olefinic signals at δ 4.938 (2'-H) and 5.061. Independent proof for the location of the label came from the ^{13}C NMR spectrum which showed a marked diminution of peak intensity for the signal at δ 124.435. This signal must be assigned to C-30' since a β-shift of −99 ppb was observed for C-31' (δ 131.263) and γ-shifts were observed for the (*E*)- and the (*Z*)-methyl groups at C-31' (−63 and −21 ppb, respectively).‡

Thus, the deuterium label from C-4 of the precursor **1d** is retained in the 30'-position, *i.e.* in the double bond corresponding to the DMAPP starter unit, but not in any of the remaining double bonds generated from IPP in the elongation process. This is in striking contrast to the situation observed for the mevalonate pathway in eukaryotic organisms where the same H-atom of IPP (H_{Re}) is lost both in the isomerization to DMAPP and in the elimination step of the elongation process.[11] An IPP isomerase has been partially purified from *E. coli*;[12] if DMAPP is generated in the organism mainly through the action of this enzyme, then one has to conclude that in *E. coli* different H-atoms are lost from IPP in the isomerization and in the elongation process. The alternative that IPP and DMAPP may be formed independently from a common intermediate of the new pathway seems less plausible but cannot be dismissed on the basis of our results.

The difference in the metabolic origin observed in this study for the olefinic hydrogens of ubiquinone may prove relevant in connection with hitherto unexplained reports based on natural abundance ^{2}H NMR analysis of cyclic monoterpenes from higher plants which had disclosed a substantial relative enrichment of deuterium at the carbon atom stemming from C-2 of the starter DMAPP unit with respect to the other vinylic hydrogen atom of the geranyl pyrophosphate precursor.[13]

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Notes and References

† Mass spectral analysis of the sample of **6** derived from **1c** showed a cluster of M^+ ions corresponding to the addition of up to eight deuterium atoms per molecule.

‡ A β-shift of −108 ppb was also observed for the signal at δ 26.795 which can now be assigned to C-29'; additional shifted signals at δ 134.918 ($\Delta\delta$ = +7 ppb) and 124.292 ($\Delta\delta$ = −6 ppb) are most probably due to the two olefinic carbons of the adjacent C_5 unit.

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