Pergamon

Tetrahedron Letters 39 (1998) 8021–8022

TETRAHEDRON LETTERS

Biosynthesis of isoprenoids in *Escherichia coli*: Retention of the methyl H-atoms of 1-deoxy-D-xylulose

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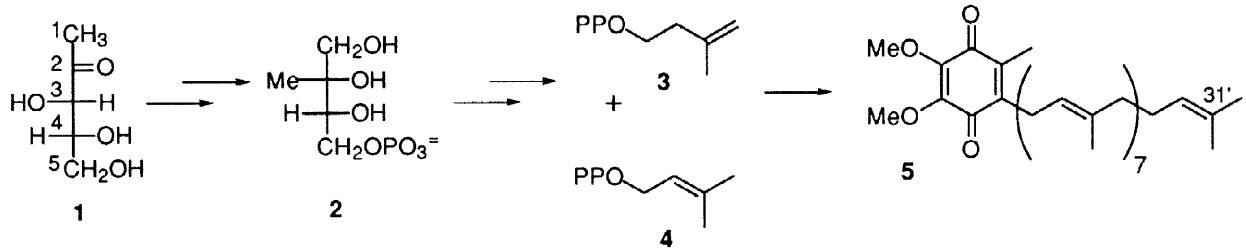
Received 31 July 1998; revised 17 August 1998; accepted 18 August 1998

Abstract: Investigation of isoprenoid biosynthesis in *E. coli* using [1,1,1-$^2H_3$]-1-deoxy-D-xylulose shows the methyl group of 1-deoxyxylulose to be incorporated into the methyl groups of ubiquinone originating from the methyl group of IPP and the (Z)-methyl group of DMAPP, and that this incorporation involves no loss of methyl hydrogen atoms.

Keywords: Biosynthesis; isoprenoids; labeling; vitamins.

Since the first demonstration that 1-deoxy-D-xylulose (**1**) is incorporated in a mevalonate independent fashion into the prenyl chains of ubiquinone and menaquinone in *E. coli*,[1] this ketopentose (or its 5-phosphate ester) has also been shown to serve as an intermediate in the biosynthesis of isoprenoids in higher plants.[2-5] The next intermediate in this pathway appears to be the branched precursor (**2**), recently shown to be formed in *E. coli* from the 5-phosphate ester of **1** by an NADPH-dependent reductoisomerase.[6] Deuterium labeling of **2** (as the nonphosphorylated form) has demonstrated that all four methylene hydrogen atoms are incorporated into ubiquinone in *E. coli*.[7] Otherwise nothing is known about the subsequent reactions or intermediates in the new pathway. In order to place limits on the mechanistic possibilities, we have investigated the fates of the hydrogen atoms of 1-deoxyxylulose (**1**) in its conversion to ubiquinone (**5**) in *E. coli*. Studies with specifically deuterated precursors have so far shown the fates of the H-atoms from 3-, 4-, and 5-positions.[1-8] A previous experiment utilizing **1** bearing deuterium in the 1-position resulted in a 90% specific incorporation into the methyl groups of ubiquinone originating from the methyl group of IPP (**3**) and the (Z)-methyl group of DMAPP (**4**, i.e. all methyl groups of the prenyl chain except the (E)-31'-methyl group).[1] However, because this experiment employed a monodeuterated methyl group ([1-^{1}H]-**1**), the possibility remained that hydrogen atom loss might occur from this position during the course of the new biosynthetic pathway (e.g. via an elimination



0040-4039/98/$ - see front matter
PII: S0040-4039(98)01791-2

reaction). In the present communication we describe an experiment using trideuteriomethyl-labeled 1-deoxyxylulose that demonstrates the incorporation of the intact methyl group.

The required [1,1,1-$^2H_3$]-1-deoxy-D-xylulose was prepared from 2,3,4-tribenzyl-D-threose and CD_3MgI (>99 atom%) as previously described,[9] and fed to cultures of *E. coli* (K-12 strain).[8,10] The ubiquinone thus produced (2.5 mg) displayed only two signals in the ^2H-NMR spectrum (1.56 and 1.70 ppm) in a 7:1 ratio, indicating that the deuterium was restricted to the methyl positions of the prenyl chain. Integration of the 500 MHz ^1H-NMR spectrum showed no deuterium to be present in the (E)-31'-methyl group (1.679 ppm), but the integrals of the remaining methyl groups of the prenyl chain (1.738 ppm, 1.57-1.60 ppm) were diminished to 30% of their normal values. With the exception of the (E)-31'-methyl signal, which was asymmetrical due to a γ-shift of -3 ppb, no ^2H-shifted signals were detected by ^1H-NMR, ruling out the presence of deuterium in the form of CH_2D or CHD_2.

Further evidence that the deuterium was incorporated exclusively as CD_3 groups came from the ^{13}C-NMR spectrum. As is expected for CD_3 groups, the signals of the deuterium-bearing carbons were not observable due to a combination of ^2H-^{13}C coupling, very long relaxation times, and the lack of nOe enhancement from bonded ^1H-atoms. The observed signals from the residual nondeuterated prenyl methyl groups (with the exception of the (E)-31'-methyl group) displayed no ^2H-shifts and were attenuated to one third of their normal intensities. The deuterium labeling pattern was also manifested in ^2H-shifted signals with β-shifts of -80 ppb for the quaternary olefinic carbons (ca. 135 ppm), and in the γ-shifts of +25 observed for the olefinic methine signals (ca. 124 ppm) and -45 ppb for the signals of the methylene groups adjacent to the quaternary olefinic carbons (ca. 40 ppm). In addition, the (E)-31'-methyl signal (25.672 ppm) was accompanied by a signal having a γ-shift of -65 ppb. The observed ratio of the ^2H-shifted to the nonshifted ^{13}C-signals was approximately 2:1.

The conclusion that the labeling pattern observed by NMR spectroscopy is a result of the 70% specific incorporation of intact CD_3 groups is further supported by mass spectrometry (EI), which shows a series of molecular ions corresponding to the incorporation of up to eight CD_3 units. A previous report has indicated the retention of up to three ^2H-atoms from the CD_3 group of [2,3,3,3-$^2H_4$]-lactate in *E. coli* ubiquinone biosynthesis, but molecules bearing only one or two ^2H-atoms predominated in that study.[10]

Acknowledgments. We thank Prof. Duilio Arigoni for his interest in this work and for many stimulating discussions.

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