Biosynthesis of vitamins B_1 and B_6 in *Escherichia coli*: concurrent incorporation of 1-deoxy-D-xylulose into thiamin (B_1) and pyridoxol (B_6)

Klaus Himmeldirk,[a] Isaac A. Kennedy,[a] Robert E. Hill,[b] Brian G. Sayer[a] and Ian D. Spenser*[a]†

[a] *Department of Chemistry, McMaster University, Hamilton, Ontario, Canada L8S 4M1*
[b] *Department of Pathology, McMaster University, Hamilton, Ontario, Canada L8N 4Z5*

It is shown by ^{13}C NMR spectroscopy that, in *Escherichia coli* mutant WG2, the C-2,-3 bond of [2,3-$^{13}C_2$]-1- deoxy-D-xylulose 2 enters C-4,-5 of the thiazole unit of thiamin (B_1) 1 and C-2,-3 of pyridoxol (B_6) 3, providing the first direct evidence that the intact C_5 chain of 1-deoxy-D-xylulose is incorporated concurrently into each of the two B Vitamins.

It has been shown that the carbon skeleton of pyridoxol (Vitamin B_6) (**3**, unstarred) is constructed from a C_2 unit and two C_3 units derived from glucose.[1] The C_2 unit enters C-2′,-2 of pyridoxol, while the C_3 units enter C-3,-4,-4′ and C-6,-5,-5′. It has been shown further that the presence of 1-deoxy-D-xylulose (**2**, unstarred) caused a decrease in the level of incorporation of label from D-[1,2,3,4,5,6-$^{13}C_6$]glucose into C-2′,-2 and into C-3,-4,-4′, of pyridoxol, relative to that into the other three carbon atoms, C-6,-5,-5′, whose level of ^{13}C enrichment remained unimpaired.[2] This shows that 1-deoxy-D-xylulose lies on the route from glucose into the C_5 unit, C-2′,-2,-3,-4,-4′, of pyridoxol. Deuterium from [1-$^2H_3$,5-(*RS*)-$^2H_1$]-1-deoxy-D-xylulose was shown by ^2H NMR spectroscopy to enter the methyl group, C-2′ and the hydroxymethyl group, C-4′, of pyridoxol in *E. coli* mutant WG2 in the ratio 3 : 1, corresponding to the distribution of deuterium in the precursor. This result made it likely, but did not prove unequivocally, that the C_5 unit, C-2′,-2,-3,-4,-4′, of pyridoxol was generated from the intact C_5 chain of the precursor.[3]

We now present direct evidence that in *E. coli* mutant WG2 the intact C_5 chain of 1-deoxy-D-xylulose supplies the C_5 unit, C-2′,-2,-3,-4,-4′, of pyridoxol.

In the formation of the C_5 unit, C-2′,-2,-3,-4,-4′, of pyridoxol from glucose, *i.e.*, of the C_5 unit that is affected by the presence of 1-deoxy-D-xylulose, the only carbon–carbon bond that is newly generated is the C-2,C-3 bond. It is therefore essential, in evaluating the status of 1-deoxy-D-xylulose as a precursor of this C_5 unit, to prove that the C-2,-3 bond of the substrate is transferred intact into the product. Accordingly, a sample of [2,3-$^{13}C_2$]-1-deoxy-D-xylulose[4] **2** was prepared.

Five 1 dm^3 cultures of *E. coli* mutant WG2 were each incubated, with D-xylose (0.5 g) as the general carbon source, in the presence of [2,3-$^{13}C_2$]-1-deoxy-D-xylulose[4] **2** (200 mg) and of 4-hydroxy-L-threonine (100 mg) and L-threonine[5] (20 mg).

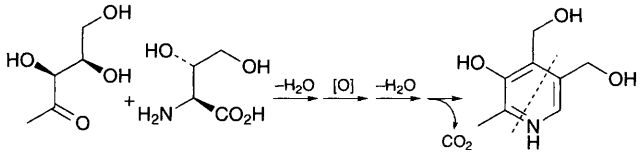
Pyridoxol hydrochloride **3** was isolated from the culture fluid of each 1 dm^3 culture after addition of natural abundance pyridoxol hydrochloride (2.5 mg) as carrier, and was purified by column and thin layer chromatography. The samples were combined and the product purified by sublimation in a high vacuum.[6]

The ^{13}C NMR spectrum of the sample of pyridoxol hydrochloride **3** shows satellites in the signals due to C-2 (δ 144.7, $^1J_{C\text{-}2,\text{-}3}$ 72.8 Hz) and C-3 (δ 154.7, $^1J_{C\text{-}2,\text{-}3}$ 72.8 Hz) and at no other site [Fig. 1 (*a*),(*b*)]. Thus the C-2,-3 bond of the precursor **2** had entered intact, and since this is the only bond that is newly generated in the course of the formation of 1-deoxy-D-xylulose from glucose it follows that the intact C_5 chain of 1-deoxy-D-xylulose had been incorporated into the C_5 unit, C-2′,-2,-3,-4,-4′, of pyridoxol.

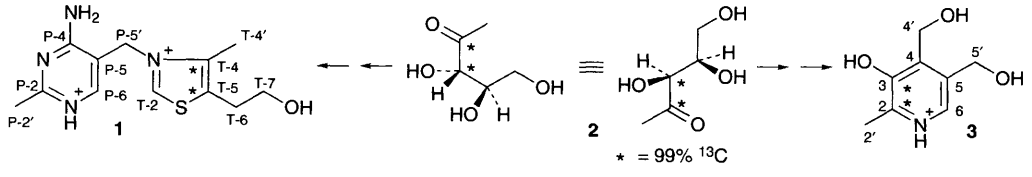
We have recently presented proof that the remaining fragment of pyridoxol, the C_3N unit N-1,C-6,5,5′, is generated intact from a C_3N unit originating by decarboxylation of 4-hydroxy-L-threonine.[5] Thus, the origin of the entire skeleton of Vitamin B_6 is now accounted for in terms of two precursors, 1-deoxy-D-xylulose and 4-hydroxy-L-threonine.‡ A chemically and biochemically rational mechanism for the derivation of pyridoxol from these two precursors, has been advanced.[7]

1-Deoxy-D-xylulose (**2**, unstarred) has been reported to serve as the precursor of yet another B vitamin: Evidence has been presented which suggests that it supplies the C_5 chain, C-4′,-4,-5,-6,-7, of the thiazole unit of thiamin (Vitamin B_1) (**1**, unstarred): Deuterium from [1-$^2H_3$,5-(*RS*)-$^2H_1$]-1-deoxy-D-xylulose was shown[8] to be incorporated into the thiazole unit of thiamin in *E. coli* and it was concluded on the basis of a statistical evaluation of the mass spectrometric fragmentation pattern of the deuterium enriched thiamin that incorporation had taken place with maintenance of four deuterium atoms within the thiazole nucleus. This led to the inference that the precursor had been incorporated as an intact unit into the C_5-chain, C-4′,-4,-5,-6,-7 of the thiazole unit.[8]

Earlier, it had been concluded on the basis of the interpretation of mass spectrometric fragmentation patterns that the C_5-chain, C-4′,-4,-5,-6,-7, of the thiazole unit arises by union of two glucose-derived fragments, a C_3 unit, giving rise to



Scheme 2



Scheme 1

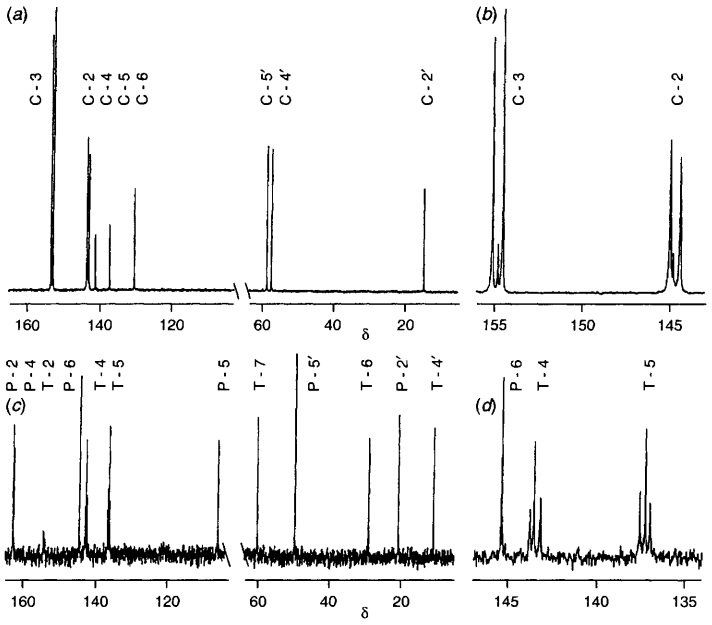
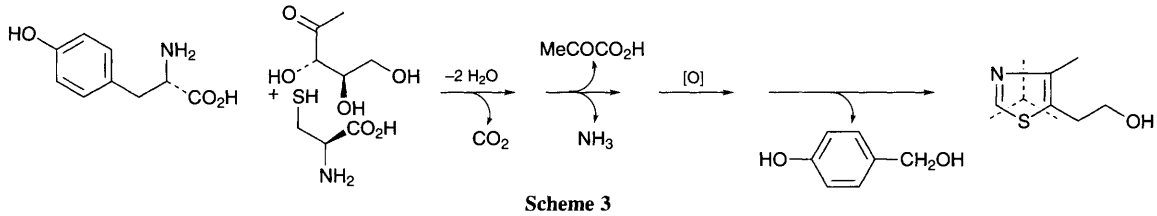


Fig. 1 125.776 MHz proton decoupled ^{13}C NMR spectra of (*a*), (*b*) pyridoxol hydrochloride (**3**) (in 100 μl D_2O) and (*c*), (*d*) thiamin chloride hydrochloride **1** (in 100 μl D_2O), isolated from *E. coli* B WG2 after incubation with [2,3-$^{13}C_2$]-1-deoxy-D-xylulose **2**. The spectra were acquired on a Bruker DRX 500 spectrometer, operating at 11.74 T, using a 2.5 mm microprobe, with a 90° pulse width (8 μs), spectral width 28985.5 Hz, recycle time (*a*) 10.56 s, (*c*) 2.56 s. Initial memory size was 32 K, which was zero-filled to 64 K before Fourier transformation, giving a final digital resolution of 0.88 Hz per data point. (*a*) and (*c*): low and high frequency regions of the spectra. (*b*) and (*d*): Expanded δ 135–155 spectral regions of the spectra (*a*) and (*c*), respectively.



Scheme 3

C-5,-6,-7, and a C_2 unit, giving rise to C-4′,-4.[9] These results are consistent with the intermediacy of 1-deoxy-D-xylulose, which in *E. coli* and other bacteria originates by condensation of pyruvic acid and D-glyceraldehyde, catalysed by pyruvate dehydrogenase (EC 1.2.4.1).[10,11]

We now present ^{13}C NMR evidence in support of the derivation of the C_5 chain, C-4′,4,5,6,7, of the thiazole moiety, from the intact C_5 chain of 1-deoxy-D-xylulose.

Thiamin chloride hydrochloride **1** was isolated from the combined cell mass from the five incubations, whose culture fluid had yielded pyridoxol. The following procedure was used: The cells were suspended in 0.1 mol dm^{-3} hydrochloric acid. Thiamin pyrophosphate chloride (cocarboxylase) (3 mg) was added as carrier and the cells were lysed by boiling for 1 h. Acid phosphatase (taka-diastase) treatment of the extract, followed by ion exchange chromatography (Amberlite CG-50), size exclusion chromatography (Sephadex G-10) and silica gel chromatography yielded the product (contaminated with silica gel) (6 mg). This was dissolved in D_2O and kept at 65 °C for 2 d to ensure complete exchange of the acidic proton at C-2 of the thiazole unit.

The ^{13}C NMR spectrum of the sample of thiamin chloride hydrochloride **1** (deuteriated at T-2 by exchange with D_2O) shows satellites in the signals due to C-4 (δ 143.6, $^1J_{C\text{-}4,\text{-}5}$ 73.4 Hz) and C-5 (δ 137.3, $^1J_{C\text{-}4,5}$ 73.4 Hz) of the thiazole moiety and at no other site [Fig. 1 (*c*),(*d*)]. As before, the C-2,-3 bond of 1-deoxy-D-xylulose **2** had entered intact, showing that the intact C_5 chain of the precursor had been incorporated into the C_5 unit, C-4′,-4,-5,-6,-7, of the thiazole moiety§ of thiamin.

These results establish that in *E. coli* the two vitamins are biosynthesized concurrently and that 1-deoxy-D-xylulose serves as a direct precursor of a different skeletal segment of each.

A research grant from the Institute of General Medical Sciences, U.S. Public Health Service (Grant GM 50778, to I. D. S.) is gratefully acknowledged. We thank Richard Pauloski for skilled technical assistance.

Footnotes

† E-mail spenser@mcmail.cis.mcmaster.ca

‡ The biogenetic anatomy of pyridoxol in *E. coli*. Derivation from 1-deoxy-D-xylulose and 4-hydroxy-L-threonine (Scheme 2).

§ The biogenetic anatomy of the thiazole moiety of thiamin in *E. coli*. Derivation from 1-deoxy-D-xylulose, L-tyrosine and a sulfur source (presumably L-cysteine) (Scheme 3).

References

1 R. E. Hill, B. G. Sayer and I. D. Spenser, *J. Chem. Soc., Chem. Commun.*, 1986, 612.
2 I. A. Kennedy, R. E. Hill, R. M. Pauloski, B. G. Sayer and I. D. Spenser, *J. Am. Chem. Soc.*, 1995, **117**, 1661.
3 R. E. Hill, B. G. Sayer and I. D. Spenser, *J. Am. Chem. Soc.*, 1989, **111**, 1916.
4 I. A. Kennedy, T. Hemscheidt, J. F. Britten and I. D. Spenser, *Can. J. Chem.*, 1995, **73**, 1329.
5 E. Wolf, R. E. Hill, B. G. Sayer and I. D. Spenser, *J. Chem. Soc., Chem. Commun.*, 1995, 1339.
6 R. E. Hill, F. J. Rowell, R. N. Gupta and I. D. Spenser, *J. Biol. Chem.*, 1972, **247**, 1869.
7 R. E. Hill and I. D. Spenser, *Nat. Prod. Rep.*, 1995, **12**, 555.
8 S. David, B. Estramareix, J.-C. Fischer and M. Therisod, *J. Chem. Soc., Perkin Trans. 1*, 1982, 2131.
9 R. H. White, *Biochemistry*, 1978, **17**, 3833.
10 A. Yokota and K. Sasajima, *Agric. Biol. Chem.*, 1984, **48**, 149.
11 A. Yokota and K. Sasajima, *Agric. Biol. Chem.*, 1986, **50**, 2517.

Received, 1st February 1996; Com. 6/00769D