calcium chloride and distilled from phosphorus pentoxide. Neopentane, Phillips 99%, was used without further purification. The bromine employed in this work was Mallinckrodt analyzed grade and was used without further purification. K_2CO_3 obtained from Fisher was ground to a powder and pumped on a vacuum line, with heating at 150 °C for 24 h prior to use.

Photolysis Experiments. All reactions were carried out in 30-mL-capacity Pyrex pressure tubes fitted with Teflon O-ring sealed needle valves. Reaction mixtures were degassed three times by a freeze–thaw technique, alternately freezing and evacuating (<0.1 μ) at −196 °C and thawing to ambient temperatures. The sealed pressure tube was placed in a Pyrex water circulating bath maintained at 14–15 °C and irradiated with a 400-W medium-pressure mercury arc at a distance of 10 cm through two layers of Pyrex glass and 5 cm of water. Irradiation times of 1.0–2.5 h were employed, and reactants were stirred with a Teflon-covered magnetic stir bar. Absolute product analyses were obtained by gas chromatography, and if necessary by ^{1}H NMR, employing internal standards in both cases. Products were identified by comparison of GC retention times and/or ^{1}H NMR spectra of authentic samples. If gas chromatography was used the products were first washed with 5% aqueous sodium bisulfite and 5% aqueous sodium bicarbonate and dried with anhydrous Na_2SO_4.

I. Bromine in the Presence of K_2CO_3. (a) Bromine (0.80 mmol), CH_2Cl_2 (78.25 mmol), *neo*-C_5H_{12} (3.50 mmol), and K_2CO_3 (4.95 mmol) were irradiated for 1.5 h; the reaction mixture is heterogeneous throughout. The color of the reaction mixture turned from red to orange. The product mixture was analyzed by GC: $CHBrCl_2$ (0.61 mmol), neo-$C_5H_{11}Br$ (0.011 mmol); r = 0.067. (b) Bromine (1.20 mmol), CH_2Cl_2 (78.25 mmol), *neo*-C_5H_{12} (3.50 mmol), and K_2CO_3 (7.40 mmol) were irradiated for 2.0 h. The reaction mixture remained heterogeneous throughout. The color change from red to orange. The product mixture was analyzed by GC: $CHBrCl_2$ (0.93 mmol), *neo*-$C_5H_{11}Br$ (0.0178 mmol); r = 0.071. (c) Bromine (0.95 mmol), CH_2Cl_2 (78.25 mmol), *neo*-C_5H_{12} (3.76 mmol), and K_2CO_3 (5.80 mmol) were irradiated for 2.0 h. The reaction mixture remained heterogeneous throughout. The color changed from red to orange. The product mixture was analyzed by GC: $CHBrCl_2$ (0.81 mmol), *neo*-$C_5H_{11}Br$ (0.015 mmol); r = 0.064.

II. Bromine in the Presence of Succinimide (the WEZ Experiment). Succinimide (2.00 mmol), CH_2Cl_2 (24.5 mmol), *neo*-C_5H_{12} (2.17 mmol), and bromine (1.00 mmol) were irradiated for 2.15 h. No color change was observed and the reaction mixture remained heterogeneous. The product was analyzed by GC: *neo*-$C_5H_{11}Br$ (0.0075 mmol), $CHBrCl_2$ (0.066 mmol); r = 0.24.

III. Bromine in the Presence of Succinimide and K_2CO_3. Succinimide (2.00 mmol), CH_2Cl_2 (26.5 mmol), *neo*-C_5H_{12} (1.65 mmol), K_2CO_3 (6.00 mmol), and bromine (1.00 mmol) were irradiated for 2.15 h. The reaction mixture remained heterogeneous throughout the irradiation period. The color change observed was red to a lighter shade of red. The product mixture was analyzed by GC: *neo*-$C_5H_{11}Br$ (0.0106 mmol), $CHBrCl_2$ (0.416 mmol); r = 0.067.

IV. Bromine with and without Succinimide; Paired Reactions. (a) Without Succinimide. Bromine (1.95 mmol), CH_2Cl_2 (46.95 mmol), and *neo*-C_5H_{12} (1.97 mmol) were irradiated for 1.25 h. The reaction mixture was homogeneous throughout the irradiation period. The product was analyzed by GC: *neo*-$C_5H_{11}Br$ (0.016 mmol), $CHBrCl_2$ (0.163 mmol); r = 0.39. **(b) With Succinimide.** Bromine (1.95 mmol), succinimide (0.61 mmol), methylene chloride (46.95 mmol), and *neo*-C_5H_{12} (3.27 mmol) were irradiated for 1.25 h. The initial reaction was homogenous and became heterogenous after ~45 min. The product was analyzed by GC: *neo*-$C_5H_{11}Br$ (0.013 mmol), $CHBrCl_2$ (0.10 mmol); r = 0.31. Succinimide analysis by ^{1}H NMR: 0.60 mmol, δ 2.65, s 4 H.

Acknowledgment. We thank the National Science Foundation for financial support of this work.

Registry No. NBS, 128-08-5; H_2, 1333-74-0; D_2, 7782-39-0; HBr, 10035-10-6; C_6H_6, 71-43-2; CH_2Cl_2, 75-09-2; $CHCl_3$, 67-66-3; *neo*-C_5H_{12}, 463-82-1; isobutane, 75-28-5; butane, 106-97-8; succinimidyl radical, 24344-83-0; butadiene, 106-99-0; 3,3-dimethyl-1-butene, 558-37-2.

Communications to the Editor

Biosynthesis of Vitamin B_6: Incorporation of a C–N Unit Derived from Glycine

Agnieszka Iwanow,[1,2] Robert E. Hill,[3] Brian G. Sayer,[1] and Ian D. Spenser*[1]

Departments of Chemistry and Pathology
McMaster University
Hamilton, Ontario, Canada L8S 4M1

Received October 11, 1983

We have presented evidence for the occurrence, in *Escherichia coli* B mutant WG 2, which closely resembles the wild type, of two pathways to pyridoxol (**2**) (vitamin B_6), one major and one minor.[4] In the major pathway the entire carbon skeleton of pyridoxol is derived from glycerol in a specific manner:[5-7] five of its eight C atoms (C-2′, -3, -4′, -5′, -6) are derived from the primary carbon atoms of glycerol, the other three (C-2, -4, -5) from the secondary carbon atom of glycerol. On the basis of these findings, we proposed a chemically rational scheme for the major route to pyridoxol in *E. coli*, from three triose precursors.[5]

In the minor route, which can be conveniently studied in *E. coli* B mutant WG 3 in which the major route is blocked, the C_3 fragment of pyridoxal (**1**), C-5′, -5, -6, is not derived as a unit from glycerol. Two of it C atoms, C-5′ and C-5, are supplied by C-2 and C-1, respectively, of glycolaldehyde,[6] and preliminary evidence suggested that C-6 arises from a one-carbon unit which can, in turn, originate from C-2 of glycerol.[4]

We now present evidence that, in mutant WG 3, C-6 of pyridoxal is derived from C-2 of glycine. Carbon 2 of glycine does, indeed, originate from C-2 of glycerol[8] and also serves as a source of one-carbon units.[8] However, the incorporation of C-2 of glycine into pyridoxal does not take place via a one-carbon unit but by way of an intact glycine-derived C–N unit that gives rise to C-6, N-1 of pyridoxal.

In two separate tracer experiments (experiments 1 and 2), cultures of *E. coli* B WG 3 (1 L, 1.5 g/L glycerol, 50 mg/L glycolaldehyde)[9] were incubated in the presence of [2-^{14}C]glycine (250 μCi, New England Nuclear). Pyridoxal (**1**) was isolated[10]

(1) Department of Chemistry.
(2) On leave of absence from the Department of Organic Chemistry, University of Warsaw, Poland.
(3) Department of Pathology.
(4) Vella, G. J.; Hill, R. E.; Mootoo, B. S.; Spenser, I. D. *J. Biol. Chem.* **1980**, *255*, 3042–3048.
(5) Hill, R. E.; Rowell, F. J.; Gupta, R. N.; Spenser, I. D. *J. Biol. Chem.* **1972**, *247*, 1869–1882.
(6) Hill, R. E.; Horsewood, P.; Spenser, I. D.; Tani, Y. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1622–1627.
(7) Hill, R. E.; Miura, I.; Spenser, I. D. *J. Am. Chem. Soc.* **1977**, *99*, 4179–4181.
(8) Neuberger, A. In "Comprehensive Biochemistry"; Florkin, M., Stotz, E. H., Eds.; Neuberger, A., Van Deenen, L. L. M., Vol. Eds.; Elsevier: Amsterdam, 1981; Vol. 19A, pp 257–303.
(9) Tani, Y.; Dempsey, W. B. *J. Bacteriol.* **1973**, *116*, 341–345.
(10) Dempsey, W. B. *Biochim. Biophys. Acta* **1972**, *264*, 344–353.
(11) Korytnyk, W.; Ikawa, M. In "Methods in Enzymology"; MacCormick, D. B., Wright, D. L., Eds.; Academic Press: New York, 1970; Vol. 18A, p 558.
(12) Satisfactory spectral and analytical data were obtained for the new compound obtained in this reaction.

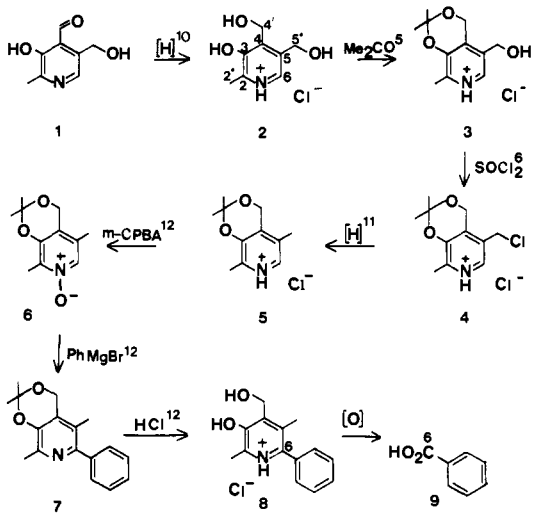
0002-7863/84/1506-1840$01.50/0

Table I. Incorporation of [2-^{14}C]Glycine into Pyridoxal in *E. coli* B WG 3

products	C-atoms of pyridoxal	experiment 1		experiment 2	
		SA[a]	RSA[b]	SA[a]	RSA[b]
3,4'-*O*-isopropylidenepyridoxol hydrochloride (**3**)	all	1.45 ± 0.02	100 ± 1	2.07 ± 0.03	100 ± 2
5'-deoxy-3,4'-*O*-isopropylidenepyridoxol *N*-oxide (**6**)	all	1.44 ± 0.02	99 ± 2	2.11 ± 0.03	101 ± 2
5'-deoxy-3,4'-*O*-isopropylidene-6-phenylpyridoxol (**7**)	all	1.45 ± 0.03	100 ± 2	2.06 ± 0.02	99 ± 2
5'-deoxy-6-phenylpyridoxol hydrochloride (**8**)	all	1.46 ± 0.02	101 ± 2	2.01 ± 0.03	97 ± 2
benzoic acid (**9**)	C-6	1.34 ± 0.02	92 ± 2	2.02 ± 0.02	98 ± 2
N-benzoyl-α-naphthylamine	C-6	1.35 ± 0.02	93 ± 2	1.94 ± 0.03	94 ± 2

[a] SA = specitic activity (dpm per mmol) × 10^{-4}. [b] RSA = relative specific activity (%) (3,4'-*O*-isopropylidenepyridoxol hydrochloride = 100%).

Scheme I



and converted into pyridoxol hydrochloride (**2**) after dilution with inactive carrier (383 and 315 mg, respectively). The labeled samples of pyridoxol were degraded by the reaction sequence shown in Scheme I, which permits assay of label at C-6. All activity within the pyridoxal derived from [2-^{14}C]glycine was localized at C-6 (Table I).

In a further experiment (experiment 3) a culture of *E. coli* B WG 3 (6 × 1 L, 1.5 g/L glycerol, 50 mg/L glycolaldehyde) was incubated in the presence of intramolecularly doubly enriched (2-^{13}C,^{15}N)glycine (90 atom % ^{13}C, 99 atom % ^{15}N, Merck Sharp & Dohme Isotopes) (30 mg/L). Pyridoxal was isolated and converted into pyridoxol hydrochloride after dilution with 6 mg of natural abundance carrier. A section of the downfield region of the ^{13}C NMR spectrum of the purified[5] labeled product is shown in Figure 1A. Comparison of the spectrum of the pyridoxol hydrochloride derived from (2-^{13}C,^{15}N)glycine with that of a natural abundance sample[7] (Figure 1B) indicates that, as expected on the basis of the results of experiments 1 and 2, only the signal due to C-6 of the labeled sample shows enhancement due to enrichment in ^{13}C. This is clearly shown by a difference spectrum (Figure 1C), obtained by subtraction of the two spectra.

In the difference spectrum the enriched C-6 signal appears as a doublet, due to ^{13}C-6,^{15}N coupling ($J_{^{13}C\text{-}6,^{15}N}$ 7.0 ± 1.0 Hz).[13] It is evident that the ^{15}N–^{13}C unit of the administered glycine had been incorporated intact into pyridoxal. Glycine is thus identified as a precursor in the "glycolaldehyde" pathway to pyridoxal.

The possibility that glycine, together with glycolaldehyde, might play a role in the biosynthesis of vitamin B_6 in *E. coli* mutants was first noted as long as 25 years ago[14] on the basis of growth studies. The present results place these early observations into biosynthetic focus.

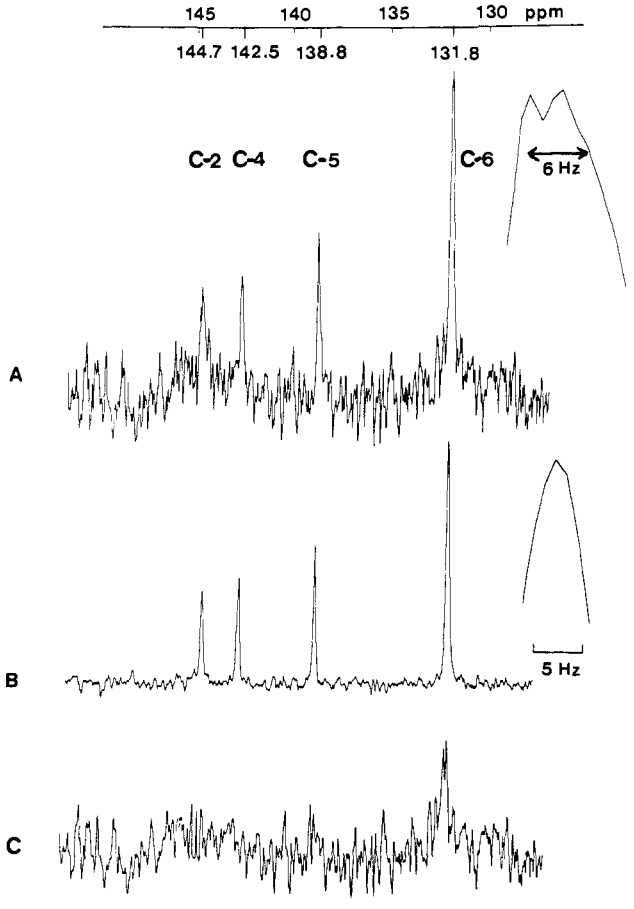


Figure 1. Sections, between δ 120 and 150, of (A) the proton noise decoupled (PND) ^{13}C NMR spectrum (149 577 scans) of the ^{13}C,^{15}N-enriched sample of pyridoxol hydrochloride (4 mg/50 μL D_2O) derived from (2-^{13}C,2-^{15}N)glycine (inset, expansion of the crest of the C-6 signal (δ 131.8) to show broadening and multiplicity), (B) the PND ^{13}C NMR spectrum (70 000 scans) of a natural abundance sample of pyridoxol hydrochloride (6 mg/50 μL D_2O) (inset, expansion of the crest of the C-6 signal, on the same scale as the expansion in spectrum A (5 Hz = 0.08 ppm)), (C) the difference spectrum (A minus B). The ^{13}C NMR spectra were recorded on a Bruker WM 250 spectrometer operating at 5.872 T and 62.9 MHz in the pulse Fourier transform mode at ambient temperature, using a 2.5-mm microprobe. Acquisition time 0.88 s, 2.13 Hz/data point; 2-μs pulses.

Acknowledgment. This work was supported by grants from the Medical Research Council of Canada (to R.E.H.) and the Natural Sciences and Engineering Research Council of Canada (to I.D.S.). We thank Richard Pauloski for skilled technical assistance.

Registry No. 1, 66-72-8; **2**, 65-23-6; **2**·HCl, 58-56-0; **3**, 6953-28-2; **4**, 6562-92-1; **5**, 88855-12-3; **6**, 83492-45-9; **7**, 88855-13-4; **8**, 88855-14-5; **9**, 65-85-0; *N*-benzoyl-α-naphthamide, 634-42-4; glycine, 56-40-6; carbon, 7440-44-0; nitrogen, 7727-37-9.

(13) Multiplicity is observable also in the expanded C-6 signal in the spectrum of the enriched sample (Figure 1A, inset). The broadening in the nonenriched C-2 signal (Figure 1A) is presumably due to coupling of natural abundance ^{13}C-2 to ^{15}N, as a consequence of a high level of ^{15}N incorporation.

(14) Morris, J. G. *J. Gen. Microbiol.* **1959**, *20*, 597–604.