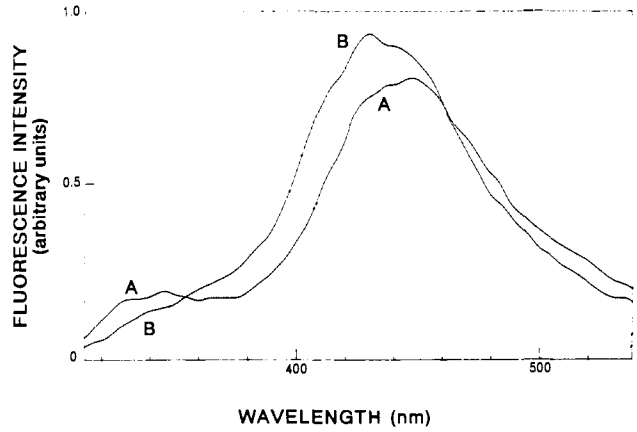

Figure 2. Luminescence dependence on pressure for ester **1** (10^{-6} M) in CHF_3 at 28 °C with excitation at 282 nm. Curve A: emission spectrum at p = 102.0 bar; maxima 350 and 446 nm. Curve B: emission spectrum at p = 46.3 bar; maximum 430 nm. Curves A and B are corrected for changes in relative absorbance efficiency.

energy have been reported. Explanations for these anomalous phenomena generally include factors such as the dependence of intersystem crossing rate on excitation energy, the formation of different solvation sites at low temperatures, and the existence of different conformers in the ground state. Since the $n\pi^*$ state of the ester **1** is high in energy,[7] it is unlikely that phosphorescence from the triplet can account for our observation that low-energy excitation causes enhanced TICT emission. In rigid media, the existence of different solvation sites or different conformers are satisfactory explanations for the anomalous behavior of closely related TICT-forming systems.[10,11] However, we observed excitation energy dependence in fluid densities of CHF_3 ranging from gas-like to liquid-like. We suggest that the excitation dependence for **1** in CHF_3 at 28 °C may be related to differential hydrogen bonding of the ester functionality. An equilibrium distribution of at least two species which differ in the extent to which the ester functionality is hydrogen-bonded would rationalize our observations.

Figure 2 depicts the emission of **1** in CHF_3 at 28 °C as a function of pressure. An increase in pressure and, therefore, an increase in the dielectric constant of the medium,[3] led to a decrease in the intensity and a red-shift of the long wavelength TICT emission. This was accompanied by an increase in the intensity and no shift in the short wavelength planar emission. Normally, with an increase in solvent polarity the TICT band both shifts to the red and gains in relative intensity.[7] The observed decrease in TICT intensity with increasing pressure suggests that the increase in viscosity of the medium with pressure[3] has an effect on the kinetics of relaxation to the TICT species on the excited-state surface. In fact, this TICT emission intensity dependence on pressure represents further experimental verification of the "twist hypothesis", which dictates hydrodynamic control[7] in the dual fluorescence of **1**.

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Registry No. 1, 10287-53-3; CHF_3, 75-46-7.

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Biosynthesis of Vitamin B_6: Incorporation of D-1-Deoxyxylulose

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We have shown[3] that the C_8 skeleton of pyridoxol (vitamin B_6) is derived in toto from the carbon atoms of glucose and, furthermore, that the only two carbon–carbon bonds of the pyridoxol skeleton that are formed de novo in the course of its biosynthesis from glucose are the bonds C-2,3 and C-4,5. This finding confirmed inferences that were drawn from earlier tracer experiments,[4-7] which showed that the C_3 units of pyridoxol, C-3,-4,-4′ and C-5′,-5,-6, were derived from intact triose phosphate generated from glucose by the normal glycolytic sequence and that the C_2 unit, C-2′,-2, of pyridoxol was generated from one such triose phosphate by loss of a terminal carbon atom. We now present evidence that an intact pentose derivative, D-1-deoxyxylulose (**2**), gives rise to the C_5 unit, C-2′,-2,-3,-4,-4′, of pyridoxol (**3**), i.e., of the unit generated from the C_2 plus one of the C_3 precursors.

In separate experiments cultures of *Escherichia coli* B WG2 were incubated, as described earlier,[4] with D-glucose as the general carbon source, in the presence of D-1-deoxy[1,1,1-$^2H_3$,(*RS*)-5-$^2H_1$]xylulose[8] (**2**) (experiment 1) and L-1-deoxy[1,1,1-$^2H_3$,-(*RS*)-5-$^2H_1$]xylulose[8] (experiment 2), respectively. Pyridoxol hydrochloride was isolated from each culture after addition of natural abundance pyridoxol hydrochloride (2.5 mg) as carrier and purified by column and thin-layer chromatography, followed by high vacuum sublimation.

The ^2H NMR spectra of the isolated samples of pyridoxol hydrochloride (ca. 1.7 mg in 50 μL of methanol, saturated solution) were recorded on a Bruker AM 500 spectrometer (Figure 1).

The spectra of the two samples (Figure 1 (parts B and D)) were different.

The spectrum of the sample of pyridoxol hydrochloride in methanol, from the *E. coli* B WG2 culture incubated with deuteriated L-1-deoxyxylulose (experiment 2) (Figure 1D), was identical with the natural abundance deuterium spectrum of the solvent (Figure 1E). Evidently, deuterium from this substrate had not been incorporated into pyridoxol.

The spectrum of the sample of pyridoxol hydrochloride, obtained from the incubation with deuterium-labeled D-1-deoxyxylulose (experiment 1) (Figure 1B), showed three signals. One of these, at δ 2.54 ppm, is readily assignable to the C-methyl group, C-2′

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(8) D-1-Deoxy[1,1,1-$^2H_3$,(*RS*)-5-$^2H_1$]xylulose (**2**) was synthesized in six steps from D-arabinose, by a published method.[9] The product **2** was obtained, in the final step of the synthesis, by acid hydrolysis of D-1-deoxy-3,5-*O*-benzylidene[1,1,1-$^2H_3$,(*RS*)-5-$^2H_1$]xylulose (**1**), whose purity and deuterium content was determined by ^1H and ^2H NMR spectroscopy: ^1H NMR (CCl_4) δ (ppm) 3.95 (1 H, H-4), 4.06 (0.4 H, H-5β), 4.20 (0.6 H, H-5α), 4.25 (1 H, H-3), 5.60 (1 H, PhC*H*); ^2H NMR (CCl_4) δ (ppm) 2.26 (3 ^2H, CD_3), 4.09 (0.61 ^2H, D-5β), 4.23 (0.43 ^2H, D-5α); ^2H content at the C-methyl group 100%; at 5β 61%; at 5α 43%; ratio: ^2H at CD_3/^2H at (5β plus 5α) = 3/(1 × (0.61 + 0.43)) = 3:1.04. L-1-Deoxy[1,1,1-$^2H_3$,(*RS*)-5-$^2H_1$]xylulose was obtained analogously, from L-arabinose. In each of the two experiments, a culture of *E. coli* B WG2 was incubated in the presence of D-glucose as the general carbon source with the deuterium-labeled samples of 1-deoxyxylulose. Expt. 1: D-deoxy[1,1,1-$^2H_3$,(*RS*)-5-$^2H_1$]xylulose (1 g), D-glucose (1 g). Expt. 2: L-1-deoxy[1,1,1-$^2H_3$,(*RS*)-5-$^2H_1$]xylulose (0.7 g), D-glucose (0.7 g).

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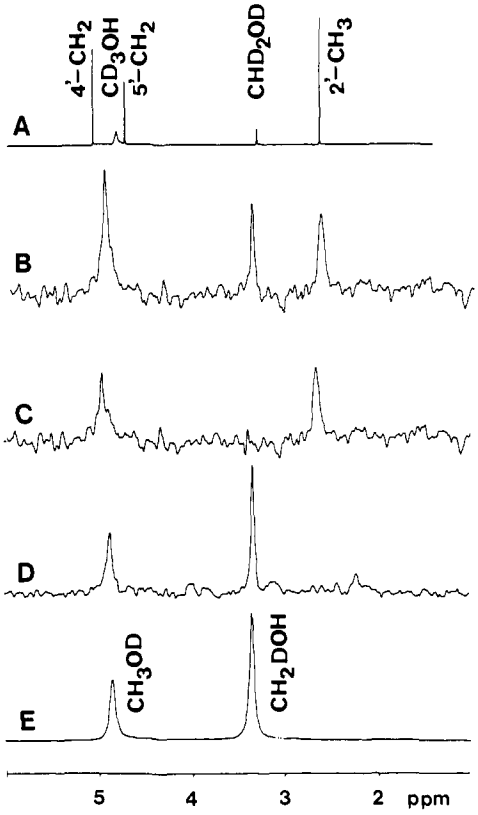
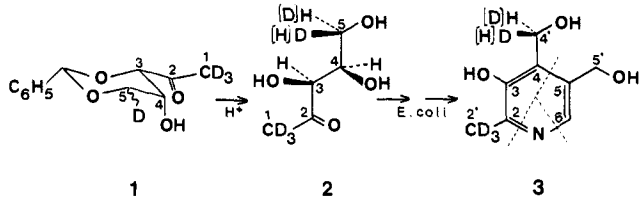


Figure 1. (A) Low-frequency region of the 500.13 MHz ^1H NMR spectrum of pyridoxol hydrochloride (in deuteriomethanol). (B) 76.775 MHz ^2H NMR spectrum of pyridoxol hydrochloride (ca. 1.7 mg in 50 μL of methanol) obtained from *E. coli* B WG2 after incubation with D-1-deoxy[1,1,1-$^2H_3$,(*RS*)-5-$^2H_1$]xylulose. (C) Difference spectrum (B minus E). (D) 76.775 MHz ^2H NMR spectrum of pyridoxol hydrochloride (ca. 1.7 mg in 50 μL of methanol) obtained from *E. coli* B WG2 after incubation with L-1-deoxy[1,1,1-$^2H_3$,(*RS*)-5-$^2H_1$]xylulose (experiment 2). (E) 76.775 MHz ^2H NMR spectrum (natural abundance) of methanol. The spectra were determined on a Bruker AM 500 spectrometer. Spectral parameters: spectral width, 2000 Hz; memory size, 16 K; pulse flip angle, 45°. To obtain adequate signal-to-noise ratio in the deuterium spectra (B and D) more than 25 000 transients were required.

Scheme I



(c.f., ^1H NMR spectrum of pyridoxol hydrochloride, Figure 1A). The second signal, at δ 3.30 ppm, corresponds to the natural abundance deuterium signal of the -CDH_2 group of the solvent (c.f., natural abundance ^2H NMR spectrum of methanol, Figure 1E). The third signal, at δ 4.95 ppm, located at a chemical shift corresponding both to the C-4′ hydroxymethyl protons of pyridoxol HCl (c.f., Figure 1A) as well as to that of the -OD group of the solvent (c.f., Figure 1E), is in fact a composite of both, as shown by the difference spectrum (Figure 1C, i.e., B minus E).

Thus, deuterium from the deuteriated sample of D-1-deoxyxylulose entered the predicted sites, C-2′ and C-4′, and only the predicted sites, of pyridoxol hydrochloride. Furthermore, since L-1-deoxyxylulose did not deliver deuterium into the vitamin, the incorporation of deuterium is stereochemically controlled.

These results lead to the inference that the intact C_5 skeleton of D-1-deoxyxylulose (**2**) enters pyridoxol (**3**) to supply the C_5 unit, C-2′,-2,-3,-4,-4′. If carbon-carbon cleavage of the precursor molecule, into a C_2 and a C_3 unit, had occurred prior to entry into pyridoxol, then deuterium would have been incorporated not only into C-2′ and C-4′, as observed, but also into C-5′ (c.f., ref 3).

Because of the superposition of signals at δ 4.95 ppm in the ^2H NMR spectrum of labeled pyridoxol hydrochloride in methanol (Figure 1B) and the unfavorable signal-to-noise ratio in the difference spectrum (Figure 1C) the ratio, signal area at δ 2.54 ppm (CD_3)/signal area at δ 4.95 ppm (CDH-4′), cannot be determined with precision. Intact incorporation of precursor into pyridoxol demands that this ratio be 3 (see ref 8). The observed value (Figure 1C) can be estimated to be <3 but >2.

Even though it is now demonstrated that the intact carbon skeleton of D-1-deoxyxylulose enters the C_5 unit, C-2′,-2,-3,-4,-4′, of pyridoxol, the question still remains whether the compound itself, or the corresponding 4-oxo derivative, lies on the direct route into pyridoxol from the C_2 and C_3 units, pyruvate and dihydroxyacetone phosphate, that give rise to the C-2′,-2 and C-3,-4,-4′ moieties, respectively.[6,7]

It is of interest that D-1-deoxyxylulose, which is now shown to be implicated in the biosynthesis of vitamin B_6 in *E. coli*, also serves as a precursor of the thiazole unit of vitamin B_1 in the same organism[9] and that a D-1-deoxypentulose has been postulated as an intermediate of the biosynthesis of vitamin B_2.[10]

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Electron-Transfer Reactions in the Marcus Inverted Region. Charge Recombination versus Charge Shift Reactions

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The recent experimental verification[1] of the Marcus inverted region[2] has stimulated renewed theoretical interest in electron-transfer processes. Kakitani and Mataga[3] have described a model in which partial solvent dielectric saturation of ionic species involved in electron-transfer reactions results in a negligible decrease in rate in the inverted region for reactions in which two neutral species yield two ionic species (termed charge separation reactions, CS). The model also predicts a pronounced inverted region for charge recombination reactions (CR) in which two ions yield two neutral species and intermediate behavior for charge shift (CSH)

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