The Conversion of 3-Hydroxy-2,4,5-trihydroxymethylpyridine into Pyridoxine by *Kloeckera apiculata*

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Kloeckera apiculata, a vitamin B-6-dependent yeast, grows in the presence of 3-hydroxy-2,4,5-trihydroxymethylpyridine in vitamin B-6-free media. On a molar basis the growth-promoting activity of this compound is approximately one-tenth that of other forms of vitamin B-6. [$G-^3H$]3-Hydroxy-2,4,5-trihydroxymethylpyridine is converted into radioactive vitamin B-6 compounds of the same specific radioactivity by growing cultures of *K. apiculata*.

The biological synthesis of vitamin B-6 has not yet been elucidated. Studies on the incorporation of various ^{14}C -labelled precursors into pyridoxine in *Escherichia coli* (Hill & Spenser, 1973) and *Saccharomyces fragilis* (Schroer & Frieden, 1973) show that the C-2'-methyl group of pyridoxine probably arises from a more oxidized carbon atom which becomes reduced at a later stage of synthesis. The report by Argoudelis (1968) that 3-hydroxy-2,4,5-trihydroxymethylpyridine permits the growth of *Saccharomyces carlsbergensis* in the absence of vitamin B-6 is therefore of interest. Further studies to determine whether this compound gives rise to a product that can substitute for pyridoxal 5'-phosphate, or whether it undergoes conversion into pyridoxal 5'-phosphate, have not been reported. The first logical step in the conversion of 3-hydroxy-2,4,5-trihydroxymethylpyridine into pyridoxal 5'-phosphate would be the reduction of the 2'-hydroxymethyl group to a 2'-methyl group, i.e. reduction to pyridoxine. In our laboratory it has been found that growth of the vitamin B-6-dependent yeast, *Kloeckera apiculata*, is supported by 3-hydroxy-2,4,5-trihydroxymethylpyridine in the absence of vitamin B-6. Further, it has been shown that [$G-^3H$]3-hydroxy-2,4,5-trihydroxymethylpyridine is converted into 3H -labelled vitamin B-6 compounds by growing cells of *K. apiculata*.

Experimental

Materials

[$G-^3H$]pyridoxine hydrochloride was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. All inorganic chemicals and lactic acid (A.R. grade), nicotinic acid, thiamin hydrochloride and glucose (Laboratory Reagent grade), biotin, D-pantothenic acid, meso-inositol, DL-methionine and L-asparagine (Biochemical grade) were obtained from BDH Chemicals Ltd., Poole, Dorset, U.K. Unlabelled pyridoxine hydrochloride, pyridoxal hydrochloride, pyridoxamine dihydrochloride, pyridoxal 5'-phosphate,

and pyridoxamine 5'-phosphate were from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey KT2 7BH, U.K. *m*-Chloroperoxybenzoic acid was from Ralph N. Emanuel Ltd., Wembley, Middx., U.K. The casein hydrolysate was a vitamin-free, acid-hydrolysed preparation of casein, known as 'casamino acids', purchased from Difco Laboratories, Detroit, Mich., U.S.A. *Kloeckera apiculata* (National Collection of Yeast Cultures no. 245) was obtained from The Brewing Industry Research Foundation, Nutfield, Redhill, Surrey, U.K. Crystallized bovine plasma albumin was from Armour Pharmaceutical Co., Eastbourne, Sussex, U.K. The basal medium for *Kloeckera apiculata* was the same as that described by Barton-Wright (1961) for the microbiological assay of the vitamin B-6 complex with *Kloeckera brevis*, now renamed *K. apiculata* (Lodder, 1971).

Methods

Measurement of radioactivity. Liquid samples (1, 10 or 100 μ l) were placed in 5 ml of liquid-scintillation mixture (100g of naphthalene, 5g of 2,5-diphenyloxazole made up to 1 litre in 1,4-dioxan), and counted to an error of 1% or less in a Beckman LS 200B liquid-scintillation system. The efficiency of counting was 27.5%.

Measurement of protein. Protein was measured by the method of Lowry *et al.* (1951) bovine plasma albumin being used as the standard.

Microbiological measurement of vitamin B-6. Vitamin B-6 was measured microbiologically by the method of Barton-Wright (1961).

Synthesis of 3-hydroxy-2,4,5-trihydroxymethylpyridine hydrochloride. 3-Hydroxy-2,4,5-trihydroxymethylpyridine hydrochloride was prepared as described by Bedford *et al.* (1963), with two modifications. In the oxidation of 3-acetoxy-4,5-di(acetoxymethyl)-2-methylpyridine (Scheme 1, compound II) to its *N*-oxide (compound III), *m*-chloroperoxybenzoic acid was used in place of perbenzoic

Scheme 1. Chemical synthesis of 3-hydroxy-2,4,5-trihydroxymethylpyridine (V) from pyridoxine (I)

(II), 3-Acetoxy-4,5-di(acetoxymethyl)-2-methylpyridine; (III), 3-acetoxy-4,5-di(acetoxymethyl)-2-methylpyridine 1-oxide; (IV), 3-acetoxy-2,4,5-tri(acetoxymethyl)pyridine. Procedures (small numbers above arrows): 1, Pyridine/acetic acid (1:1, v/v), 3h, 100°C, free base released with Na_2CO_3 ; 2, *m*-chloroperoxybenzoic acid in CHCl_3 ; 3, heated under reflux for 2h with acetic anhydride; 4, heated under reflux for 45min with 4M-HCl. Abbreviation: Ac, acetyl.

acid. The final product was not recrystallized; it was purified by column chromatography on Dowex 50 (H^+ form) with a concave gradient of 0–6M-HCl.

Synthesis of $[G\text{-}^3\text{H}]$ 3-hydroxy-2,4,5-trihydroxymethylpyridine hydrochloride. The sequence of reactions used for the synthesis of unlabelled 3-hydroxy-2,4,5-trihydroxymethylpyridine hydrochloride, and shown in Scheme 1, were adapted to the micro-scale preparation of the ^3H -labelled compound.

$[G\text{-}^3\text{H}]$ Pyridoxine hydrochloride (1 mCi, 0.137mg) was purchased as a sterile 1ml aqueous solution. This was evaporated to dryness by placing the opened vial in an evacuated desiccator over P_2O_5 in the dark. By using 1ml quantities of a mixture of pyridine and acetic anhydride (1:1, v/v), the contents of the vial were washed into a graduated 25ml tube until the total volume was 5ml. The tube was fitted with a condenser, and the solution was heated for 3h at 100°C on a boiling-water bath. After standing overnight at room temperature (15–20°C), the pyridine and acetic anhydride were removed by rotary evaporation. Diethyl ether (10ml) and a few drops of water were added to the residual oil. Solid Na_2CO_3 was then added and triturated with a glass rod, in order to neutralize any remaining acetic anhydride or acetic acid, and to convert the product into its free base (compound II). The ether solution, containing excess of solid Na_2CO_3 , was dried over anhydrous MgSO_4 , filtered and evaporated under N_2 . At this stage the product (compound II) always contained some pyridine, as judged from the smell. A solution of *m*-chloroperoxybenzoic acid (0.5g) in chloroform (5ml) was added to the impure residue of compound (II), and left for 24h at

room temperature in a 25ml graduated glass-stoppered tube. The solution was then treated with saturated NaHCO_3 (15ml), which was sufficient to neutralize and extract all the organic acids. Portions (10 μl each) of the chloroform and aqueous layers were taken for the determination of ^3H radioactivity. The aqueous layer and the chloroform layer always contained an approximately equal total amount of radioactivity (about 2.7×10^8 c.p.m.), i.e. the maximum possible yield of the *N*-oxide (III) at this stage was 50%. The chloroform layer was separated, washed once with water, dried over anhydrous MgSO_4 and evaporated to dryness under N_2 . The product (compound III) was a trace of oil, which contained small crystals of the impurity pyridine *N*-oxide. This was heated under reflux for 2h with acetic anhydride (8ml) to form 3-acetoxy-2,4,5-tri(acetoxymethyl)pyridine (compound IV). The acetic anhydride was removed by rotary evaporation and compound (IV) was hydrolysed to compound (V) by heating under reflux for 45min with 4M-HCl (10ml). Water and HCl were removed by rotary evaporation and the product was applied to a column (3cm $\times$ 20cm) of Dowex 50 (H^+ form). A concave gradient of 0–6M-HCl (produced from 600ml of water and 500ml of 6M-HCl) was passed through the column, and 10ml fractions were collected. Samples (10 μl) of each fraction were taken for the assay of ^3H . The hydrochloride of 3-hydroxy-2,4,5-trihydroxymethylpyridine (compound V) was eluted at $V_e = 340\text{ml}$, in a total volume of 60ml. ^3H -labelled by-products, which were eluted earlier, accounted for about half of the total ^3H . The chief by-product, which was unidentified, and did

not support the growth of *K. apiculata*, was eluted at $V_e = 110\text{ml}$. In this system, pyridoxine is eluted at $V_e = 420\text{ml}$; if it were present, it would be well separated from the product. Fractions containing the product were dried by rotary evaporation, and the product was stored in a desiccator under decreased pressure over KOH in the dark. The product was chromatographically homogeneous, and showed the same behaviour as authentic 3-hydroxy-2,4,5-trihydroxymethylpyridine hydrochloride in the following systems: (1) paper chromatography in butan-1-ol/acetic acid/water (4:1:1, by vol.); (2) column chromatography on Dowex 50 (H^+ form) with 0.6M-HCl as described above; (3) column chromatography on Dowex 50 (H^+ form) with a combined pH-concentration gradient of ammonium formate (Tiselius, 1972). In each of these systems, 3-hydroxy-2,4,5-trihydroxymethylpyridine is well separated from pyridoxal, pyridoxine, pyridoxamine and their phosphates, with the exception that there is some overlap with pyridoxal on the second Dowex column. The i.r. spectrum of the product was identical with that of authentic 3-hydroxy-2,4,5-trihydroxymethylpyridine hydrochloride. The mass spectrum of the hydrochloride of compound (V) showed a mass ion peak at 185, as expected for the free base $\text{C}_8\text{H}_{11}\text{O}_4\text{N}$. The specific radioactivity of the product was 1.05Ci/mmol , which indicates that 30% of the ^3H of the original radioactive pyridoxine was labilized during the preparation. This is in accordance with the manufacturer's claim that about 70% of the ^3H is located in position 6 of the pyridine ring. ^3H atoms in any other position of the molecule would be more labile, especially under acid conditions, and they are presumably lost during the preparation.

Growth of culture. Growth was followed by measurement of the E_{540}^{cm} of cell suspensions. The relationship between E_{540} and dry cell weight was determined in separate experiments by the procedure of Meynell & Meynell (1970).

A loop of *K. apiculata* from a stock slope was suspended in sterile water (5ml). The cells were packed by centrifugation and resuspended in sterile water (5ml). This suspension was added to the basal medium (500ml), which was incubated overnight at 30°C with forced aeration. Growth under these conditions in the absence of exogenous vitamin B-6 resulted in a resting cell population equivalent to $10\text{--}20\mu\text{g}$ dry wt./ml. This slight growth is due to the contamination of the basal medium with vitamin B-6, equivalent to $20\text{--}40\text{ng}$ of pyridoxine/litre.

Compounds under investigation for vitamin B-6 activity were then added to the stationary cell culture, and the E_{540} was measured at intervals of 10–15min.

Preparation of cell extracts of *K. apiculata*.

Basal medium (1litre), containing $[\text{G-}^3\text{H}]$ pyridoxine hydrochloride ($10\mu\text{Ci}$; 6.7nmol), or $[\text{G-}^3\text{H}]$ 3-hydroxy-2,4,5-trihydroxymethylpyridine hydrochloride ($100\mu\text{Ci}$; 67.0nmol) was inoculated with *K. apiculata* and incubated at 30°C with forced aeration. During growth (at a cell density of approx. $280\mu\text{g}$ dry cell wt./ml), or 1h after the end of growth (at $380\mu\text{g}$ dry cell wt./ml) the cells were collected by centrifugation at 0°C for 15min at $12000g$ (MSE High-Speed 18 centrifuge, $6 \times 250\text{ml}$ angle rotor no. 69179, at 9000rev./min). The cells were resuspended in basal growth medium (250ml) and collected again by centrifugation. The washed cells were resuspended in phosphate buffer (10ml) (approx. $0.1\text{M-KH}_2\text{PO}_4$ adjusted to pH7.2 with NaOH solution and diluted to 0.05M) and the suspension was passed three times through a French pressure cell at 137.5MPa (20000lb/in^2). Cell debris was removed by centrifugation at $9000g$ (Christ Junior 15000 centrifuge, $6 \times 25\text{ml}$ rotor at 12000rev./min), the supernatant volume was adjusted to 10ml with $0.05\text{M-KH}_2\text{PO}_4$ buffer, pH7.2, and samples were taken for the determination of protein, radioactivity and total vitamin B-6 by microbiological assay.

Separation of protein-bound vitamin B-6 and free vitamin B-6 in extracts of *K. apiculata*. The cell extract (about 10ml) was placed on a column ($2.8\text{cm} \times 37\text{cm}$) of Sephadex G-200 equilibrated with $0.05\text{M-KH}_2\text{PO}_4$ buffer, pH7.2, and the column was eluted with the same buffer. Column fractions (10ml) were assayed for radioactivity and for E_{280} . The column effluent showed two separate peaks of radioactivity. The earlier peak ($V_e = 82\text{ml}$, total volume 70ml) also contained most of the protein, and vitamin B-6 in this fraction was designated 'protein-bound vitamin B-6'. The later peak ($V_e = 210\text{ml}$, total volume 70ml) was designated 'free vitamin B-6'.

Pyridoxine hydrochloride, pyridoxine 5'-phosphate, pyridoxal hydrochloride, pyridoxal 5'-phosphate, pyridoxamine dihydrochloride and pyridoxamine 5'-phosphate (1mg of each) were added to the free vitamin B-6 fraction, which was adjusted to pH10 with NaOH solution, then placed on a column ($2.5\text{cm} \times 8\text{cm}$) of Dowex 1 (Cl^- form). Then 0.01M-NaOH (200ml) was passed through the column, followed by 0.1M-HCl . Radioactivity corresponding to vitamin B-6 emerged from the column with the acid front and was collected in a total volume of 150ml. Although some fractionation of vitamin B-6 compounds occurred at this stage, the total radioactive eluate was pooled and dried by rotary evaporation. The product was stored in the dark for 24h at decreased pressure over KOH to remove the final traces of HCl, then analysed by chromatography on a column ($0.9\text{cm} \times 40\text{cm}$) of Dowex 50 (H^+ form) with a combined pH-concentration gradient of ammonium formate as described by Tiselius (1972). The radioactivity of each fraction

(5 ml) was measured and vitamin B-6 compounds were detected and identified by u.v. spectroscopy.

This column does not separate 3-hydroxy-2,4,5-trihydroxymethylpyridine and pyridoxal. The column fractions containing 3-hydroxy-2,4,5-trihydroxymethylpyridine and/or pyridoxal were combined and the total radioactivity was determined. To remove ammonium formate, the mixture was chromatographed on Dowex 1 (Cl^- form) as described above, and pyridoxal and 3-hydroxy-2,4,5-trihydroxymethylpyridine were collected in a volume of 20 ml; this was dried by rotary evaporation and the residue was dissolved in 0.1 M-NaOH (5 ml). Solid NaBH_4 (100 mg) was added gradually over a period of 2 min with stirring, to convert the pyridoxal into pyridoxine. The mixture was acidified to pH 3.4 with 2 M- H_2SO_4 and the 3-hydroxy-2,4,5-trihydroxymethylpyridine and pyridoxine were separated on a column (0.9 cm $\times$ 40 cm) of Dowex 50 (H^+ form) by the method of Tiselius (1972) as described above. The ratio of 3-hydroxy-2,4,5-trihydroxymethylpyridine to pyridoxine determined by radioactivity from this separation was taken to represent the original ratio of 3-hydroxy-2,4,5-trihydroxymethylpyridine to pyridoxal.

Analysis of protein-bound vitamin B-6. Pyridoxine hydrochloride, pyridoxine 5'-phosphate, pyridoxal hydrochloride, pyridoxal 5'-phosphate, pyridoxamine dihydrochloride, and pyridoxamine 5'-phosphate (1 mg of each) were added to the protein-bound vitamin B-6 fraction, followed by trichloroacetic acid to a final concentration of 5% (w/v). The precipitated protein was removed by centrifugation and the supernatant was shaken with an equal volume of diethyl ether to extract the trichloroacetic acid. The aqueous layer was then adjusted to pH 10 with NaOH solution and placed on a column (2.5 cm $\times$ 8 cm) of Dowex 1 (Cl^- form). The analysis was then performed as described above for free vitamin B-6.

Results and Discussion

Growth of K. apiculata at the expense of 3-hydroxy-2,4,5-trihydroxymethylpyridine

Under the conditions described, the maximum rate of growth of *K. apiculata* was supported by 0.02 μmol of pyridoxine, pyridoxal or pyridoxamine/litre. Higher concentrations of any form of vitamin B-6 caused no further increase in the growth rate, and lower concentrations supported slower growth rates. At this maximum growth rate, the time required for doubling the dry cell mass was 75 min. At concentrations higher than 0.0125 μmol /litre, pyridoxine, pyridoxal and pyridoxamine also supported a dry-cell-mass doubling time of 75 min for the first 4 h after the start of growth, but slower rates were then observed.

The shortest time for the doubling of dry cell mass supported by 3-hydroxy-2,4,5-trihydroxymethylpyri-

dine was 110 min, with the compound at a concentration of 0.2 μmol /litre; higher concentrations caused no further increase in growth rate. In the range 0.125–0.2 μmol of 3-hydroxy-2,4,5-trihydroxymethylpyridine/litre the differences in growth rate became manifest about 4 h after the start of growth.

In the microbiological assay of vitamin B-6 with *K. apiculata*, 8 nmol of 3-hydroxy-2,4,5-trihydroxymethylpyridine/litre, or 0.8 nmol of pyridoxine/litre support growth to a dry cell mass of 84 μg /ml. At higher concentrations pyridoxine supports growth even more efficiently so that 3.5 nmol of pyridoxine/litre supports growth to a final dry cell mass of 374 μg /ml, and 35 nmol of 3-hydroxy-2,4,5-trihydroxymethylpyridine/litre supports growth to a final dry cell mass of 240 μg /ml. In these growth studies, the lag period was always 75 min and it showed no dependence on the nature or concentration of the added compound.

Nevertheless, 3-hydroxy-2,4,5-trihydroxymethylpyridine can completely satisfy the vitamin B-6 requirement of *K. apiculata*. Cultures of this organism have been subcultured for more than a year in our laboratory on vitamin B-6-free media supplemented with 3-hydroxy-2,4,5-trihydroxymethylpyridine. On transfer to media containing pyridoxine they show the same rate of growth as cultures maintained on pyridoxine.

According to Lodder (1971) the cells of *K. apiculata* are apiculate (lemon-shaped), ovoid, or elongate, single, in pairs, or occasionally in groups of three or four; wide variations in cell size, i.e. (1.4–5.3) $\mu\text{m} \times$ (2.6–12.2) μm , are also encountered. Under the present growth conditions such wide variations in shape and size have not been observed. Normal bipolar budding occurred during growth at the expense of pyridoxine, or 3-hydroxy-2,4,5-trihydroxymethylpyridine, and the fully grown, slightly apiculate cells measured (4.2–4.4) $\mu\text{m} \times$ (2.6–2.8) μm (grown on pyridoxine), and (4.0–4.2) $\mu\text{m} \times$ (2.2–2.4) μm (grown on 3-hydroxy-2,4,5-trihydroxymethylpyridine), as determined by electron microscopy.

Analysis of vitamin B-6 compounds in cells of K. apiculata

Table 1 shows the concentrations of vitamin B-6 compounds in the cells of *K. apiculata* under different conditions of growth. The measurement of radioactivity and the microbiological determination of vitamin B-6 in the cell extract before the Sephadex chromatography showed that the specific radioactivity of the total vitamin B-6 was 70% of that claimed for the purchased pyridoxine. It appears therefore that 30% of the ^3H of the labelled pyridoxine became labilized when it was metabolized by *K. apiculata*, and when it was converted chemically into 3-hydroxy-2,4,5-trihydroxymethylpyridine. Probably the remaining 70% is located entirely in position 6 of the pyridine ring. The

quantity of vitamin B-6 was therefore calculated from total radioactivity of each column fraction, by using a specific radioactivity of 1.05 Ci/mmol.

During growth with pyridoxine, all the known forms of vitamin B-6 are found in *K. apiculata*. The highest concentration is shown by pyridoxamine (20.4 pmol/mg of protein) and the concentration of pyridoxine is relatively low (1.6 pmol/mg of protein). The presence of 4-pyridoxic acid 5'-phosphate may be an artefact, resulting from the photo-oxidation of pyridoxal 5'-phosphate (Reiber, 1972). However, the constancy of its occurrence in the free vitamin B-6 fraction of cells grown with pyridoxine, and its absence from all the other systems analysed, some of which contained relatively high concentrations of pyridoxal 5'-phosphate, suggest that it is a normal constituent of cells grown with pyridoxine. All the known forms of vitamin B-6 were also present in stationary cells grown with pyridoxine. The concentration of pyridoxamine 5'-phosphate, however, was markedly lower and it was detected only in the protein-bound vitamin B-6 fraction, whereas the concentration of pyridoxine was relatively high. Under these conditions, the extent of growth is still limited by the concentration of pyridoxine in the medium (starting concn. 6.7 nmol/litre). Since the intracellular concentration of pyridoxine in stationary cells (43.5 pmol/mg of protein) was as high as 90% of that (48.1 pmol/mg of protein) in growing cells, it is concluded that the intracellular concentration of vitamin B-6 was not in excess of that required for growth, and a small decrease in this concentration prevented further cell budding. On the other hand, the active coenzyme form of vitamin B-6 is pyridoxal 5'-phosphate, which showed a higher concentration in stationary cells than in growing cells.

During and after growth with 3-hydroxy-2,4,5-trihydroxymethylpyridine, not all vitamin B-6 compounds were present in detectable quantities. In both stationary and growing cells, the predominant compound was 3-hydroxy-2,4,5-trihydroxymethylpyridine; thus the total concentration of true vitamin B-6 compounds (11.4 pmol/mg of protein in growing cells; 17.3 pmol/mg of protein in stationary cells) was lower, whereas the total concentration, including 3-hydroxy-2,4,5-trihydroxymethylpyridine, was

higher than in cells grown with pyridoxine. In view of this relatively large accumulation of 3-hydroxy-2,4,5-trihydroxymethylpyridine in the cells, the restricted ability of *K. apiculata* to use 3-hydroxy-2,4,5-trihydroxymethylpyridine as a supplement for true vitamin B-6 is not due to a slow rate of penetration of this compound into the cell.

These results show that *K. apiculata* converts 3-hydroxy-2,4,5-trihydroxymethylpyridine into other known forms of vitamin B-6. The high intracellular concentration of 3-hydroxy-2,4,5-trihydroxymethylpyridine raises the question of whether this compound might also substitute for true vitamin B-6, in addition to supporting growth by conversion in vitamin B-6. In this case, the oxidation and phosphorylation of 3-hydroxy-2,4,5-trihydroxymethylpyridine would be expected to produce a derivative that could replace pyridoxal 5'-phosphate. There is no evidence for the production of any such compound from 3-hydroxy-2,4,5-trihydroxymethylpyridine. Probably 3-hydroxy-2,4,5-trihydroxymethylpyridine is first converted into pyridoxine by reduction of the 2'-hydroxymethyl group. The reduction of an alcohol group to an alkyl group in living organisms is found in the reduction of ribonucleotides to deoxyribonucleotides (Reichard, 1967); the reduction of 5,10-methylenetetrahydrofolate (the dehydration product of hydroxymethyltetrahydrofolate) to methyltetrahydrofolate (Cathou & Buchanan, 1963); and, if the keto tautomer of 3-hydroxy-2,4,5-trihydroxymethylpyridine is considered, a plausible mechanism would be that shown in Scheme 2, which is analogous to the reduction of a hydroxy acid derivative in the synthesis of fatty acids (White *et al.*, 1973).

Since *K. apiculata* does not synthesize vitamin B-6 from simple precursors and it depends entirely on an external source of this vitamin, it is not possible to perform the necessary isotopic-tracer experiments, or to obtain mutants to test the possibility that 3-hydroxy-2,4,5-trihydroxymethylpyridine is a true biological precursor of vitamin B-6. For studies of this kind, it would be necessary to use organisms such as *Saccharomyces marxianus* (Pardini & Argoudelis, 1968), *Saccharomyces fragilis* (Schroer & Frieden, 1973), or *E. coli* (Dempsey, 1966), which are known to synthesize vitamin B-6 from simple precursors.

Scheme 2. Possible conversion of 3-hydroxy-2,4,5-trihydroxymethylpyridine into pyridoxine

(VI), Keto form of 3-hydroxy-2,4,5-trihydroxymethylpyridine; (VII), keto form of pyridoxine. Reactions: 1, dehydration; 2, hydrogenation.

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