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Synthesis and Biological Activity of Homologs of Pyridoxal and Pyridoxamine

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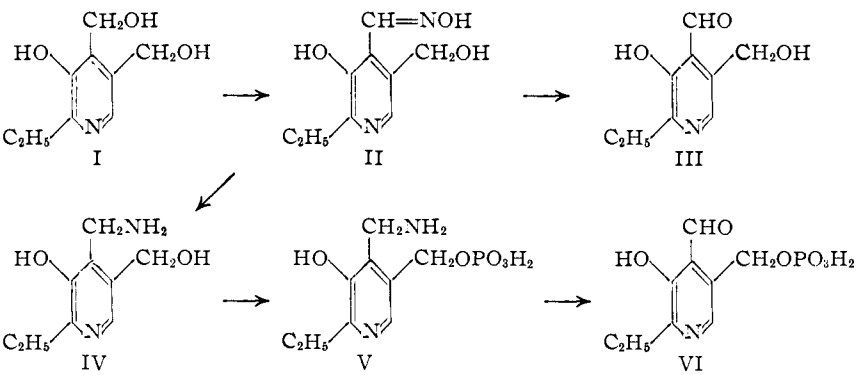
The synthesis of 2-ethyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine (ω-methylpyridoxal), of 2-ethyl-3-hydroxy-4-aminomethyl-5-hydroxymethylpyridine (ω-methylpyridoxamine) and of their corresponding 5-phosphoric acid esters is described. These homologs of vitamin B_6 are without growth-promoting activity for yeast, but act as antagonists of vitamin B_6 for this organism. Under the conditions tested they have growth-promoting activity for lactic acid bacteria varying from 1 to 34% that of vitamin B_6 itself.

A homolog of pyridoxine, 2-ethyl-3-hydroxy-4,5-bis-(hydroxymethyl)-pyridine (ω-methylpyridoxine (I)), synthesized by Harris and Wilson[1] was found by Rabinowitz and Snell[2] to be the most potent antagonist of vitamin B_6 for yeast so far tested. For this reason, the corresponding homologs of pyridoxal and pyridoxamine and their phosphoric acid esters become of considerable interest.



Compound I was oxidized with manganese dioxide at room temperature and the product, 2-ethyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine (ω-methylpyridoxal (III)), isolated as its oxime (II). II was converted to III with nitrous acid. II on hydrogenation in the presence of platinum catalyst gave 2-ethyl-3-hydroxy-4-aminomethyl-5-hydroxymethylpyridine (ω-methylpyridoxamine (IV)). IV was treated with phosphorus pentoxide in 85% phosphoric acid to give 2-ethyl-3-hydroxy-4-aminomethyl-5-pyridylmethylphosphoric acid (ω-methylpyridoxamine phosphate (V)), which on oxidation with manganese dioxide yielded 2-ethyl-3-hydroxy-4-formyl-5-pyridylmethylphosphoric acid (ω-methylpyridoxal phosphate, (VI)). The structures of the products are based on analogy with the reactions of pyridoxine, which are almost identically followed.

The effects of these compounds on growth vary remarkably with the test organism and the conditions, and will be considered in greater detail elsewhere. Results of a preliminary screening (Table I) show each of the unphosphorylated compounds to act as vitamin B_6 antagonists for yeast. For lactic acid bacteria they show growth-promoting activity similar to but of a lower order than that of the corresponding forms of vitamin B_6.

Experimental[3,4]

Oxime of 2-Ethyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine (II).—A generous sample of 2-ethyl-3-hydroxy-4,5-bis-(hydroxymethyl)-pyridine hydrochloride (I), m.p. 188–189°, was supplied by Dr. Karl Folkers of Merck and Co., Inc. To 0.915 g. of I in 22.5 ml. of 0.67 *N* hydrochloric acid was added 8.7 g. of powdered manganese dioxide. The mixture was shaken occasionally during two days at room temperature. Heyl[5] has reported the oxidation of

(1) S. A. Harris and A. N. Wilson, THIS JOURNAL, **63**, 2526 (1941).

(2) J. C. Rabinowitz and E. E. Snell, *Arch. Biochem. Biophys.*, **43**, 399, 408 (1953).

(3) All melting points are corrected.

(4) Microanalyses in part by Dr. A. Elek.

(5) D. Heyl, THIS JOURNAL, **70**, 3434 (1948).

TABLE I

COMPARATIVE ACTIVITIES OF ω-METHYLHOMOLOGS OF VITAMIN B_6 IN PROMOTING GROWTH OF MICROÖRGANISMS

Compound	Activity[a] for *Saccharomyces carlsbergensis* 4228[b] (pyridoxine = 100)	*Streptococcus faecalis* 8043[d] (pyridoxamine = 100)	*Lactobacillus casei* 7469[e] (pyridoxal = 100)	*Lactobacillus delbrueckii* 9649[f] (pyridoxamine phosphate = 100)
ω-Methylpyridoxine (I)	0.0[c]	<0.1	<0.1	...
ω-Methylpyridoxal (III)	.0[c]	1.6	34	...
ω-Methylpyridoxamine (IV)	.0[c]	1.4	<0.1	<0.1
ω-Methylpyridoxamine phosphate (V)	.0	2.0		10
ω-Methylpyridoxal phosphate (VI)	.0	0.11		<0.1

[a] The form of vitamin B_6 used as standard was that most active for each organism, and is indicated below the species name. Where shapes of the dose–response curves differ, average values are given. ω-Methylpyridoxal and the phosphoric acid esters were sterilized by filtration and added to previously autoclaved media. We are indebted to Dr. Beverly Guirard and Mr. R. P. Sandman for some of the assays. [b] Pyridoxal, pyridoxamine and pyridoxine are almost equally active for this organism; their phosphate esters are essentially inactive. [c] When tested in the presence of sub-optimal vitamin B_6, these compounds stimulate growth at low concentrations, then become inhibitory as their concentration is increased (*cf.* reference 2). Inhibition is alleviated by increasing the concentration of vitamin B_6. [d] Pyridoxamine, pyridoxal and pyridoxamine phosphate have similar activity for this organism; other forms of vitamin B_6 are comparatively inactive. [e] Of the several forms of vitamin B_6, only pyridoxal has significant activity for this organism. [f] Only pyridoxamine phosphate has high activity for this organism; unphosphorylated forms of vitamin B_6 are essentially inactive.

pyridoxine to pyridoxal with manganese dioxide at 60–70°, which conditions are also used by Heyl, *et al.*,[6] for the preparation of II. The reaction mixture was filtered and the manganese dioxide washed with a small amount of water. To the combined filtrates, 8 g. of sodium acetate trihydrate and 2 g. of hydroxylamine hydrochloride were added, and the mixture was heated on a steam-bath for two minutes. A crystalline precipitate was obtained almost immediately. After an hour at room temperature, the oxime II was filtered off, washed with water and dried; yield 0.484 g. (59%), m.p. 205–206° dec. Further recrystallizations from aqueous ethanol gave silky needles, m.p. 212–212.5° dec. Heyl, *et al.*,[6] report 225–226°.

Anal. Calcd. for $C_9H_{12}N_2O_3$ (196.2): C, 55.1; H, 6.2; N, 14.3. Found: C, 55.1; H, 6.1; N, 14.35.

2-Ethyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine Hydrochloride (III).—II (392 mg.) was treated with nitrous acid as described by Harris, *et al.*,[7] but using a 0.4 molar excess of sodium nitrite, and the reaction mixture concentrated to dryness in vacuum. To the residue 20 ml. of absolute ethanol was added, the insoluble sodium chloride filtered off and washed with a small amount of absolute ethanol, and the filtrates evaporated to dryness in vacuum. The process was repeated to remove a further small amount of sodium chloride. The sirupy residue was dissolved in 20 ml. of 0.3 *N* hydrochloric acid, heated on a steam-bath for 15 minutes, filtered and concentrated to dryness in vacuum. On trituration with acetone the resulting sirup crystallized, yielding 366 mg. (84%) of III. The product was recrystallized by dissolving in a small amount of water, concentrating to near dryness and adding acetone. It had no definite melting point but gradually softened and darkened from 150°. Like pyridoxal, it gave an intense yellow color in sodium hydroxide and its absorption spectrum on a molar basis was almost identical with that of pyridoxal at *p*H 1, 6.8 and 10.

Anal. Calcd. for $C_9H_{12}NO_3Cl$ (217.7): C, 49.7; H, 5.6; N, 6.4. Found: C, 49.2; H, 5.6; N, 6.2.

2-Ethyl-3-hydroxy-4-aminomethyl-5-hydroxymethylpyridine Dihydrochloride (IV).—To 20 ml. of 95% ethanol were added 1.6 ml. of 6 *N* hydrochloric acid and 0.3 g. of platinic oxide. The mixture was hydrogenated at 27° and 1 atm. until no more hydrogen was taken up. A solution of II (0.62 g.) in 50 ml. of ethanol was introduced into the hydrogenation flask and hydrogenation continued until 174 ml. of hydrogen had been taken up by the sample. The catalyst was filtered off, and the filtrate concentrated to dryness in vacuum. The resulting sirup solidified on scratching and was purified by dissolving in ethanol, adding ether to turbidity, scratching to induce crystallization, then adding additional ether in small portions until no further turbidity was observed; yield 0.634 g. (74%) of IV monohydrate. From the mother liquors an additional 51 mg. (6%) was obtained. The product was further purified by boiling in 5 ml. of *n*-propyl alcohol, adding water dropwise until the solid was completely dissolved, and cooling, whereby it crystallized in needles, m.p. 164–166°. The hydrochloride also had a tendency to crystallize in thick clumps melting at 99–102° but this could be avoided by seeding with the needles. The amine gave an orange color with ninhydrin similarly as pyridoxamine.

Anal. Calcd. for $C_9H_{16}N_2O_3Cl_2 \cdot H_2O$ (273.2): C, 39.6; H, 6.6; N, 10.3. Found: C, 40.05; H, 6.9; N, 10.9.

This compound (R_f 0.63) was readily separable from pyridoxamine (R_f 0.55) on paper chromatograms with 77% ethanol as the developing solvent. This test showed the compound to be free of pyridoxamine; bioautographs showed the growth zone with *S. faecalis* induced by it to coincide with that of the higher homolog.

2-Ethyl-3-hydroxy-4-aminomethyl-5-pyridylmethylphosphoric Acid (V).—The monohydrate of IV (0.634 g.) was phosphorylated and the product purified according to the procedure of Peterson, *et al.*,[8] for the preparation of pyridoxamine phosphate and yielded a total of 0.363 g. (53%) of V dihydrate which crystallized in fine white silky needles. On treatment with ninhydrin the product gave the orange color characteristic of pyridoxamine or its phosphate.

Anal. Calcd. for $C_9H_{15}O_6N_2P \cdot 2H_2O$ (298.2): C, 36.2; H, 6.4; P, 10.4. Found: C, 36.5; H, 6.4; P, 11.0.

2-Ethyl-3-hydroxy-4-formyl-5-pyridylmethylphosphoric Acid (VI).—The dihydrate of V (0.180 g.) was oxidized with manganese dioxide and purified as described by Peterson, *et al.*,[9] for the preparation of pyridoxal phosphate. Two yellow zones separated on the resin, the slower of which was lyophilized to yield a total of 100 mg. (56%) of VI dihydrate obtained as a fluffy, lemon-yellow mat with no apparent crystalline form.

Anal. Calcd. for $C_9H_{12}O_6NP \cdot 2H_2O$ (297.2): C, 36.4; H, 5.4; P, 10.4. Found: C, 36.9; H, 5.4; P, 10.4.

Growth Studies.—Cultural conditions for *S. carlsbergensis*, *S. faecalis* and *L. casei* were those used by Rabinowitz and Snell.[2,10] *Lactobacillus delbrueckii* (ATCC #9649) is representative of cultures that require pyridoxamine phosphate or pyridoxal phosphate for growth, and was grown as described by Peters, *et al.*,[11] but with pyridoxamine phosphate and D-alanine omitted from the medium, which was supplemented with 5 mg. per 10 ml. of histidine. The comparative activities of various naturally-occurring forms of vitamin B_6 for these organisms, summarized in the footnotes of Table I, have been considered elsewhere.[2,12–14]

AUSTIN, TEXAS

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