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# Synthesis and Enzymological Activity of 3-Hydroxy-2-*n*-propyl-4,5-pyridinedimethanol

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The conversion of 3-hydroxy-2-ethyl-4,5-pyridinedimethanol and 3-hydroxy-2-isopropyl-4,5-pyridinedimethanol to their corresponding aldehydes by yeast pyridoxine dehydrogenase was described by Melius and Marshall.[1] Also 3-hydroxy-2-methyl-6-chloro-4,5-pyridinedimethanol was found to be oxidized by the enzyme with an activity of the order of that for the *i*-Pr analog. The rate of reaction for the Me and Et analogs was about 4 times that for the *i*-Pr analog and the hydroxychloro compound. In the present report the synthesis and yeast pyridoxine dehydrogenase action on 3-hydroxy-2-*n*-propyl-4,5-pyridinedimethanol (VIII) is described.

The synthesis of VIII involved an initial condensation of ethyl-*n*-butyropyruvate and cyanoacetamide to form 4-carbethoxy-3-cyano-6-*n*-propyl-2-pyridone (I).[2] I was then carried through a sequence of reactions involving nitration,[3] chlorination, reduction with $SnCl_2$,[4] reduction with Pd-$H_2$,[3] hydrolysis with HCl,[3] diazotization, and reduction with $NaBH_4$,[6] to give finally VIII. Thus a modification of the reduction of the $NO_2$ group was utilized here, in which $SnCl_2$ was used in place of Fe which had been utilized in the preparation of the *i*-Pr analog.

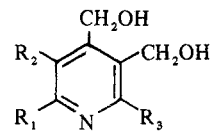
The pyridoxine dehydrogenase enzyme used here was a preparation described by Morino and Sakamoto.[2] The assay of enzymatic activity toward VIII is given in Table I and compared with the activities of the compds prepared and studied by Melius and Marshall.[1]

## Experimental Section†

**4-Carbethoxy-3-cyano-6-*n*-propyl-2-pyridone (I)** was prepd refluxing a soln of the Na salt of ethyl *n*-butyropyruvate[5] (208 g; 1.0 mole) and cyanoacetamide (92 g; 1.1 moles) in abs EtOH (1400 ml) for 3 hr. After standing at room temp overnight, the reaction mixt was chilled and treated with an ice cold soln made up by dilg concd HCl (200 ml) to 1200 ml with ice and $H_2O$. The crude product thus pptd was washed thoroughly with $H_2O$ before being crystd from aq EtOH (3000 ml; (60:40) EtOH-$H_2O$) to give 152 g (65%) of I, mp 146–148°.

†Melting points are corrected and were determined in a Mel-Temp apparatus (Laboratory Devices, Cambridge, Mass.) Microanalyses were by Galbraith Laboratories, Knoxville, Tenn.

**Table I.** Enzyme Activity of Analogs

| Compound | $R_1$ | $R_2$ | $R_3$ | PDH activity, % |
|---|---|---|---|---|
| |  | | | |
| 1 Pyridoxine | Me | OH | H | 100 |
| 2 ω-Methylpyridoxine[a] | Et | OH | H | 95 |
| 3 *i*-Pr analog[a] | *i*-Pr | OH | H | 25 |
| 4 Cl analog[a] | Me | OH | Cl | 22 |
| 5 *n*-Pr analog VIII | *n*-Pr | OH | H | 5 |

[a]Data for these PDH activity estimates were obtd from Melius and Marshall.[1]

**4-Carbethoxy-3-cyano-6-*n*-propyl-5-nitro-2-pyridone (II).** Compd I (23.5 g; 0.1 mole) was nitrated with $HNO_3$-$Ac_2O$, essentially as described by Wuest,[3] to give, after recrystn from 50% aq EtOH, 18.5 g (66.3%) of II, mp 163–164°.

**4-Carbethoxy-2-chloro-5-nitro-6-*n*-propylnicotinonitrile (III).** Compd II (27.9 g; 0.1 mole) and $PCl_5$ (22.9 g; 0.11 mole) were mixed and heated together at 125 ±5° for 2 hr. $POCl_3$ was removed *in vacuo* before the residue was triturated with crushed ice until solidification was complete. Recrystn of the crude product from abs EtOH gave 21.2 g (71%) of III, mp 48–50°.

**5-Amino-2-chloro-4-carbethoxy-6-*n*-propylnicotinonitrile (IV).** Compd III (29.8 g; 0.1 mole), suspended in $Et_2O$ was treated with a freshly filtered soln of $SnCl_2$ (78 g) in concd HCl (165 ml) in a manner analagous to that described by Greene and Montgomery.[4] The crude product was recrystd from abs EtOH (625 ml) to give 23 g (86%) of IV, mp 168–169°.

**5-Amino-4-carbethoxy-6-*n*-propylnicotinonitrile (V).** Hydrogenation of IV (26.8 g; 0.1 mole) over 5% Pd-$BaCO_3$[3] and work-up of the reaction mixt gave 10.5 g (45%) of V after recrystn from abs EtOH, mp 132–133°.

**3-Amino-2-*n*-propylpyridine-4,5-dicarboxylic Acid (VI).** Hydrolysis of V (15.6 g; 0.067 mole) with concd HCl[3] gave 9.0 g (60%) of the dicarboxylic acid VI, mp 215–216° dec.

**3-Hydroxy-2-*n*-propylpyridine-4,5-dicarboxylic Acid (VII).** Compd VI (8.3 g; 0.037 mole) was diazotized at 80° in aq soln to give 4.1 g (49.4%) of VIII, mp 230–232°.

**3-Hydroxy-2-*n*-propyl-4,5-pyridinedimethanol Hydrochloride (VIII).** Reduction of the 2 $CO_2H$ groups of VII (2.25 g; 0.01 mole) with $NaBH_4$-$AlCl_3$ as described by Blackwood[6] gave the *n*-Pr analog of pyridoxine hydrochloride (0.94 g; 41%); mp 210–212° dec. *Anal.* ($C_{10}H_{15}NO_3 \cdot HCl$) C, H, N.

**Enzymatic assays** were carried out as described by Melius and Marshall.[1] The yeast pyridoxine dehydrogenase (PDH) activity was measured by the reaction of phenylhydrazine with the aldehyde produced from the pyridoxine and its analogs.[7]

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