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Synthesis of Vitamin B<sub>6</sub> Derivatives. III.  
3-(4-Formyl-3-hydroxy-2-methyl-5-pyridyl)propionic Acid,  
An Analog of Pyridoxal Phosphate (I)

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The preparation and properties of a new analog of pyridoxal phosphate, 3-(4-formyl-3-hydroxy-2-methyl-5-pyridyl)propionic acid and of the corresponding 4-aminomethyl compound are described. The n.m.r. spectrum of the new aldehyde is compared with those of 5-deoxypyridoxal and pyridoxal phosphate. The broad aldehyde proton peak of 5-deoxypyridoxal is sharpened by heating, suggesting an equilibrium between two geometrical isomers. Mass spectra of the new aldehyde and of 5-deoxypyridoxal are analyzed. The electronic absorption spectrum is nearly the same as that of 5-deoxypyridoxal. The new aldehyde undergoes rapid transamination with amino acids, and the corresponding aminomethyl compound can be reoxidized to the aldehyde by heating with glyoxylic acid in aqueous solution.

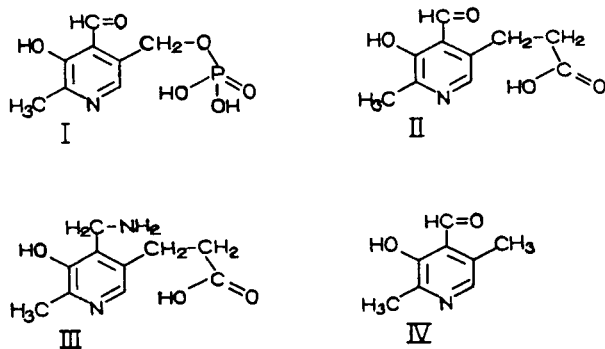
Pyridoxal phosphate (I) serves as a coenzyme for many different enzymic transformations, mostly involving amino acids. The role of the phosphate group in the catalytic action of this substance is unclear, but it is often assumed that it serves simply as a "handle" for the binding of the coenzyme to the protein portion of the enzyme. The synthesis of a new analog, 3-(4-formyl-3-hydroxy-2-methyl-5-pyridyl)propionic acid (or  $\alpha^5$ -carboxymethyl-5-deoxypyridoxal, II) has been reported by Tomita and Metzler (3). An improved preparation of this compound is described. The corresponding 4-aminomethyl derivative (III), an analog of pyridoxamine phosphate has also been made, and some chemical and spectroscopic properties of both

that II binds at the active site of an apotransaminase more weakly than does pyridoxal phosphate and that the enzyme containing the modified coenzyme is almost without catalytic activity on the normal substrates (4). It is a weak competitive inhibitor of tryptophanase (5) and also binds weakly to muscle apophosphorylase *b* (6).

Compound (II) was prepared by oxidation of the corresponding 4-hydroxymethyl compound. Several conditions for the oxidation of such 4-pyridinemethanols were examined, the most satisfactory of which was the use of manganese dioxide in chloroform or in a chloroform dioxane mixture. Oxidation in such solvents rather than in water is advantageous because the product can be isolated more easily. Stirring of a suspension of 5-deoxypyridoxol and manganese dioxide in chloroform at room temperature for 22 hours gave 5-deoxypyridoxal (IV) in 74% yield (7). Oxidation of 3-(3-hydroxy-4-hydroxymethyl-2-methyl-5-pyridyl)propionic acid in a chloroform dioxane mixture at room temperature for 72 hours produced II in 30% yield. For these oxidations, it is important to use freshly prepared manganese dioxide (prepared according to the procedure of Mancera *et al.* (8,9)) and to stir vigorously.

Reaction of II with hydroxylamine gave the oxime in 76% yield. This was reduced by catalytic hydrogenation to 3-(4-aminomethyl-3-hydroxy-2-methyl-5-pyridyl)propionic acid (or  $\alpha^5$ -carboxymethyl-5-deoxypyridoxamine, III) in 59% yield.

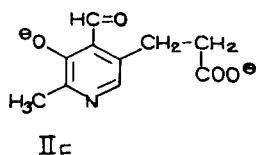
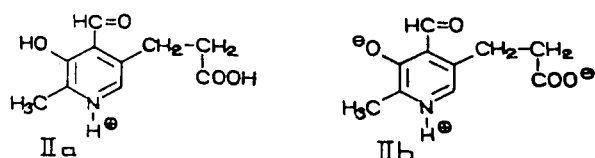
Electronic absorption spectra of aqueous solutions of three of the ionic forms of II are shown in Fig. 1. If only the total charge is considered, four different ionic forms



compounds are presented. Since II is similar to pyridoxal phosphate in geometry and in charge distribution in neutral aqueous solutions, it should be of great interest to test it with enzymes normally containing pyridoxal phosphate. Unpublished experiments from this laboratory indicate

exist, each bearing one less proton than the preceding form. These will be designated here as  $H_3P$ ,  $H_2P$ ,  $HP$  and  $P$  respectively, and the  $pK_a$  values relating them as  $pK_1$ ,  $pK_2$  and  $pK_3$ . The spectra of  $H_3P$  and  $P$  are directly measurable at low and high  $pH$ , respectively, whereas that of  $H_2P$  is represented approximately by that of a solution at  $pH$  6.1. The precise spectrum of  $HP$  shown in Figure 1 was evaluated using a digital-computer program devised by Nagano (10).

The close similarity of these spectra to those of IV at similar values of  $pH$  (10,11) suggests that structures IIa, IIb and IIc, respectively, account for the longer wavelength



maxima in the spectra (at 342, 380 and 392  $m\mu$ , respectively, for  $H_3P$ ,  $HP$  and  $P$ ). As with IV, an equilibrium with hydrated forms, lacking the free carbonyl group but otherwise corresponding to IIa, IIb and IIc must also exist. The hydrated forms account for the shorter wavelength peaks (at 295, 327 and 303  $m\mu$ , respectively) (11,12). The value of  $pK_3$ , describing the dissociation of  $HP$  to  $P$ , was evaluated from the change of the spectrum with  $pH$  as  $8.23 \pm 0.01$  and from a titration curve as 8.15. The values of  $pK_1$  and  $pK_2$  were estimated from a titration curve as 3.7 and 4.6. The major part of the change in spectrum between  $H_3P$  and  $HP$  is centered around a  $pH$  of 3.8.

The n.m.r. spectrum of II in deuterium oxide is shown in Fig. 2, and the spectra of II and of IV are compared in Table I. The spectrum of IV shows four peaks in alkaline solution and three peaks in acid solution. The one-proton peaks at -638 c.p.s. (acid) and -613 c.p.s. (alkaline) and at -492 c.p.s. (acid) and -431 c.p.s. (alkaline) can be assigned confidently to the aldehyde- and  $C_6$ -protons, respectively by comparison with the data reported by Korytnyk and Singh (13) for pyridoxal and pyridoxal-5-phosphate (Table I). Similar assignments can be made for II. The six-proton peak of IV at -164 c.p.s. in acid solution and the two three-proton peaks at -147 and -136 c.p.s. in alkaline solutions are due to the 2- and 5-methyl groups. The -147 c.p.s. peak in alkaline solution probably represents the 2-methyl group, the shift

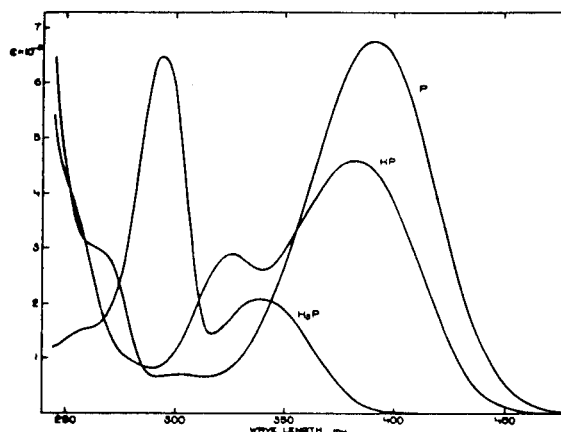


Figure 1. Electronic absorption spectrum of three ionic forms of  $\alpha^5$ -carboxymethyl-5-deoxy pyridoxal (II) in water.  $H_3P$  represents the most highly protonated form (see text).

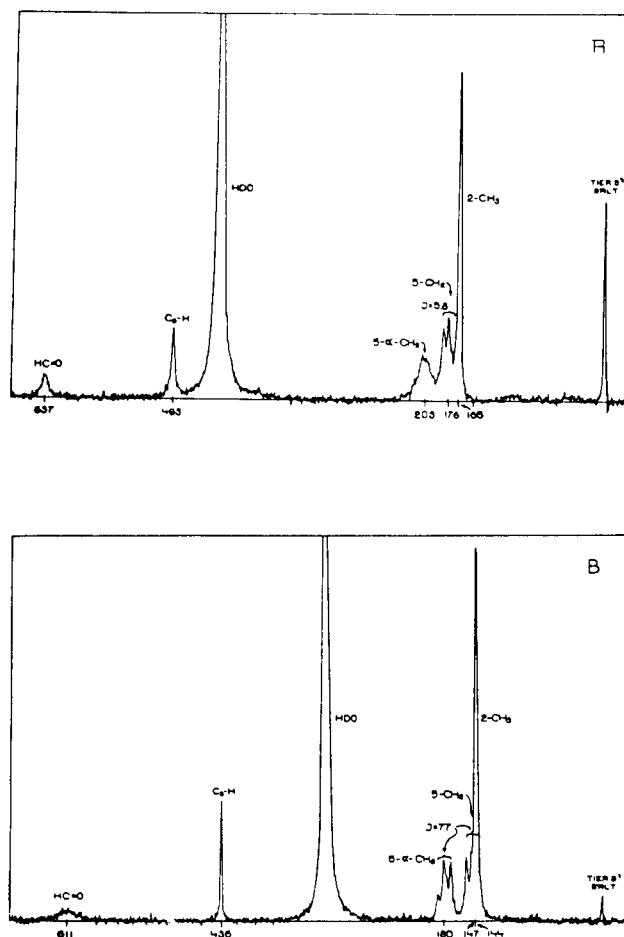


Figure 2. N.M.R. spectrum of compound II in  $D_2O$ .  
(A) In acid solution (in DCl).  
(B) In alkaline solution (in NaOD).

TABLE I  
N.M.R. Spectra of Aldehydes in D<sub>2</sub>O (a)

	2-CH <sub>3</sub>			CHO or Hemiacetal				
	Acid	Alkaline	Difference	Acid	Alkaline	Difference		
IV	-164	-147	17	-638	-613	25		
II	-165	-144	21	-637	-611	26		
Pyridoxal (b)	-159	-139	20	-402	-425	23		
				-404		-22		
Pyridoxal-5'-phosphate (b)	-156	-140.5	15.5		-622			

	C <sub>6</sub> -H			5-CH <sub>2</sub> (or -CH <sub>3</sub> )			5-α-CH <sub>2</sub> (See Structure II)	
	Acid	Alka- line	Differ- ence	Acid	Alka- line	Differ- ence	Acid	Alkaline
IV	-492	-431	61	-164	-136	28		
II	-493	-436	57	-176	-147	29	-203	-180
Pyridoxal (b)	-492	-442	50	-315	-289	26		
Pyridoxal-5'- phosphate (b)	-492	-457	35	-303	-297	6		
				-306	-303	3		

(a) Expressed in units of c.p.s. at 60 Mc. The internal standard was Tier's Salt for compounds II and IV. All spectra were obtained using a Varian HR-60 n.m.r. spectrometer. Solutions were about 30 mg. of compounds in 500 μl of N-DCl or N-NaOD. (b) Data of Korytnyk and Singh (13), who used 1,4-dioxane as the internal standard.

TABLE II  
Principal Peaks in Mass Spectra of Aldehydes

Mass Numbers and intensities (in parentheses)  
relative to that of highest peak = 100

IV	151 (M <sup>+</sup> , 100), 123 (13), 122 (46), 94 (17), 82 (22).
II	209 (M <sup>+</sup> , 71), 191 (39), 181 (11), 163 (100), 150 (37), 136 (39), 135 (33), 122 (59).

of +17 c.p.s. from its position in acid corresponding to that observed for the 2-methyl group of pyridoxal and pyridoxal phosphate (Table I). The peak at -136 c.p.s. is somewhat broadened, presumably through coupling with the C<sub>6</sub>-proton. Application of decoupling techniques led to a sharpening of this peak, confirming this hypothesis.

In compound II the two-proton triplet peaks at -176 c.p.s. and -203 c.p.s. (acid) and at -147 c.p.s. and -180 c.p.s. (alkaline) must represent the methylene groups of the 5-propionic acid side-chain. The methylene group β to

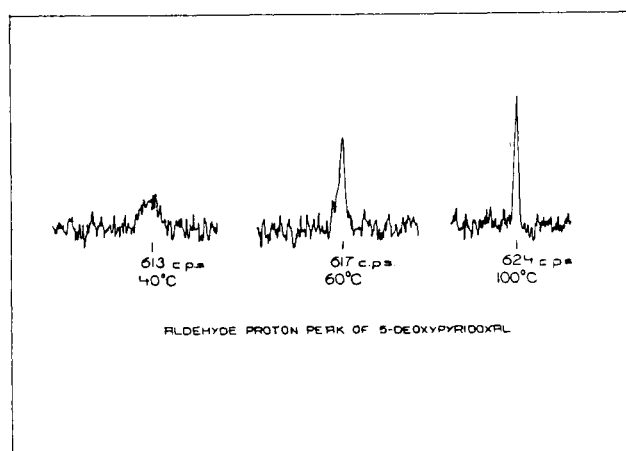
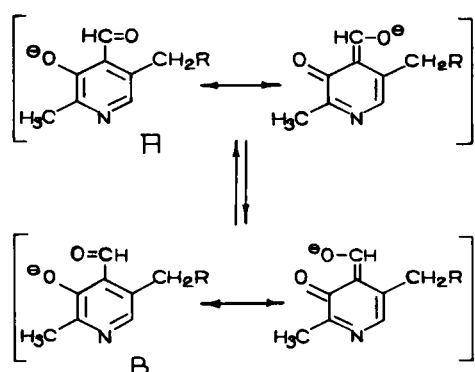


Figure 3. N.M.R. peak of the aldehyde proton of 5-deoxy-pyridoxal at three different temperatures.

the carboxyl and adjoining the ring is of similar character to the 5-methyl group of IV, and we have therefore assigned the peaks at  $-176$  c.p.s. (acid) and  $-147$  c.p.s. (alkaline) to it. Significantly, in pyridoxal phosphate (I) all the differences of the chemical shifts between acid and alkaline solutions ( $2\text{-CH}_3$ ,  $\text{C}_6\text{-H}$  and  $5\text{-CH}_2\text{-}$ ) are less than for pyridoxal, for II, or for IV.

The broad peaks of the aldehyde protons of these compounds in alkaline solutions may be explained by the equilibrium between the two structures, A and B. The alde-

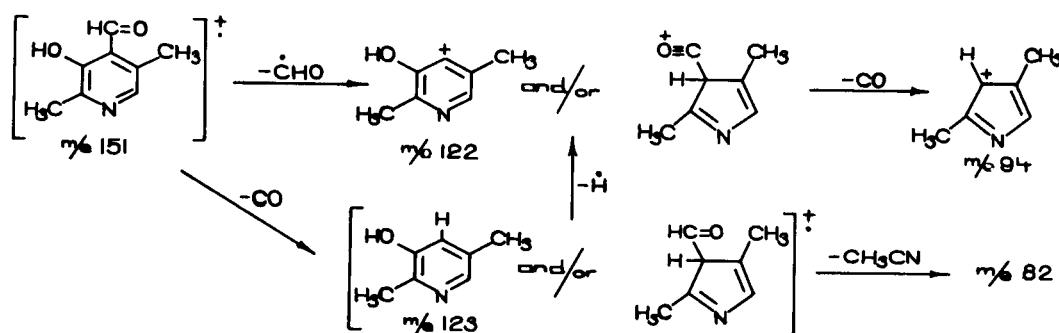


hyde proton peak for IV is sharper at higher temperatures, as would be predicted (Fig. 3).

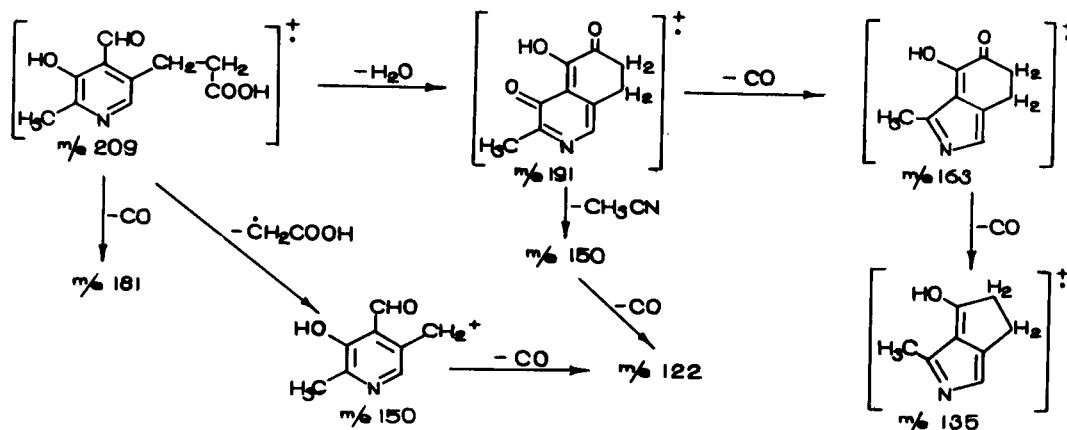
In acid solution, the ratios of the areas of the peaks of the aldehyde- to the  $\text{C}_6$ -protons are 0.78 and 0.58, respectively, for IV and for II. Korytnyk and Singh (13) were unable to locate the peak of the aldehyde proton in acid solutions in pyridoxal phosphate. These results are difficult to interpret because the electronic absorption spectra of all three compounds in acid are nearly identical, and from these a ratio of free aldehyde to hydrated forms of only about 0.3 in acid solutions has been estimated (11, see also Fig. 1). However, the spectral measurements were made at a concentration of about  $10^{-4}$  M and the n.m.r. measurements at about 0.3 M.

The positions of the principal peaks in the mass spectra of IV and II are shown in Table II. Five of these are present for IV, the largest being the molecular ion peak at  $m/e$  151. Metastable peaks were observed corresponding to a loss, from the molecular ion, of the radical  $\text{CHO}\cdot$  and carbon monoxide, respectively, to yield peaks at  $m/e$  122 and 123, the former predominating. The loss of  $\text{CHO}\cdot$  is reported to be a minor pathway of cleavage of benzaldehyde (14) as is the loss of CO to form the radical cation of ben-

SCHEME I



SCHEME II



zene. The loss of CO is the major pathway of fragmentation of phenol (15); hence, for deoxyripyridoxal two possible pathways of loss of CO and two possible structures for the resulting ion of  $m/e$  123 can be proposed (Scheme I). Metastable ion peaks exist corresponding to the loss of hydrogen from  $m/e$  123 to yield  $m/e$  122 and of acetonitrile to yield  $m/e$  82. The latter cleavage is similar to that reported by DeJongh *et al.* (16) for  $\alpha^4,3$ -*O*-isopropylidene-isopyridoxal and related compounds. The peak at  $m/e$  94 results from loss of CO from  $m/e$  122, an observation supported by the presence of the expected metastable ion peak.

The mass spectrum of II shows eight major peaks, the base peak being that of  $m/e$  163. Metastable ion peaks are seen for the loss of water from the molecular ion of  $m/e$  209 to give  $m/e$  191 and for the loss of CO and of acetonitrile from  $m/e$  191 to give  $m/e$  163 and  $m/e$  150, respectively. The ethyl ester of II fragments in a similar manner, but with the loss of ethanol rather than water. Proposed structures are shown in Scheme II. Metastable ion peaks are also present to support the loss of CO and of  $\cdot\text{CH}_2\text{COOH}$  from the molecular ion to give  $m/e$  181 and  $m/e$  151, respectively. The first of these cleavages is similar to that seen with IV, but the intensity of the  $m/e$  181 peak is relatively less than that of the corresponding  $m/e$  123 peak for IV if allowance is made for the extensive conversion of II to the ion of  $m/e$  163. The cleavage of II to the pyridyl cation of  $m/e$  150 corresponds to that often observed with pyridyl compounds (13). A metastable ion peak was also present for the loss of acetonitrile from  $m/e$  191 to yield  $m/e$  150. The peaks at  $m/e$  135 and 122 result from expulsion of carbon monoxide from  $m/e$  163 and 150, respectively. The appropriate metastable ion peaks being present.

That the peaks at  $m/e$  150 and  $m/e$  122 both contained two different fragments (see Scheme II) was confirmed by a high resolution mass spectrum of II.

Calculated Mass	Empirical Formula (Mass)
150.0304	C <sub>8</sub> H <sub>6</sub> O <sub>3</sub> (150.0317)
150.0539	C <sub>8</sub> H <sub>8</sub> O <sub>2</sub> N (150.0555)
122.0365	C <sub>7</sub> H <sub>6</sub> O <sub>2</sub> (122.0368)
122.0598	C <sub>7</sub> H <sub>8</sub> ON (122.0606)

Like pyridoxal and deoxyripyridoxal, II reacts rapidly with aqueous solutions of amino acids, apparently undergoing transamination to III. The reverse reaction of III with  $\alpha$ -ketoacids to form II does not proceed readily, the equilibrium evidently favoring the amine. However, III reacts rapidly at 50° with 0.1 *M* sodium glyoxylate (at pH 5.2 with 0.002 *M* aluminum ammonium sulfate) to be converted in over 90% yield to II (17).

## EXPERIMENTAL

All melting points were uncorrected. The n.m.r. spectra were obtained at 60 Mc. using Varian A-60 and HR-60 instruments calibrated by standard methods. The internal standard was Tiers' salt. Mass spectra were determined with an Atlas Mat mass spectrometer with an ionizing potential of 70 e.v. The UV spectra were measured at 25° with a Cary 15 spectrophotometer.

### 3-(4-Formyl-3-hydroxy-2-methyl-5-pyridyl)propionic Acid (II)

One gram (4.7 mmole) of 3-(3-hydroxy-4-hydroxymethyl-2-methyl-5-pyridyl)propionic acid, prepared by the method of Tomita *et al.* (18) or of Korytnyk (19) and powdered finely, was suspended together with 10 g. of freshly prepared manganese dioxide (8,9) in a mixture of 100 ml. of chloroform and 100 ml. of dioxane in a 500 ml. flask and stirred at room temperature. After 72 hours of stirring the reaction mixture was filtered. The residue was washed with a 1:1 mixture of chloroform and dioxane (50 ml. portions) 5 – 10 times, until the washings were colorless. The combined filtrate and washings were evaporated to dryness under reduced pressure, and the residue was extracted by alternately triturating and refluxing with about 10 ml. of absolute ethanol several times over a period of one-half hour. The residual manganese dioxide was removed by filtration through a medium sintered glass filter. The filtrate was again evaporated to dryness and the residue was recrystallized several times from a minimum (2–3 ml.) of hot absolute ethanol. The yield of II after the recrystallization was 0.25 g. (30%), m.p. 191–192°.

*Anal.* Calcd. for C<sub>10</sub>H<sub>11</sub>O<sub>4</sub>N: C, 57.41; H, 5.30; N, 6.70. Found: C, 57.50; H, 5.35; N, 6.73.

$\nu$  max (in KBr): 1695 cm<sup>-1</sup> (COOH); 1653 cm<sup>-1</sup> (CHO). Thin layer chromatography: R<sub>f</sub> = 0.59 (*n*-BuOH: Acetic acid: H<sub>2</sub>O = 4:1:5, silica gel G, location of compounds with Gibb's reagent (1% dichloroquinone chloroimide in benzene).

### Oxime of II.

Sixty mg. (0.3 mmole) of II, 70 mg. (1 mmole) of hydroxylamine hydrochloride, 82 mg. (1 mmole) of sodium acetate and 50 ml. of ethanol were refluxed in a 100 ml. flask for 3 hours. After cooling, the reaction mixture was filtered and the residue was washed with ethanol. The combined filtrate and washings were evaporated under reduced pressure, and the residue was recrystallized from about 5 ml. of ethanol to yield 50 mg. (76%) of the oxime of II, m.p. 211–212°.

*Anal.* Calcd. for C<sub>10</sub>H<sub>12</sub>O<sub>4</sub>N<sub>2</sub>: C, 53.57; H, 5.39. Found: C, 53.47; H, 5.57.

### 3-(4-Aminomethyl-3-hydroxy-2-methyl-5-pyridyl)propionic Acid Dihydrochloride (III)

A solution of 0.23 g. (1 mmole) of the oxime of II in 50 ml. water and 2 ml. of concentrated hydrochloric acid was hydrogenated over 0.30 g. of 10% palladium on activated charcoal at room temperature and atmospheric pressure. Two moles of hydrogen per mole of oxime were consumed in about an hour. The catalyst was filtered off and washed with water. The combined filtrate and washings were taken to dryness under vacuum, and the residue was recrystallized from methanol and ether.

*Anal.* Calcd. for C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>O<sub>3</sub>·2HCl: C, 42.41; H, 5.70; N, 9.90. Found: C, 42.21; H, 5.83; N, 9.58.

$\lambda$  max (H<sub>2</sub>O, pH 8.08), 321 m $\mu$ ,  $\epsilon$  = 8.1 x 10<sup>3</sup>, 249 m $\mu$ ,  $\epsilon$  = 4.5 x 10<sup>3</sup>.  $\nu$  max (in KBr), 1686 cm<sup>-1</sup> (CO).

### Reaction of Aldehyde, II, with Leucine Ethyl Ester.

A solution of II (10<sup>-4</sup> *M*) and leucine ethyl ester (0.24 *M*) in 0.1 *M* cacodylic acid buffer at pH 6.8 was examined spectrophotometrically.

metrically at various times. After 2.5 hours at 25°, the change in the spectrum indicated that about 20% of the aldehyde had been converted to the amine, III. The reaction continued until the absorption bands of the aldehyde (and Schiff's base) disappeared almost completely. The change in the spectrum is almost identical to that observed with 5-deoxypyridoxal, which was shown by chromatographic identification of products to yield 5-deoxypyridoxamine.

#### Reaction of Amine, III, with Glyoxylic Acid.

The reaction of amine, II, with  $\alpha$ -keto acids is not expected to proceed to completion because of an unfavorable equilibrium constant. The reaction with glyoxylic acid, is an exception (17). A solution of III, ( $10^{-4}$  M) and sodium glyoxylate (0.1 M) containing  $2 \times 10^{-3}$  M aluminum ammonium sulfate as a catalyst in 0.1 M acetate buffer at pH 5.2 yielded about 90% of aldehyde, II, in 20 hours at 50° as judged by the change in the spectrum.

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