at 134–135° and a mixture m.p. was 131–132° (lit.,[7] 138–139°). The infrared spectra (Nujol mull) for synthetic and isolated products were identical.

Isolation of VII.—This compound was isolated from rabbit urine in low yield by the procedure of Paul, *et al.*[2c] Comparison with the photochemically produced product is given below.

Photochemical Product of 5-Nitro-2-furaldehyde Diacetate.—Dry 5-nitro-2-furaldehyde diacetate (IX) (100 g.) was irradiated with frequent mixing in flat-sided Roux bottles which had been flushed with nitrogen and stoppered. About one hour of midday sunlight caused the white solid to turn green. Irradiation in air resulted in yellowing and decreased the yield. The green crystals were dissolved in 500–600 ml. of warm (about 50°) ethanol, then 600 ml. of water and 1500–2000 ml. of ethyl acetate were added. The yellow water–alcohol layer was separated and the brown-red ethyl acetate layer washed once with about 100 ml. of water. The combined aqueous layers were extracted twice with equal volumes of ethyl acetate to remove residual IX yielding an aqueous phase with absorption maxima at 400 and 300 mμ. The ratio of absorbances at 400:300 mμ is 2.1:1. 1-Isopropylidene-2-acetylhydrazine (1 g.) and 1 ml. of concd. HCl/500 ml. of solution were added. After standing overnight in the refrigerator, the solution was concentrated *in vacuo* to yield a brownish-yellow solid with absorption maxima at 415 and 320 mμ; recrystallization from water did not give a pure product. VII isolated from urine[2c] yielded upon hydrolysis in dilute HCl at 70° a product which absorbed at 400 mμ, and which corresponded in R_f in the butanol:ethanol:NH_4OH solvent with that of the photochemically produced VIII. Both of these compounds had R_f 0.5 and both VII compounds had R_f 0.20. Solutions of either, on standing for 0.5 hr. at room temperature with an equal volume of 0.2% 2,4-dinitrophenylhydrazine in 2 *N* HCl, yielded red derivatives with R_f 0.27 and $\lambda_{max.}$ 462 mμ. An infrared spectrum (KBr pellet) of isolated VII showed no nitro group absorption and bands at 5.75, 5.95 and 6.9 μ.

Synthesis and Biological Activity of Pyridoxine Analogs

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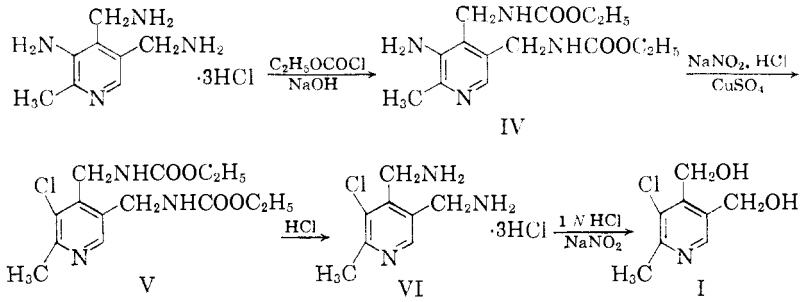
The syntheses of three new analogs of pyridoxine have been described. On biological evaluation in rats, 3-chloro-4,5-bis(hydroxymethyl)-2-methylpyridine hydrochloride (I) was a weak vitamin B_6 antagonist compared with deoxypyridoxine.

In the course of the synthesis of analogs of merimine[1] for testing as ataractic agents, several intermediates were prepared which could serve equally well for the preparation of compounds related to pyridoxine. Since these intermediates were available, they were so used in the hope of preparing analogs of vitamin B_6 heretofore not reported[2] in the literature.

The three new analogs described in this paper are 3-chloro-4,5-bis-(hydroxymethyl)-2-methylpyridine hydrochloride (I), 3-hydroxy-4,5-bis(hydroxymethyl)pyridine hydrochloride (II, demethylpyridoxine) and 3-amino-4,5-bis(hydroxymethyl)pyridine hydrochloride (III).



The two aliphatic amino groups of the starting compound, 3-amino - 4,5 - bis(aminomethyl) - 2 - methylpyridine trihydrochloride,[3] were blocked by carbethoxylation.[1] The intermediate IV, thus obtained, on diazotization afforded 4,5-bis(carbethoxyaminomethyl)-3-chloro-2-methylpyridine (V). Hydrolysis of V with concentrated hydrochloric acid gave VI. Diazotization and hydrolysis of VI with sodium nitrite and 1 *N* hydrochloric acid produced a low yield of I. This reaction was also carried out in dilute sulfuric acid to give I in a low yield, but the product contained impurities which could not be completely eliminated.

Another useful intermediate was 5-aminocinchomeronic acid (VII), prepared by acid hydrolysis of 5-amino-N-benzylcinchomeronimide.[1]

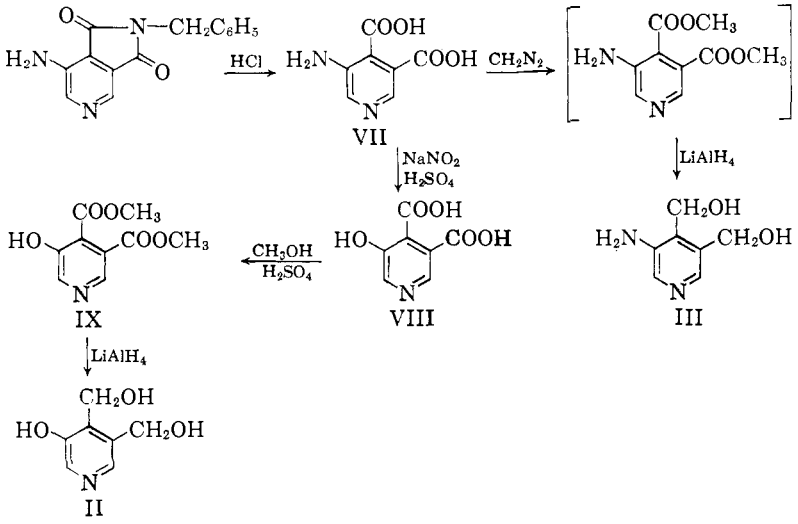
The acid VII was also obtained by decarboxylating 3-amino-2,4,5-pyridinetricarboxylic acid[1] in acetic anhydride. The hydroxy acid VIII was formed from VII by the method of Reed and Shive.[4] Esterification of VIII with methanol gave IX and this on reduction with lithium aluminum hydride in tetrahydrofuran led to II. The

(1) S. M. Gadekar, J. L. Frederick, J. Semb and J. R. Vaughan, Jr., *J. Org. Chem.*, **26**, 468 (1961).

(2) While this manuscript was in preparation, B. van der Wal, Th. J. de Boer and H. O. Huisman, *Rec. trav. chim.*, **80**, 203 (1961), described the synthesis of nor-vitamin B_6(II).

(3) J. H. Mowat, F. J. Pilgrim and G. H. Carlson, *J. Am. Chem. Soc.*, **65**, 954 (1943).

(4) L. J. Reed and W. Shive, *ibid.*, **68**, 2740 (1946).



intermediate VII was also used for the preparation of III; it was converted to the diester with diazomethane. The ester was reduced to III *in situ* with lithium aluminum hydride in refluxing tetrahydrofuran.[5]

Biological Evaluation.[6]—Three compounds, I, II, and VI, were tested; compound III was not tested because of insufficient material. Young male rats weighing about 40 g. were placed on a vitamin B_6 deficient diet[7] containing 30% casein for 16 to 17 days during which time their vitamin B_6 reserves were depleted and they began to show typical signs of vitamin B_6 deficiency, such as growth retardation, acrodinia, spastic gait, etc. At this time the compounds were administered subcutaneously in water solution at concentrations of 100–1000 μg./day or 2500 μg./day. Two control groups received a known vitamin B_6 antagonist, 4-deoxypyridoxine, and a known vitamin B_6 active compound, pyridoxine, respectively. A third control group remained untreated. The effect of the various treatments on the course of vitamin B_6 deficiency was determined. Pertinent data are summarized in Table I. In a subsequent experiment (groups 6 and 7, Table I) varying amounts of pyridoxine were administered with I. The following conclusions may be drawn from these results:

1. The administration of 10 μg. of pyridoxine/day under these

(5) We did not encounter the problem of separating a mixture of products as described by van der Wal, *et al.*,[2] perhaps because we carried out the reduction at a higher temperature.

(6) The biological studies were done by E. C. DeRenzo.

(7) L. R. Cerecedo, J. R. Foy and E. C. DeRenzo, *Arch. Biochem.*, **17**, 397 (1948).

experimental conditions led to a prompt increase in growth rate and disappearance of all deficiency signs within one week.

2. Untreated controls maintained their body weight and survived for at least two weeks. The symptoms of vitamin B_6 deficiency became progressively more severe before death but no epileptiform fits were observed.

3. The administration of 50 or 100 μg. of deoxypyridoxine per day did not induce convulsive seizures but promptly led to weight loss and death within one week.

4. When administered at a dose of 2500 μg./day I promptly induced weight loss and death within a few days. All rats which received I at a dose of 1000 or 2500 μg. displayed severe convulsive seizures about one hour after administration. The animals were very sensitive to sound or touch which aggravated the severity of the seizures. The simultaneous administration of 25 μg. of pyridoxine and I prevented the development of seizures, restored growth rate and caused the disappearance of other deficiency symptoms.

5. In comparison with deoxypyridoxine, I may be considered a weak vitamin B_6 antagonist. On the basis of effect on body weight and mortality, it was found roughly one-twentieth to one-fiftieth as active as deoxypyridoxine. On the other hand, considering its ability to induce epileptiform fits, I would appear to be exerting a specific effect on vitamin B_6 dependent enzymes in the central nervous system, an effect not exhibited by deoxypyridoxine.

6. The other two compounds, II and VI, were inert in vitamin B_6 deficient rats. The results with II are in agreement with those reported by Pol and Klein Obbink.[8]

Experimental[9]

3-Amino-4,5-bis(carbethoxyaminomethyl)-2-methylpyridine (IV).—A solution of 55.1 g. (0.200 mole) of 3-amino-4,5-bis(aminomethyl)-2-methylpyridine trihydrochloride in 200 ml. of water was treated with 120 ml. of 5 *N* sodium hydroxide and the resultant light yellow solution was cooled to about 8–10°. With stirring, this solution was treated gradually with 43.4 g. (0.400 mole) of ethyl chlorocarbonate, simultaneously adding 80 ml. of 5 *N* sodium hydroxide to maintain the reaction mixture slightly on the alkaline side. After completing the addition of chlorocarbonate and alkali, the cooling bath was removed and the reaction was allowed to come to room temperature. A copious white precipitate that had formed was recooled in an ice bath and then filtered off, washed with cold water, and dried in an oven; yield, 42.3 g. (68%), m.p. 190–191°. Recrystalliza-

(8) G. Pol and H. J. Klein Obbink, *Rec. trav. chim.*, **80**, 217 (1961).

(9) All melting points are uncorrected. The ultraviolet spectra were determined in 0.1 *N* hydrochloric acid.

TABLE I

EFFECT OF PYRIDOXINE AND PYRIDOXINE ANALOGS ON RATS SHOWING TYPICAL VITAMIN B_6 DEFICIENCY SYMPTOMS

Group	Treatment (μg./rat/day)	Rats	Av. body wt. (g.) at start of treatment	Av. body wt. (g.) at death or end of treatment	Av. survival time (days) after treatment	Remarks
(1)	None	11	72	63	14	7 Rats survived for 17 days when they were discarded.
(2)	Pyridoxine (10 μg.)	11	72	127	—	Symptoms disappeared within one week; growth rate restored. Treatment discontinued after 15 days.
(3)	Deoxypyridoxine (50–100 μg.)	11	73	58	6	All rats promptly lost weight and died within one week.
(4)	(I) (100–1000 μg.)	4	80	80	18	When given 1000 μg. all rats displayed convulsions.
(5)	(I) (2500 μg.)	7	70	62	3	All rats showed convulsions after administration of (I) and died within a few days.
(6)	(I) (2500 μg.) plus Pyridoxine (10 μg.)	4	73	69	2	One rat survived, growth rate was restored and it appeared normal. Treatment discontinued after 12 days.
(7)	(I) (2500 μg.) plus Pyridoxine (25 μg.)	4	76	117	—	No convulsive seizures observed. All rats apparently normal after 12 days treatment.
(8)	(II) (2500 μg.)	7	69	73	13	One rat died on the 13th day. Remaining 6 showed severe deficiency signs and were discarded after 17 days.
(9)	(VI) (2500 μg.)	7	69	63	16	Average value for 3 rats. Remaining 4 with severe deficiency signs were discarded after 17 days.

tion of a sample from ethanol afforded silky needles melting at 195–196°; λ_{max} shoulder at 245, 319; log ϵ 3.667, 3.847.

Anal. Calcd. for $C_{14}H_{22}N_4O_4$: C, 54.2; H, 7.15; N, 18.1. Found: C, 54.1; H, 7.30; N, 18.0.

3-Chloro-4,5-bis(carbethoxyaminomethyl)-2-methylpyridine (V).—To a solution of 16 g. (0.052 mole) of 4,5-bis(carbethoxyaminomethyl)-3-amino-2-methylpyridine in 40 ml. of concentrated hydrochloric acid and 30 ml. of water was added a warm solution of 22 g. (0.083 mole) of copper sulfate pentahydrate in 40 ml. of water. The mixture was cooled to −2° in a salt-ice bath and a solution of 6.3 g. (0.093 mole) of sodium nitrite in 25 ml. of water was added dropwise with stirring over a period of 1 hr. The mixture was allowed to come to room temperature and was then warmed to 50° until evolution of nitrogen ceased. After saturating with hydrogen sulfide, the copper sulfide was filtered off and the clear filtrate chilled and made basic with 5% sodium bicarbonate, yielding a tacky solid. A crystalline product was obtained by redissolving this solid in hydrochloric acid (decolorizing charcoal) and reprecipitating with sodium hydroxide; yield, 16 g. (93%), m.p. 102–106°. Recrystallization from 30% ethanol gave a white crystalline solid; m.p. 114–114.5° after drying; λ_{max} 282, log ϵ 3.927.

Anal. Calcd. for $C_{14}H_{20}ClN_3O_4$: C, 50.9; H, 6.12; Cl, 10.8; N, 12.8. Found: C, 50.7; H, 6.12; Cl, 10.8; N, 12.9.

4,5-Bis(aminomethyl)-3-chloro-2-methylpyridine Trihydrochloride (VI).—A solution of 3.3 g. (0.010 mole) of 4,5-bis(carbethoxyaminomethyl)-3-chloro-2-methylpyridine in 50 ml. of concentrated hydrochloric acid was heated over a steam bath for 48 hr. and evaporated *in vacuo* to dryness. The solid was triturated with 15 ml. of ethanol and the product was filtered off; yield, 2.8 g. (95%); m.p. 290–292° (dec. with darkening above 250°). Recrystallization of 300 mg. of this material from 5 ml. of ethanol and 1 ml. of water (decolorizing charcoal), and addition of 1 ml. of alcoholic hydrogen chloride gave a yellow precipitate which was air dried; yield, 200 mg. (67.8%); m.p. 292–295° (dec. with darkening above 235°); λ_{max} 282.5, log ϵ 3.732.

Anal. Calcd. for $C_8H_{12}ClN_3 \cdot 3HCl$: C, 32.6; H, 5.10; Cl, 47.8; N, 14.2. Found: C, 32.7; H, 5.11; Cl, 47.8; N, 13.9.

3-Chloro-4,5-bis(hydroxymethyl)-2-methylpyridine Hydrochloride (I).—A solution of 1.5 g. (0.0050 mole) of 4,5-bis(aminomethyl)-3-chloro-2-methylpyridine trihydrochloride in 20 ml. of water and a solution of 3.5 g. (0.051 mole) of sodium nitrite in 20 ml. of water were added simultaneously to a hot (approx. 90°) 500 ml. solution of 1 *N* hydrochloric acid with stirring. After the reaction was complete the mixture was evaporated to dryness under reduced pressure. The residue was extracted with 3 × 30 ml. of boiling absolute ethanol and the extracts were chilled. Any inorganic salts which separated were filtered off. The filtrate was evaporated to dryness *in vacuo*, and the remaining oily residue was redissolved in ethanol and the solution acidified with a few drops of alcoholic hydrogen chloride. Addition of ether precipitated a yellow crystalline product which was filtered off and air dried; yield, 0.60 g. (53%); m.p. 89–106° (dec.). Recrystallization from ethanol and alcoholic hydrogen chloride (decolorizing charcoal) gave 25% of white product melting at 164–164.5°; λ_{max} 282.5, log ϵ 3.874.

Anal. Calcd. for $C_8H_{10}ClNO_2 \cdot HCl \cdot 0.5H_2O$: C, 41.2; H, 5.19; Cl, 30.5; N, 6.01. Found: C, 41.0; H, 5.13; Cl, 30.8; N, 5.90.

5-Aminocinchomeronic Acid Hydrate (VII). Method A.—A suspension of 2.53 g.

(0.0100 mole) of N-benzyl-5-aminocinchomeronimide in 50 ml. of 5 *N* hydrochloric acid was refluxed for 12 hr. The dark-brown solution was decolorized with charcoal and the filtrate was adjusted to pH 3 with ammonium hydroxide and cooled in an ice bath for several hours. No solid precipitated. The solution was evaporated to a brown residue which was redissolved in 25 ml. of concd. hydrochloric acid and the acid solution was refluxed for an additional 8 hr. This time on adjusting the solution to the same pH, a light-yellow solid precipitated on cooling; yield 1.0 g. (50%), m.p. 242–244°. A sample recrystallized from water, melted at 243–245° (dec.). A mixture with an analytical sample of 5-aminocinchomeronic acid monohydrate was not depressed in melting point.

Method B.—A suspension of 3.93 g. (0.0150 mole) of 3-aminopyridine-2,4,5-tricarboxylic acid in 30 ml. of acetic anhydride was refluxed for 5 hr. The dark-brown solution was evaporated *in vacuo* to a dark residue which was then refluxed with 60 ml. of concentrated hydrochloric acid for 12 hr. The contents were decolorized with charcoal and the filtrate was evaporated to a yellow solid which was dissolved in 15 ml. of water. The aqueous solution was adjusted to pH 3 with ammonium hydroxide, whereupon a pale yellow solid separated; yield 0.54 g. (18%), m.p. 243–245° (dec.); λ_{max} 345 mμ; log ϵ 3.176.

Anal. Calcd. for $C_7H_6N_2O_4 \cdot H_2O$: C, 42.0; H, 4.03; N, 14.0. Found: C, 41.7; H, 3.98; N, 14.1.

Dimethyl 5-Hydroxycinchomeronate (IX).—To a mixture of 5.0 g. (0.025 mole) of 5-hydroxycinchomeronic acid[4] in 250 ml. of reagent grade methanol was added dropwise 30 ml. of concd. sulfuric acid. The resulting clear solution was refluxed for 24 hr. and then evaporated under diminished pressure to a syrup. With the temperature of the syrup kept below 10°, 60 ml. of water was added and the solution was rendered basic by gradual addition of solid sodium bicarbonate. The precipitated sodium sulfate was filtered off and the aqueous layer was salted with sodium chloride. The mixture was thoroughly extracted with ethyl acetate and the organic layer, after drying over anhydrous magnesium sulfate, was evaporated to a solid residue which on trituration with ligroin gave 1.55 g. (29%) of a solid melting at 129–133°. As the free ester base could not be crystallized conveniently it was converted to the hydrochloride in 54% yield; m.p. 195–197°; λ_{max} 302, log ϵ 3.496.

Anal. Calcd. for $C_9H_9NO_5 \cdot HCl$: C, 43.8; H, 4.06; N, 5.67; Cl, 14.4. Found: C, 43.6; H, 4.26; N, 5.58; Cl, 14.3.

4,5-Bis(hydroxymethyl)-3-hydroxypyridine Hydrochloride Hemihydrate (II).—To a solution of 1.00 g. (0.026 mole) of lithium aluminum hydride in 50 ml. of anhydrous tetrahydrofuran was added dropwise another solution of 1.0 g. (0.0048 mole) of dimethyl 5-hydroxycinchomeronate in 25 ml. of tetrahydrofuran. The resulting light-yellow mixture was refluxed for 8 hr. and allowed to stand at room temperature overnight. The excess lithium aluminum hydride was decomposed with 100 ml. of water and the solid was filtered off. The filtrate was saturated with carbon dioxide and evaporated under reduced pressure to a solid residue. The residue was extracted with 2 × 100 ml. of boiling ethanol. The filtrate was acidified with alcoholic hydrogen chloride and evaporated to 5 ml. *in vacuo*. On addition of ether, followed by chilling, a brown precipitate formed; yield, 0.75 g. (81%); m.p. 120–125°. Recrystallization of this product from ethanol (decolorizing charcoal) and alcoholic hydrogen chloride gave a tan solid; m.p. 124–126°, λ_{max} 289, log ϵ 3.755.

Anal. Calcd. for $C_7H_9NO_3 \cdot HCl \cdot 0.5\ H_2O$: C, 42.1; H,5.55; N, 7.00; Cl, 17.7. Found: C, 42.0; H, 5.32; N, 6.86; Cl, 17.9.

3-Amino-4,5-bis(hydroxymethyl)pyridine Hydrochloride (III).—A suspension of 2.0 g. (0.011 mole) of 5-aminocinchomeronic acid in 30 ml. of methanol was treated with a solution of diazomethane in ether made from 5.6 g. (0.063 mole) of N-nitrosomethylurea. The reaction mixture was swirled occasionally and allowed to stand until no more nitrogen was evolved. The suspended solid was filtered off and found to be 1.0 g. of starting material. The ether filtrate was evaporated to a brown oil from which no solid product could be obtained. This 1.0 g. of crude ester in 50 ml. of anhydrous tetrahydrofuran was added slowly to a solution of 0.38 g. (0.010 mole) of lithium aluminum hydride in 100 ml. of tetrahydrofuran. The resulting orange mixture was refluxed for 8 hr. and allowed to stand overnight at room temperature. The excess hydride was destroyed with 2 ml. of water and the insoluble salts were filtered off. The solid was washed three times with 30 ml. of boiling methanol and the combined filtrates were evaporated *in vacuo* to a tarry residue. Recrystallization of this from ethanol–ethyl acetate (decolorizing charcoal) gave a tan solid; yield 0.25 g. (34% on the basis of reacted acid); m.p. 135–140° (dec.). As purification of the base was difficult the hydrochloride was prepared. Recrystallization of a 100 mg. sample of the hydrochloride from ethanol and alcoholic hydrogen chloride yielded 40 mg. of a light tan solid; m.p. 164–165° (dec.); λ_{max} 252.5, 322.5, log ϵ 3.988, 3.941.

Anal. Calcd. for $C_7H_{10}N_2O_2 \cdot HCl$: C, 43.8; H, 5.78; N, 14.6; Cl, 18.5. Found: C, 44.1; H, 6.12; N, 14.3; Cl, 18.7.

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6-Deoxytetracyclines. II. Nitrations and Subsequent Reactions[1]

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The nitration of 6-deoxytetracyclines and the proof of structure of the resulting nitro compounds are described. The nitro groups were reduced to form amino derivatives, and these in some cases were acylated. The *in vitro* antibacterial potencies of the new compounds are compared to tetracycline.

The tetracycline antibiotics are a family of potent, broad spectrum antibacterial substances elaborated by several members of the genus

(1) Paper I: J. H. Boothe, J. J. Hlavka, J. P. Petisi, and J. L. Spencer, *J. Am. Chem. Soc.*, **82**, 1253 (1960); *cf.* J. J. Beereboom, J. J. Ursprung, H. H. Rennhard, and C. R. Stephens, *ibid.*, **82**, 1003 (1960).

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