3-Methyl-4-hydroxyisoquinoline (VI).—3-Methyl-4-methoxyisoquinoline hydrochloride (5 g.) was distilled with about 30 cc. of 48% hydrobromic acid until a concentrated solution was obtained. The crystalline hydrobromide (m. p. 232–233°) was converted to its free base, namely, 3-methyl-4-hydroxyisoquinoline (VI) by digesting with 6 *N* ammonium hydroxide. The precipitated base was recrystallized from water; m. p. 180°.

Anal. Calcd. for $C_{10}H_9NO$: C, 75.45; H, 5.70; N, 8.80. Found: C, 75.88; H, 5.84; N, 8.77.

The 3-methyl-4-methoxyisoquinoline hydrochloride was made by the catalytic reduction of 1-chloro-3-methyl-4-methoxyisoquinoline which has been described by Gabriel and Colman.[14]

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Summary

Methyl iodide reacted with vitamin B_6 to yield the methiodide quantitatively.

Vitamin B_6 methiodide on treatment with silver carbonate and vitamin B_6 on treatment with diazo methane yielded the same N-methyl-vitamin B_6 derivative. The properties, reactions, potentiometric titration data, and ultraviolet absorption data of acidic and alkaline aqueous solutions of this substance and its hydroiodide, show that the N-methyl-vitamin B_6 derivative is a phenol betaine or zwitterion.

The close similarity in the absorptions of the vitamin B_6 methiodide and hydrochloride at *p*H 2.1 to 6.6 and the close similarity in the titration data show that at *p*H 6.8 in aqueous solution, vitamin B_6 is to be regarded as existing essentially as a zwitterion in structure. In organic solvents, the β-hydroxypyridine structure appears to predominate. The interpretation of Ichiba and Michi that the production of the N-methyl derivative is due to a pyridone-methide form of vitamin B_6 is believed to be erroneous.

The absorption spectra of β-hydroxypyridine and its methiodide, O-methyl-vitamin B_6 methiodide, and 3-methyl-4-hydroxyisoquinoline were found to be in agreement with the interpretation given to the absorption spectra of vitamin B_6 methiodide and hydrochloride.

Methylation of the nitrogen atom of vitamin B_6 destroyed its biological activity.

(14) Gabriel and Colman, *Ber.*, **33**, 980 (1900).

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Chemistry of Vitamin B_6. II. Reactions and Derivatives

BY STANTON A. HARRIS

In connection with the synthesis of vitamin B_6 in this Laboratory,[1] a number of pyridine compounds related structurally to vitamin B_6 were made for the determination of their antidermatitic effect on vitamin B_6 depleted rats. The results were expected to show certain relationships between structure and activity, and these were discussed with the biological assays which have already been published.[2] Since certain of these derivatives have not been described in the literature, they are included in this paper.

Kuhn and Wendt[3] and Ichiba and Michi[4] have described a triacetate of vitamin B_6 which, after sublimation in high vacuum, tended to crystallize. The hydrochloride of vitamin B_6 triacetate (II) is described herein as a crystalline substance. The hydrochloride of vitamin B_6 diacetate (VI) has been made from the dibromomethyl derivative (V) by treatment with silver acetate in a mixture of potassium acetate and acetic acid. The 3-hydroxy group is unsubstituted in this diacetate. Both the triacetate and the diacetate were found to be as fully active[2] as vitamin B_6. Both acetates were stable in 0.01 *N* hydrochloric acid solution at 37°, but were slowly hydrolyzed in 0.01 *N* sodium hydroxide solution at the same temperature. The question of the ease of hydrolysis of these acetates is of interest in connection with the biological[2] and microbiological assays.

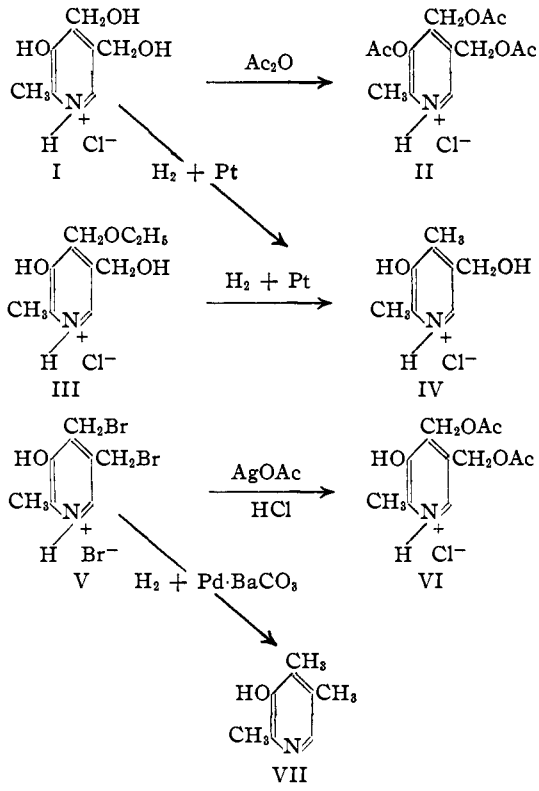
Hydrogenation of vitamin B_6 hydrochloride (I) and the 4-ethoxymethyl derivative (III) over

(1) Harris and Folkers, THIS JOURNAL, **61**, 1245 (1939); **61**, 3307 (1939).

(2) Unna, *Proc. Soc. Exptl. Biol. Med.*, **43**, 122 (1940).

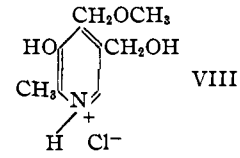
(3) Kuhn and Wendt, *Ber.*, **71**, 780 (1938).

(4) Ichiba and Michi, *Sci. Papers Inst. Phys. Chem. Research* (Tokyo), **35**, 73 (1938).



Adams platinum catalyst yielded 2,4-dimethyl-3-hydroxy-5-hydroxymethylpyridine hydrochloride (IV), which was obtained previously by Möller and collaborators,[5] presumably by direct synthesis. It is interesting to note that hydrogenation attacks the hydroxymethyl or ethoxymethyl group in the nuclear 4-position, whereas oxidation attacks the hydroxymethyl group in the nuclear 5-position with resultant lactone formation.[6,7] The dibromomethyl derivative (V) was hydrogenated over palladium and barium carbonate catalyst to give the 2,4,5-trimethyl-3-hydroxypyridine (VII). Although both the mono- and didesoxy derivatives of vitamin B_6 were inactive on vitamin B_6 depleted rats,[2] IV was weakly active for the growth of and promotion of acid formation by *Streptobacterium plantarum*,[8] whereas VII was inactive.

When vitamin B_6 base was heated in methanol at 125° for four hours, a compound, $C_9H_{13}NO_3$, was isolated as the hydrochloride. It showed one methoxyl group, no N-methyl group, a positive ferric chloride and 2,6-dichloroquinone chlorimide[9,10] reaction using a borate buffer. This latter reaction indicated that the 4-hydroxymethyl group was substituted. The product had the same melting point as the 4-methoxymethyl derivative (VIII) obtained by synthesis.



Experimental Part

2 - Methyl - 3 - acetoxy - 4,5 - bis - (acetoxymethyl)-pyridine Hydrochloride (Vitamin B_6 Triacetic Hydrochloride), II.—One gram of vitamin B_6 hydrochloride was dissolved in an equal mixture of pyridine and acetic anhydride. The solution was allowed to stand overnight, when it was heated on a steam-bath for twenty minutes and then concentrated to dryness in a vacuum. The concentration was twice repeated with absolute alcohol and then the residue was extracted with ether in which the triacetate dissolved. Addition of dry hydrogen chloride to this ether solution caused immediate crystallization. After two recrystallizations from absolute alcohol, the substance melted at 157°. It gave a negative ferric chloride test.

Anal. Calcd. for $C_{14}H_{18}NO_6Cl$: C, 50.68; H, 5.47; N, 4.22. Found: C, 50.60; H, 5.28; N, 3.96.

Hydrolysis experiments showed that this triacetate was stable in 0.01 *N* hydrochloric acid, but that it was slowly hydrolyzed in 0.01 *N* alkaline solution at 37°.

2 - Methyl - 3 - hydroxy - 4,5 - bis - (acetoxymethyl)-pyridine Hydrochloride (Vitamin B_6 Diacetate Hydrochloride) VI.—The dibromomethyl derivative (V) was treated with three equivalents of silver acetate in a 22% solution of potassium acetate in acetic acid. The mixture was heated on the steam-bath for one-half hour, filtered and concentrated until crystallization took place. It was then filtered, washed with acetone and the combined filtrates evaporated to dryness and extracted with ether. On the addition of hydrogen chloride gas, an oil separated which crystallized on the addition of alcohol. After recrystallization from absolute alcohol, the hydrochloride of vitamin B_6 diacetate melted at 160–161°; 25% yield. A mixed melting point with the triacetate hydrochloride showed a depression of 20°. Its aqueous solution gave a good ferric chloride test. Hydrolysis experiments showed that it had the same relative stability as that of the triacetate.

Anal. Calcd. for $C_{12}H_{16}O_5NCl$: C, 49.75; H, 5.56; N, 4.84. Found: C, 49.99; H, 5.82; N, 4.82.

2,4,5-Trimethyl-3-hydroxypyridine, VII.—An alcoholic solution containing 1.88 g. of the dibromo derivative (V)

(5) Möller, Zima, Jung and Moll, *Naturwiss.*, **27**, 228 (1939).

(6) Stiller, Keresztesy and Stevens, THIS JOURNAL, **61**, 1237 (1939).

(7) Harris, Stiller and Folkers, *ibid.*, **61**, 1242 (1939).

(8) Möller, *Z. physiol. Chem.*, **260**, 246 (1939).

(9) Scudi, Koones and Keresztesy, *Proc. Soc. Exptl. Biol. Med.*, **43**, 118 (1940).

(10) The reaction of vitamin B_6 with the 2,6-dichloroquinone chlorimide was negative in the presence of a borate buffer according to the investigations of Dr. J. V. Scudi in this Laboratory, *Proc. Am. Physiol. Soc.*, **168** (1940); *J. Biol. Chem.*, November (1940).

was reduced with hydrogen and palladium on barium carbonate catalyst. The theoretical amount of hydrogen was absorbed in two or three minutes. A small amount of sodium bicarbonate solution was added to complete the neutralization and the solution was evaporated to dryness, extracted with acetone and again evaporated to dryness. The solid residue was dissolved in ether from which the 2,4,5-trimethyl-3-hydroxypyridine crystallized on concentration and cooling; m. p. 178°; yield 0.28 g. (40%).

Anal. Calcd. for $C_8H_{11}ON$: C, 70.04; H, 8.09; N, 10.19. Found: C, 69.78; H, 7.85; N, 10.31.

An additional yield was obtained from the ether mother liquors on the addition of hydrogen chloride, whereupon the hydrochloride of 2,4,5-trimethyl-3-hydroxypyridine crystallized. After recrystallization from absolute alcohol, the substance melted at 216°.

Anal. Calcd. for $C_8H_{12}ONCl$: C, 55.33; H, 6.96; N, 8.06. Found: C, 55.15; H, 7.01; N, 7.91.

The Hydrochloride of 2,4-Dimethyl-3-hydroxy-5-hydroxymethylpyridine, IV.—A solution of 1 g. of vitamin B_6 hydrochloride in 140 cc. of 95% alcohol was shaken with hydrogen in the presence of 0.5 g. of Adams catalyst until three moles had been absorbed. The only product which was isolated in a crystalline form proved to be a desoxy vitamin B_6. The same compound was obtained by the reduction of the 4-ethoxy derivative of vitamin B_6. Since an ethoxy group was removed by this reduction the product must be 2,4-dimethyl-3-hydroxy-5-hydroxymethylpyridine hydrochloride. It melted at 267–268°. Möller and collaborators[5] reported a melting point of 254°.

Anal. Calcd. for $C_8H_{12}NO_2Cl$: C, 50.66; H, 6.38; N, 7.39. Found: C, 50.67; H, 6.36; N, 7.37.

2 - Methyl - 3 - hydroxy - 4 - methoxymethyl - 5 - hydroxymethylpyridine Hydrochloride, VIII.—One gram of vitamin B_6 hydrochloride was exactly neutralized with one equivalent of sodium methoxide in 20 cc. of methanol and heated in a bomb tube at 125° for four hours. The solution was evaporated to dryness and dissolved in acetone to remove the sodium chloride. Crystals were obtained which proved to be impure vitamin B_6 base. The mother liquor yielded an oily residue which was extracted with ether and treated with ethereal hydrogen chloride when a crystalline product separated which melted at 168° after two recrystallizations from absolute ethanol.

Anal. Calcd. for $C_9H_{14}O_3NCl$: C, 49.18; H, 6.42; N, 6.39; OCH_3, 14.1. Found: C, 49.02; H, 6.43; N, 6.33; OCH_3, 11.28, 11.15; NCH_3, 0.

The product gave positive ferric chloride and 2,6-dichloroquinone chlorimide reactions[9] even in the presence of a borate buffer.[10] This indicated that the 4-hydroxymethyl group was substituted thus preventing the formation of the borate complex. Mixed melting point with a known synthetic sample of 2-methyl-3-hydroxy-4-methoxymethyl-5-hydroxymethylpyridine hydrochloride (VIII) indicated that these products might be the same.

In a second experiment with six grams of vitamin B_6 hydrochloride in 60 cc. of methanol containing 1 equivalent of sodium methoxide, the solution was heated for eight hours at 130°. An insoluble precipitate was obtained indicating that condensation had taken place to some extent. The filtrate yielded an ether soluble fraction that gave a hydrochloride; yield 0.75 g. (12%); m. p. 181°. There was no depression of mixed melting point with an authentic sample of 2-methyl-3-hydroxy-4-methoxymethyl-5-hydroxymethylpyridine hydrochloride which also had a melting point of 181°. This sample for mixed melting point was kindly furnished by Dr. J. van de Kamp, who synthesized it by a series of reactions similar to those previously reported for the corresponding ethoxy derivative.[1]

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Summary

Acetylation of vitamin B_6 with acetic anhydride gave a triacetate which in turn gave a crystalline hydrochloride. Vitamin B_6 dibromide hydrobromide yielded a diacetate when treated with silver acetate which also gave a crystalline hydrochloride. Reduction of the dibromide gave 2,4,5-trimethyl-3-hydroxypyridine. Catalytic reduction of vitamin B_6 or its 4-ethoxymethyl derivative gave 2,4 - dimethyl - 3 - hydroxy - 5 - hydroxymethylpyridine. Methyl alcoholic solutions of vitamin B_6 when heated at 125° gave a small yield of the 2-methyl-3-hydroxy-4-methoxymethyl-5-hydroxymethylpyridine.

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