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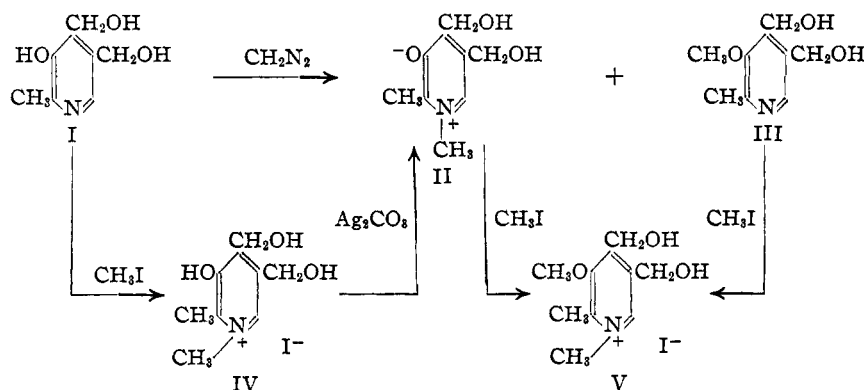
Chemistry of Vitamin B₆. I. Tautomerism*

BY STANTON A. HARRIS, T. J. WEBB AND KARL FOLKERS

The tautomerism of vitamin B₆ (I) was first reported by Keresztesy and Stevens.¹ It was stated² later that the observed changes in structure could be explained in terms of the amphoteric nature of the vitamin. Further evidence as to the nature of this tautomerism has now been obtained by the comparison of the absorption spectra and ionization constants of vitamin B₆ with those of vitamin B₆ methiodide (IV), N-methyl-vitamin B₆ betaine (II) and related compounds.

Vitamin B₆ methiodide has been made in a nearly quantitative yield from vitamin B₆ base and methyl iodide in a mixture of benzene and methyl alcohol. This quaternary salt gave neither the Gibbs color test with 2,6-dichloroquinone chlorimide, as developed³ for vitamin B₆, nor the *p*-dimethylaminobenzaldehyde reaction for α -methylpyridine methiodides. Treatment of vitamin B₆ methiodide in water solution with silver carbonate gave an N-methyl derivative of vitamin B₆ (II), which proved to be identical with one of the compounds obtained by Ichiba and Michi⁴ by the action of diazomethane on vitamin B₆. This N-methyl derivative, on treatment with methyl iodide at 110–115°,⁵ gave O-methyl-vitamin B₆ methiodide (V) which had previously been made⁶ by the action of methyl iodide on O-methyl-vitamin B₆ (III). The O-methyl-vitamin B₆ was made by the action of diazomethane on vitamin B₆ by Kuhn and Wendt,⁷ Stiller, Keresztesy

and Stevens,² and by Ichiba and Michi,⁴ the latter of whom also isolated the N-methyl derivative (II) from the reaction mixture. This reaction of diazomethane on vitamin B₆ has been repeated and the N-methyl derivative was found to be identical with the compound made from vitamin B₆ methiodide and silver carbonate. Its analyses, its solubility in water and ethanol, and its insolubility in ether and chloroform are properties expected for an internal salt. These properties and reactions (IV \rightarrow II \rightarrow V) of the N-methyl derivative are those of a phenol betaine.



The ultraviolet absorption spectra⁸ of N-methyl-vitamin B₆ betaine (II) and of vitamin B₆ methiodide (IV) in aqueous solution at different pH values are shown in Figs. 1 and 2. In addition, Fig. 2 includes the curve for vitamin B₆ at pH 4.5 for comparison with the methiodide at the corresponding pH. The striking similarity between the absorptions of the N-methyl-vitamin B₆ betaine and of the vitamin B₆ methiodide at a given pH is sufficient to indicate identity of the two structures at a given pH.⁹ This similarity is to be expected since, in the light of the above structures, each should give rise to the same ionization equilibria at a given pH. It is to be noted further that the spectra of these two compounds are similar to those of vitamin B₆ shown in Fig. 3, and especially so as regards the marked shifts in absorption which accompany changes in pH.

(8) In the figures, absorptions are represented in terms of the extinction coefficient computed on the basis of one millimole per liter; wave lengths, in Å. units.

(9) The absorption of the methiodide is doubtless increased somewhat in the region below 2400 Å. on account of the absorption of the iodide ion itself (see Scheibe, *Ber.*, **59**, 1322 (1926)).

* Data in this paper were presented to the Organic Division of the American Chemical Society at Detroit, Michigan, on September 11, 1940.

(1) Keresztesy and Stevens, *THIS JOURNAL*, **60**, 1267 (1938).

(2) Stiller, Keresztesy and Stevens, *ibid.*, **61**, 1239 (1939).

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

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

(5) This reaction is analogous to the reaction of protopapaverine with methyl iodide to give the O-methyl ether methiodide (Späth and Epstein, *Ber.*, **61**, 334 (1928)), and is quite general for phenol betaines.

(6) Kuhn and Löw, *ibid.*, **72**, 1453 (1939).

(7) Kuhn and Wendt, *ibid.*, **71**, 1534 (1938).

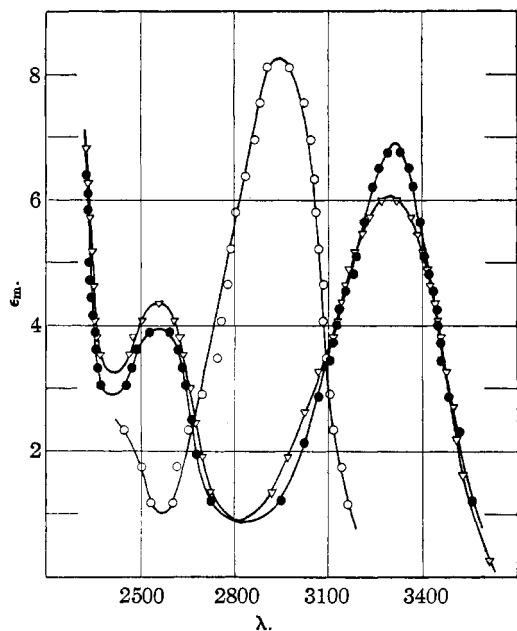


Fig. 1.—Absorption spectra⁸ for N-methyl-vitamin B₆ betaine in aqueous solution: ●, at pH 10.2; ○, at pH 2.1; ▽, at pH 6.5.

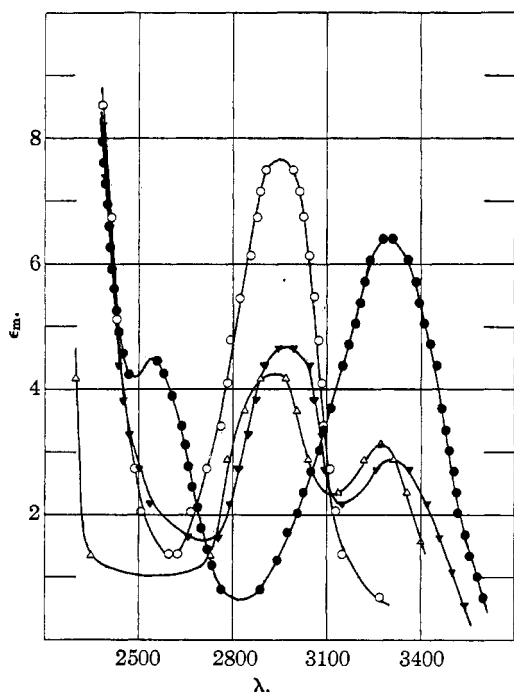


Fig. 2.—Absorption spectra⁸ for vitamin B₆ methiodide in aqueous solution: ●, at pH 10.2; ○, at pH 2.1; ▽, at pH 4.8; Δ, vitamin B₆ hydrochloride at pH 4.5.

With β -hydroxypyridine derivatives, further examples of the changes in absorption which accompany changes in pH may be given. β -Hydroxypyridine² itself showed changes resembling

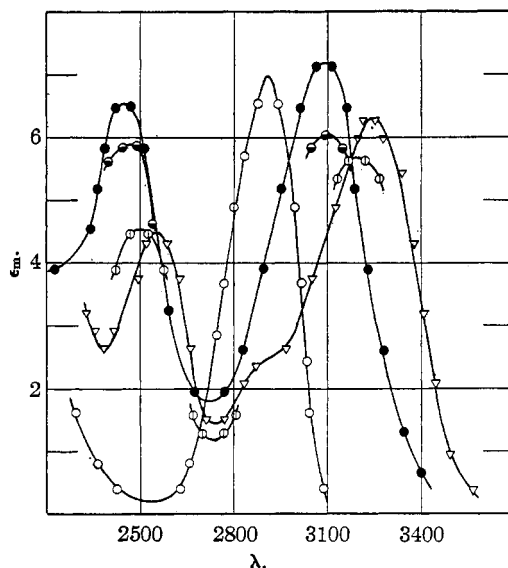


Fig. 3.—Absorption spectra⁸ for vitamin B₆ hydrochloride: ○, at pH 2.1; ▽, at pH 6.6 (0.01 *M* sodium acetate); ○, at pH 8 (0.01 *M* disodium hydrogen phosphate); ●, at pH 10.2; ●, at pH 14.

unmistakably those of vitamin B₆, in spite of the fact that the lack of additional substituents resulted in an appreciable alteration in the absorption at a given pH. With the methiodide of β -

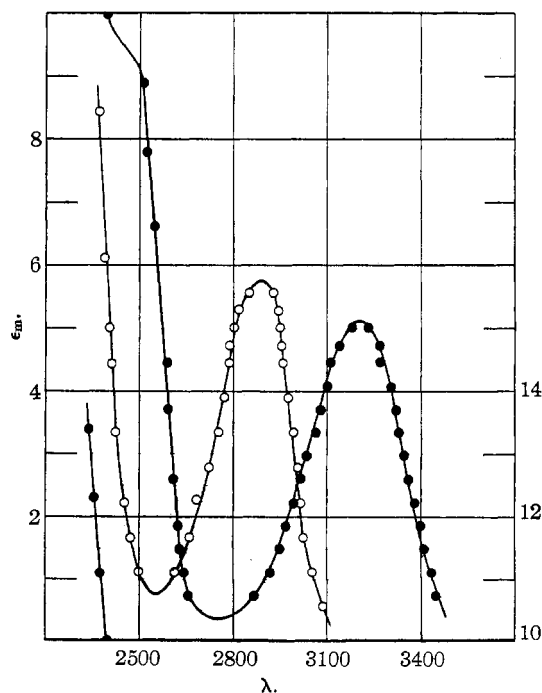


Fig. 4.—Absorption spectra⁸ for β -hydroxypyridine methiodide in aqueous solution: ●, at pH 10.2; ○, at pH 2.1. (Ordinates on the right refer only to the curve at the extreme lower left.)

hydroxypyridine, as shown in Fig. 4, the changes with pH were still unmistakably like those observed in the case of vitamin B_6 although the absorption in the neutral and alkaline range no longer closely resembles that of vitamin B_6 . The shifts of the absorption with changing pH in the case of these compounds was in sharp contrast to the O-methyl-vitamin B_6 ,² as well as its methiodide, neither of which showed any alteration in absorption as the pH was changed from 2 to 10. A single band at approximately 2800 Å. was observed for both compounds. Furthermore, it was of interest to see whether the characteristic shift would still be discernible in the case of a compound which has a completely different absorption but which retains the β -hydroxypyridine grouping. For this purpose, the absorption spectra of 3-methyl-4-hydroxyisoquinoline (VI) were measured at pH 2.1, 13, and 14. The results are shown in

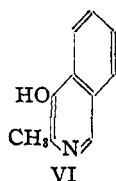


Fig. 5, which reveal clearly the expected shifts.

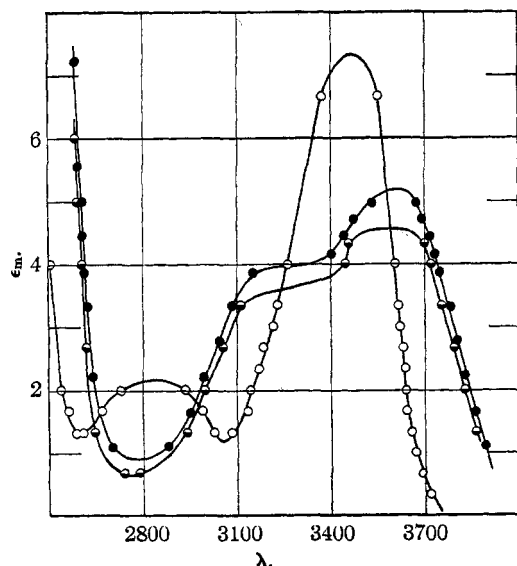
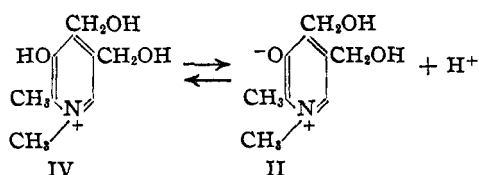


Fig. 5.—Absorption spectra⁸ for 3-methyl-4-hydroxyisoquinoline in aqueous solution: ●, at pH 14; ○, at pH 13; ○, at pH 2.1. The measurements at pH 2.1 were made in sufficient detail only to show the character of the band.

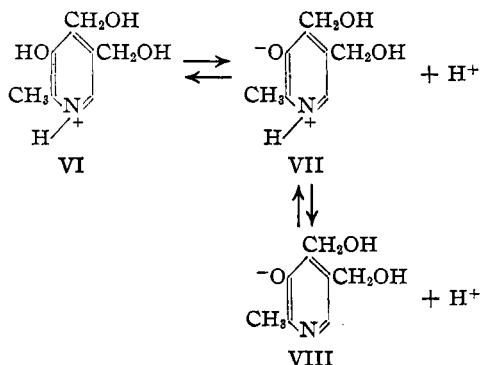
Further confirmation of the conclusions reached above as to the structural changes in vitamin B_6

and related compounds which accompany changes in pH was found in the potentiometric titrations of these compounds. Vitamin B_6 methiodide showed only one end-point in the pH range from 3 to 11.5 when titrated potentiometrically with alkali. This end-point was very pronounced, the pH changing from 7 to 9 on the addition of approximately 0.1 ml. of alkali. The mid-point of the neutralization occurred at pH 4.92. In the light of the structures above, this titration was compared with the titration with acid of the N-methyl-vitamin B_6 betaine (II). The mid-point of this titration was observed to occur at pH 4.92, in exact agreement with that found in the titration of the methiodide with alkali. The titration of the betaine with acid is assumed therefore to represent the reverse of the titration of the methiodide with alkali in accordance with the ionization equilibrium, $IV \rightleftharpoons II$.



In addition it may be mentioned that β -hydroxypyridine methiodide titrated precisely like vitamin B_6 methiodide while pyridine methiodide bound no appreciable amount of alkali in the same range of pH (3–11.5). This evidence is assumed to indicate conclusively that at intermediate values of the pH , derivatives of β -hydroxypyridine methiodide give rise to zwitterion structures.

Vitamin B_6 hydrochloride neutralized two equivalents of alkali in the range of pH from 3 to 11.5. The mid-points occurred at pH 4.72 and 8.96, respectively. The similarity between the neutralization of the first equivalent of alkali by vitamin B_6 hydrochloride and the neutralization by alkali of vitamin B_6 methiodide indicated moreover that in each of these neutralizations alkali-binding groups were involved which were practically identical. Accordingly, the equilibria, $VI \rightleftharpoons VII \rightleftharpoons VIII$, are assumed to represent the successive stages of acidic ionization of vitamin B_6 hydrochloride. The enhancement of the ionization of the phenolic hydrogen in these compounds is readily explained qualitatively in terms of the electrostatic effects arising from the net positive charge on the nitrogen atoms. The two ionization constants for vitamin B_6 hydrochloride



indicated that the maximum percentage (98.5) of the zwitterion (VII) existed at pH 6.84.

The fact that vitamin B₆ hydrochloride neutralized the second equivalent of alkali was in agreement with the liberation of the weak base VIII. Further evidence on the structural changes, VI \rightarrow VII \rightarrow VIII, resulting from the first and second neutralizations was found in the absorption spectra. The absorption of vitamin B₆ hydrochloride in water, as shown in Fig. 3, shows the characteristic change from the one maximum at 2910 Å. (ϵ_m 7) at pH 2.1 to the two maxima at 3240 Å. (ϵ_m 6.3) and 2550 Å. (ϵ_m 4.5) at pH 6.6, the latter pH corresponding closely to the observed end-point of the first neutralization in the titration of the vitamin B₆ hydrochloride. It is to be noted that in the pH range 2.1 to 6.6, corresponding to the structural changes VI \rightarrow VII, the greater part of the change in the absorption has been effected. These changes are to be compared with the absorption of N-methyl-vitamin B₆ betaine in aqueous solution as shown in Fig. 1. The maximum at 2950 Å. (ϵ_m 8.25) at pH 2.1 has changed to two maxima at 3300 Å. (ϵ_m 6.1) and 2560 Å. (ϵ_m 4.3) at pH 6.5, the latter pH in turn corresponding to the point of neutralization in the titration of the methiodide. The close similarity in these absorptions of vitamin B₆ hydrochloride and methiodide at pH 2.1 and 6.5 to 6.6, together with the titration data, serve to establish the close relationship of the equilibrium (VI \rightarrow VII) of vitamin B₆ to the equilibrium (IV \rightarrow II) of the methiodide; thus, on the basis of the zwitterion interpretation for N-methyl-vitamin B₆ betaine (II), vitamin B₆ at pH 6.6 is to be regarded as existing essentially as the zwitterion (VII). The absorption of the vitamin B₆ between pH 6.6 and 10.2 shows definite changes in that the maximum at 3230 Å. (ϵ_m 6.3) decreases and then increases in intensity progressively with a corresponding shift

in the band to 3100 Å. (ϵ_m 6.0) at pH 10.2. The maximum at 2550 Å. at pH 6.6 shifts progressively to 2470 Å. (ϵ_m 5.9) at pH 10.2. These changes in absorption between pH 6.6 and 10.2 are the result of the structural change accompanying the shift in equilibrium, VII (zwitterion) \rightarrow VIII (tertiary base). It is to be noted, moreover, that in the absorption of N-methyl-vitamin B₆ betaine (shown in Fig. 1) there is no essential change in the absorption in the range of pH 6.5 to 10.2. This behavior is in agreement with the absence of a second neutralization reaction (in this range of pH) for the methiodide, corresponding to the structural change, VII \rightarrow VIII, for vitamin B₆.

Although vitamin B₆ in neutral aqueous solution is represented by the zwitterion structure, it is of interest to consider its structure in an organic solvent. The absorption of the vitamin B₆ base in absolute ethanol is shown in Fig. 6. Since the single band exhibited is similar to the single absorption band shown by the vitamin in acid aqueous solution (Fig. 3), it follows that the β -hydroxypyridine structure (I) represents the principal structural form. Nevertheless, the isolation of the N-methyl derivative (II), besides the O-methyl ether (III) from the reaction of diazomethane on the base in a mixture of methanol and ether, suggests the presence also of the zwitterion modification in such organic solvents.

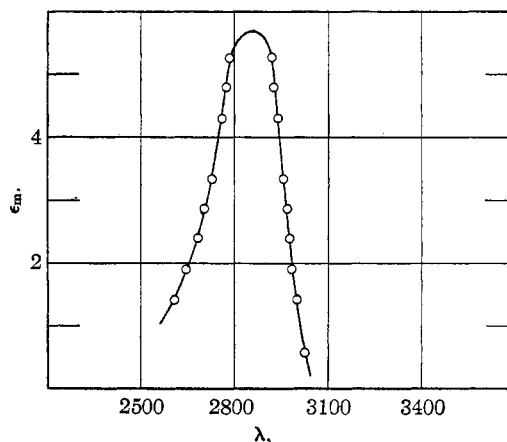
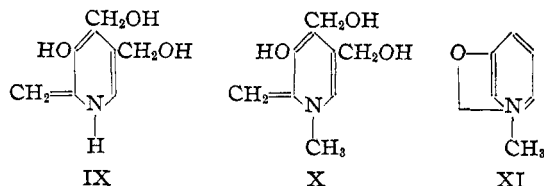


Fig. 6.—Absorption spectrum* for vitamin B₆ base in absolute ethanol.

The representation of vitamin B₆ as a zwitterion molecule in aqueous solution aided in the interpretation of the condensation reaction which occurs when a 10% aqueous solution of vitamin B₆ is heated at 125°. The nature of this reaction and the product will be described in a future paper.

Ichiba and Michi¹⁰ suggested that the tautomerism of vitamin B₆ involved the 2-methyl group as shown by the so-called "pyridone-methide" structure IX, and that the N-methyl derivative would be represented by X. This interpreta-



tion appears to be incorrect since β -hydroxypyridine, and its methiodide, which have no 2-methyl group, exhibit a tautomerism which is very similar to that of vitamin B₆ and its methiodide. Williams¹¹ prepared the N-methyl derivative of β -hydroxypyridine and suggested the betaine structure (XI).

The biological activity of the following compounds was determined in the Merck Institute for Therapeutic Research by Dr. Klaus Unna using a single dose curative assay¹² on vitamin B₆ depleted rats. Vitamin B₆ methiodide (IV) at a dose level of 1500 micrograms and the N-methyl-vitamin B₆ betaine (II) at a dose level of 2500 micrograms were completely inactive on vitamin B₆ deficient rats. The 3-methyl-4-hydroxyisoquinoline (VI) at a dose level of 2500 micrograms was completely inactive also. Thus the betaine and the methiodide showed no signs of activity at a dose level 50 times greater than the effective dose of the vitamin, demonstrating that methylation of the nitrogen atom of the vitamin B₆ molecule destroys the activity. The inactivity of the 4-hydroxyisoquinoline derivative at a dose level 50 times that of the vitamin shows that the 4- and 5-hydroxymethyl groups are essential.

Experimental Part

2 - Methyl - 3 - hydroxy - 4,5 - bis - (hydroxymethyl) - pyridine Methiodide (Vitamin B₆ Methiodide) (IV).—A solution of 0.5245 g. of vitamin B₆ base in 240 cc. of hot benzene plus 20–30 cc. of methanol was treated with 8 cc. of methyl iodide and was refluxed overnight, when crystals had separated. The solution was evaporated to one-third its volume, when the crystals were removed by filtration

and washed with ether to remove traces of iodine. The yield of vitamin B₆ methiodide was 0.965 g. (96.5%). Recrystallization from absolute alcohol raised the m. p. to 188–189°.

Anal. Calcd. for C₉H₁₄O₃NI: C, 34.74; H, 4.54; N, 4.50; (N)CH₃, 4.83. Found: C, 34.90; H, 4.42; N, 4.46; (N)CH₃, 4.46.

2 - Methyl - 3 - hydroxy - 4,5 - bis - (hydroxymethyl) - pyridine Methyl Betaine (N-Methyl-vitamin B₆ Betaine) (II).—A solution of 2 g. of vitamin B₆ methiodide in water was treated with freshly prepared silver carbonate until the iodide ion was completely removed. The solution was filtered and concentrated to dryness at the water pump and the residue was recrystallized from absolute alcohol. The total yield of N-methyl-vitamin B₆ betaine was 0.94 g. (80%). After recrystallization from absolute alcohol the melting point was 196°.

Anal. Calcd. for C₉H₁₃O₃N: C, 59.00; H, 7.15; N, 7.65. Found: C, 58.80; H, 7.13; N, 7.64.

This same compound⁴ was also prepared by the methylation of vitamin B₆ hydrochloride in methyl alcohol with diazomethane. It was separated from the O-methyl derivative of vitamin B₆ by extraction with ether in which the latter is soluble. The residue was dissolved in hot absolute alcohol, filtered with charcoal and treated with an excess of ether. The yield of N-methyl-vitamin B₆ betaine was 0.11 g., 12.3%, m. p. 180–185°. After recrystallization from absolute alcohol the melting point and mixed melting point with the sample described above was 193–195°.

2 - Methyl - 3 - methoxy - 4,5 - bis - (hydroxymethyl) - pyridine Methiodide (O-Methyl-vitamin B₆ Methiodide) (V).—This compound was first made by Kuhn and Löw⁸ by treating O-methyl-vitamin B₆ with methyl iodide in a bomb tube at 100°. The ether soluble portion from the diazomethane reaction on vitamin B₆ was treated with methyl iodide in boiling benzene solution. After two hours an oil separated which solidified on cooling and was recrystallized three times from absolute alcohol when the melting point was 124.5–126°. This melting point and the analysis showed it to be the same compound as obtained by Kuhn and Löw.⁸

Anal. Calcd. for C₁₀H₁₆O₃NI: C, 36.92; H, 4.95; N, 4.32. Found: C, 36.90; H, 5.10; N, 4.28.

This compound also was obtained by the treatment of the N-methyl derivative of vitamin B₆ with methyl iodide in a bomb tube at 110–115° for three and one-half hours. After recrystallization from absolute alcohol, the melting point and mixed melting point were the same as described above.

β -Hydroxypyridine Methiodide.—Fischer and Renouf¹³ prepared the methiodide of β -hydroxypyridine by combining the two components in a bomb tube at 100° but they did not give the melting point or the complete analysis. It has now been made by boiling a benzene solution of β -hydroxypyridine with an excess of methyl iodide. The yield from 0.1186 g. was 0.23 g., 78%, m. p. 114–116°, after crystallization from alcohol and ether or by concentrating an acetone solution.

Anal. Calcd. for C₆H₈NOI: C, 30.40; H, 3.40; N, 5.91. Found: C, 30.58; H, 3.12; N, 5.55, 5.58.

(13) Fischer and Renouf, *Ber.*, **17**, 1896 (1884).

(10) Ichiba and Michi, *Sci. Papers Inst. Phys. Chem. Research (Tokyo)*, **36**, 1 (1939).

(11) Williams, *Ind. Eng. Chem.*, **13**, 1107 (1921).

(12) Reedman, Sampson and Unna, *Proc. Soc. Exptl. Biol. Med.*, **43**, 112 (1940). By this procedure it has been shown that a single dose of 100 micrograms of vitamin B₆ (hydrochloride) cures 100% of the deficient animals within 14 days, and that a dose of 50 micrograms produces complete cures in 75% of the animals. Lower doses fail to produce complete cures, but signs of partial healing were obtained regularly with 25 micrograms.

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 C₁₀H₉NO: 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

Acknowledgments.—The authors wish to express their appreciation to Messrs. G. A. Boyack and A. N. Wilson for assistance on preparations, to Messrs. D. F. Hayman, H. S. Clark and W. Reiss for the microanalyses, and to Mr. W. A. Bastedo, Jr., for assistance on the physical measurements.

Summary

Methyl iodide reacted with vitamin B₆ to yield the methiodide quantitatively.

Vitamin B₆ methiodide on treatment with silver carbonate and vitamin B₆ on treatment with diazo methane yielded the same N-methyl-vitamin B₆.

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

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 B₆ derivative is a phenol betaine or zwitterion.

The close similarity in the absorptions of the vitamin B₆ methiodide and hydrochloride at pH 2.1 to 6.6 and the close similarity in the titration data show that at pH 6.8 in aqueous solution, vitamin B₆ 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 B₆ is believed to be erroneous.

The absorption spectra of β -hydroxypyridine and its methiodide, O-methyl-vitamin B₆ methiodide, and 3-methyl-4-hydroxyisoquinoline were found to be in agreement with the interpretation given to the absorption spectra of vitamin B₆ methiodide and hydrochloride.

Methylation of the nitrogen atom of vitamin B₆ destroyed its biological activity.

RAHWAY, N. J.

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[CONTRIBUTION FROM THE RESEARCH LABORATORY OF MERCK & CO., INC.]

Chemistry of Vitamin B₆. II. Reactions and Derivatives

BY STANTON A. HARRIS

In connection with the synthesis of vitamin B₆ in this Laboratory,¹ a number of pyridine compounds related structurally to vitamin B₆ were made for the determination of their antidermatitic effect on vitamin B₆ 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 Wendt³ and Ichiba and Michi⁴ have described a triacetate of vitamin B₆ which, after sublimation in high vacuum, tended to crystal-

lize. The hydrochloride of vitamin B₆ triacetate (II) is described herein as a crystalline substance. The hydrochloride of vitamin B₆ 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 active² as vitamin B₆. 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 biological² and microbiological assays.

Hydrogenation of vitamin B₆ 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).