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Studies of Crystalline Vitamin B_1. XVII. Synthesis of Vitamin B_1

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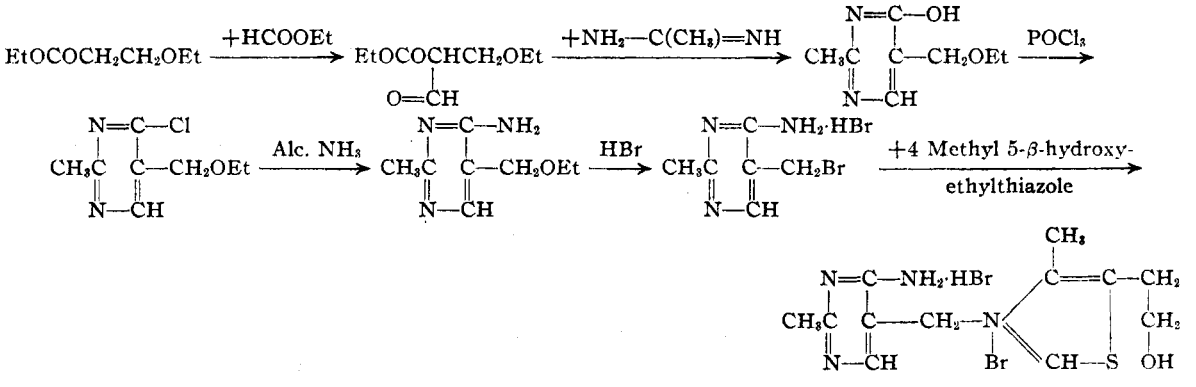
The structure of vitamin B_1 having been determined by previous identification and ultimate synthesis of several degradation products, notably 4-methyl-5-beta-hydroxyethylthiazole,[1] 2,5-dimethyl-6-aminopyrimidine,[2] 2-methyl-6-oxypyrimidine-5-methylene sulfonic acid[3] and the mode of linkage of the two nuclei indicated by the presence of quaternary nitrogen in the vitamin,[4] a synthesis of the vitamin was undertaken.

The possibilities of three principal routes of synthesis leading to the pyrimidine portion of the vitamin were explored: (1) the addition of formaldehyde to the 5-position of an appropriate pyrimidine; (2) a Curtius, Hoffmann or Loessen degradation of appropriate derivatives of 2-methyl-6-oxypyrimidine-5-acetic acid whose ethyl ester, hydrazide and amide we had prepared in excellent yields; and (3) the conversion of a 5-ethyoxymethyl-pyrimidine into the corresponding 5-halomethyl derivative.

Of these the first two proved difficult to control at one or more stages. The third offered more promise of amenability. As has been indicated in a preliminary communication,[5] a successful synthesis has been worked out according to the scheme



The final condensation step in the synthesis took place less readily than had been anticipated and numerous trials were necessary before crystalline material was obtained in a state of apparent purity although ample antineuritic potency could be demonstrated in the reaction mixtures by physiological tests. The difficulty of isolating the crystals was enhanced by their surprising solubility in alcoholic solvents. As first obtained, the crystalline bromide hydrobromide melted at 219–220° and the chloride hydrochloride at 232–234°. These were obtained by adding ether to methanol solutions. Presently, however, through the cooperation of Dr. A. G. Stein, a method was evolved whereby the vitamin was recrystallized as the bromide hydrobromide from methanol and as the chloride hydrochloride from water and ethanol. The bromide hydrobromide so obtained melted at 227–231° and the chloride hydrochloride at 248–250°. In no case is the melting point of either natural or synthetic vitamin very satisfactory as a criterion of purity as melting is preceded by decomposition. Further, melting on a hot stage under crossed Nicols reveals that in all cases a loss of birefringence occurs at about 190°, suggesting some intramolecular transformation at that temperature.

We have compared the low and high melting forms somewhat elaborately by crystallographic, spectrometric and electrometric means as well as analytically without detecting clear evidence of the presence of an impurity in the low melting crystals. Furthermore, numerous physiological tests including both curative and prophylactic experiments have likewise indicated as yet no significant deviations among the two forms of synthetic and the natural chloride. Further re-

(1) H. T. Clarke and S. Gurin, THIS JOURNAL, **57**, 1876 (1935); E. R. Buchman, *ibid.*, **58**, 1803–1805 (1936).

(2) R. R. Williams, A. E. Ruehle and J. Finkelstein, *ibid.*, **59**, 526–530 (1937).

(3) J. K. Cline, R. R. Williams, A. E. Ruehle and R. E. Waterman, *ibid.*, **59**, 530–533 (1937).

(4) R. R. Williams and A. E. Ruehle, *ibid.*, **57**, 1856–1860 (1935).

(5) R. R. Williams, *ibid.*, **58**, 1063–1064 (1936).

port will be made when these experiments are complete.[6]

Immediately after the publication of our preliminary communication of the synthesis,[7] there reached us a paper by Dr. Rudolf Grewe[8] confirming the structure previously proposed by ourselves[5] and containing the information that a synthesis of the vitamin had been achieved in recent months by Drs. Andersag and Westphal at the Elberfeld Laboratories of the I. G. Farbenindustrie A.-G. To date no publication has appeared revealing the route of this synthesis or the characteristics of the product. It is therefore impossible to compare the results with our own at this time. Happily, however, for science there appears to be no ground for dispute regarding the true constitution of the vitamin.

The yields reported herein have in many instances been improved by modifications which are still under investigation. Our synthesis has proved amenable to large scale development and has already been utilized for the production of many kilos of the vitamin on an economical basis.

Ethyl Sodioformyl-β-ethoxypropionate.—A mixture of 73 g. of ethyl β-ethoxypropionate and 40 g. of ethyl formate was dropped slowly during eight hours onto 12 g. of sodium wire covered with anhydrous ether. The yellow sodioformyl derivative thus formed appeared as a solid mass which occluded all the ether and unreacted esters. The sodioformyl derivative was used without isolation for the subsequent reaction. It must be protected from atmospheric moisture and should be used promptly as it is not very stable.

2 - Methyl - 5 - ethoxymethyl - 6 - oxypyrimidine.—To the crude sodioformyl derivative obtained above 45 g. of acetamidine hydrochloride, 100 cc. of absolute alcohol, and a solution of 12 g. of sodium in 200 cc. of absolute alcohol were added. The ether was distilled off and the mixture heated, under reflux, for sixteen hours. The contents of the flask was then cooled, neutralized with 10% acetic acid and evaporated on the steam-bath. The residue was taken up in a small amount of water and extracted repeatedly with chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate and the chloroform removed by evaporation *in vacuo*. The remaining brown gummy substance was treated with dioxane, which dissolved a portion and left a discolored white solid. The residual solid was separated, dried, and sublimed in high vacuum at 140°. The sublimate was placed in a Soxhlet extractor, extracted repeatedly with anhydrous ether and the residue again sublimed in high vacuum. The sublimate was a pure white cake of 2-methyl-5-ethoxymethyl-6-oxypyrimidine which melted at 175–176°; yield 3.5%.

Anal. Calcd. for $C_8H_{12}O_2N_2$: C, 57.11; H, 7.20. Found: C, 56.92, 56.96; H, 7.04, 6.84.

2 - Methyl - 5 - ethoxymethyl - 6 - chloropyrimidine.—One gram of 2-methyl-5-ethoxymethyl-6-oxypyrimidine was heated with 8 cc. of phosphorus oxychloride for three hours at 78°. The pyrimidine dissolved slowly and left but a small quantity of undissolved material. The excess phosphorus oxychloride was then distilled off *in vacuo*. To the residue was added a small amount of crushed ice and water and the excess acid neutralized by the addition of solid sodium bicarbonate. The dark colored solution was extracted repeatedly with chloroform and the combined chloroform extracts were dried over anhydrous sodium sulfate. The chloroform was removed *in vacuo* and the residue distilled. A fraction which boiled at 78–80° at 1 mm. was collected. On redistillation the pure chloropyrimidine boiled at 72–73° at 0.5 mm. and was obtained as a colorless oil with an odor resembling acetamide; yield 70%.

Anal. Calcd. for $C_8H_{11}N_2OCl$: C, 51.46; H, 5.94; N, 15.01; Cl, 19.01. Found: C, 50.84, 50.82; H, 5.64, 5.70; N, 14.85, 14.92; Cl, 18.95.

2 - Methyl - 5 - ethoxymethyl - 6 - aminopyrimidine.—One gram of 2-methyl-5-ethoxymethyl-6-chloropyrimidine was treated with 15 cc. of saturated alcoholic ammonia in a bomb-tube at 140° for fifteen hours. The contents of the tube were concentrated *in vacuo* and a partly crystalline residue remained. The residue was dissolved in a small amount of water, the solution made alkaline by addition of sodium carbonate and the solution then extracted repeatedly with chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate and the chloroform was removed *in vacuo*. A gummy residue containing a small amount of crystals was left. When this residue was treated with hot anhydrous ether a solid separated immediately. The solid was removed by centrifuging and discarded. The ethereal mother liquors on standing and partial evaporation of the ether deposited large crystals of the impure aminopyrimidine, which were filtered off, dried, and purified by repeated sublimation in high vacuum at 60–80°. The product, a pure white pyrimidine with an odor reminiscent of piperidine, melted at 89.5–90.5°; yield 70%. *Anal.* Calcd. for $C_8H_{13}ON_3$: C, 57.45; H, 7.58; N, 25.13. Found: C, 57.31, 57.62; H, 7.62, 7.65; N, 25.64, 25.50, 25.82.

2 - Methyl - 5 - bromomethyl - 6 - aminopyrimidine Hydrobromide.—One hundred and fifty mg. of 2-methyl-5-ethoxymethyl-6-aminopyrimidine was heated with 10 cc. of a 10% solution of anhydrous hydrobromic acid in glacial acetic acid for two hours at 100°. At the end of this time the mixture was cooled and the liquid remaining decanted from the crystals which separated out. The crystals were washed several times with anhydrous ether and then purified by dissolving in a small amount of cold methanol and reprecipitating by the addition of ether. The pure compound as obtained melts at 192–193° and is the hydrobromide of 2-methyl-5-bromomethyl-6-aminopyrimidine; yield 90%. *Anal.* Calcd. for $C_6H_9N_3Br_2$: C, 25.45; H, 3.21; N, 14.85. Found: C, 26.36, 26.55, 26.45; H, 3.50, 3.62, 3.34; N, 14.71, 14.99.

Vitamin B_1 Bromide Hydrobromide.—One hundred and fifty mg. of 2-methyl-5-bromomethyl-6-aminopyrimidine hydrobromide was heated with 150 mg. of 4-methyl-5-β-

(6) R. R. Williams and J. K. Cline, THIS JOURNAL, **59**, 216 (1937).
(7) R. R. Williams and J. K. Cline, *ibid.*, **58**, 1504–1505 (1936).
(8) R. Grewe, *Z. physiol. Chem.*, **242**, 89–96 (1936).

hydroxyethylthiazole[9] and 0.2 cc. of butanol for fifteen minutes at 120°. The pyrimidine went into solution and shortly thereafter a precipitate settled out. The reaction mixture was diluted with 1 cc. of boiling absolute ethyl alcohol and allowed to stand until no more material settled out. The alcoholic mother liquors were removed by filtration and the precipitate washed several times with small amounts of cold absolute ethyl alcohol. The precipitate then was recrystallized by dissolving in hot methanol, adding absolute alcohol to the hot solution until a permanent cloud appeared and allowing to cool slowly.

The condensation product thus obtained occurs as rosets of needles which melt at 229–231°; yield 45%.

With doses of 6 γ, cures of polyneuritis were effected in rats on a vitamin B_1-free diet. These cures endured for several days, indicating an activity equal to the natural vitamin. It appears to hold one-half molecule of water of crystallization. *Anal.* Calcd. for $C_{12}H_{17}ON_4SBr \cdot HBr \cdot 0.5\,H_2O$: C, 33.09, H, 4.51; N, 12.87. Found: C, 33.18, 32.76; H, 4.82, 4.70; N, 12.73.

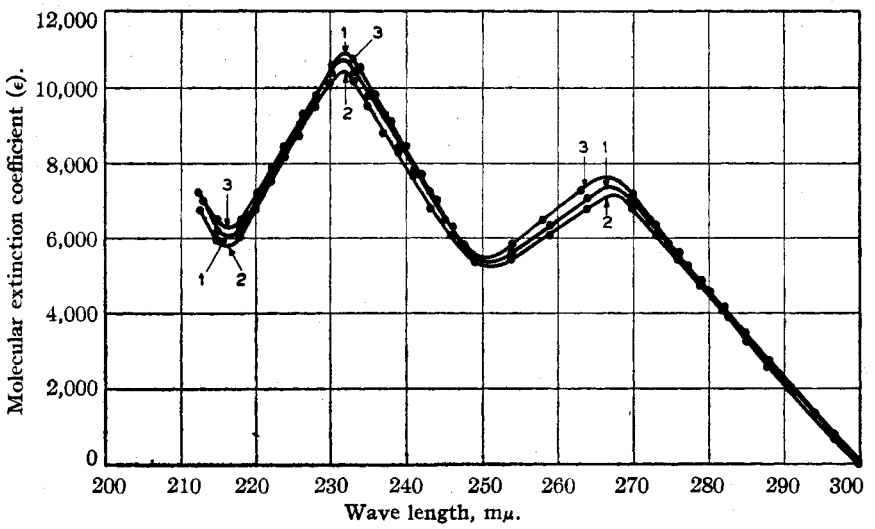


Fig. 1.—Curve 1, natural vitamin (hydrochloride); curve 2, synthetic vitamin (hydrochloride); curve 3, synthetic vitamin (hydrobromide).

Vitamin B_1 Chloride Hydrochloride.—One hundred and fifty mg. of vitamin B_1 bromide hydrobromide was dissolved in hot methanol and shaken with slight excess silver chloride for a half hour.

The silver salts were filtered off and to the hot solution absolute alcohol was added to incipient cloudiness. On cooling the vitamin hydrochloride crystallized out. To free it from traces of silver chloride it was dissolved in a little water and filtered. The filtrate was evaporated *in vacuo* to dryness, the crystalline residue was dissolved in a minimum amount of water and to the hot aqueous solution ten volumes of absolute alcohol was added. On standing the crystalline chloride hydrochloride separated melting at 248–250°; yield 90%. *Anal.* Calcd. for $C_{12}H_{17}ON_4SCl \cdot HCl \cdot 0.5H_2O$: C, 41.60; H, 5.53; N, 16.17; Cl,[10] 21.04; S,[10] 9.51. Found: C, 41.30, 41.72; H, 5.48, 5.64; N, 16.25, 16.26; Cl,[10] 21.30, 21.13; S,[10] 9.72, 9.56.

A comparison of the ultraviolet absorptions of the natural and synthetic products is shown in Fig. 1. Curative tests on polyneuritic rats have given the following results:

No. rats	Dose in γ	Cured	Recurrence of polyneuritis, days
7	3	2	6
7	4	2	5.5
7	4.5	3	6.7
12	5	7	6.3
8	5.5	7	7
9	6.0	9	5.6

We gratefully acknowledge our indebtedness to Mr. D. F. Hayman and Mr. S. Adler for the microanalyses, to Mr. A. E. Ruehle for the spectrograms, to Dr. E. R. Buchman for several samples of 4-methyl-5-β-hydroxyethylthiazole, and to Mr. H. W. Hermance for the crystallographic comparison of the synthetic and natural vitamin. Thanks are also due to Dr. R. T. Major for advice and for facilities put at our disposal. We also wish to express our appreciation to Dr. Hans Molitor and Mr. W. L. Sampson for the many physiological assays necessary in the course of the work.

Summary

1. A practical synthesis of vitamin B_1 has been described.

2. The synthesis of several new pyrimidines useful as intermediates in the synthesis of vitamin B_1 has been described.

3. The structure previously proposed for vitamin B_1 has been confirmed by synthesis.

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(9) E. R. Buchman, THIS JOURNAL, **58**, 1803 (1936).

(10) Analyses performed on anhydrous vitamin on macro scale. On account of hygroscopicity of the vitamin, Cl and S determinations on a micro scale were not very satisfactory. See Wintersteiner, Williams and Ruehle, THIS JOURNAL, **57**, 517 (1935).