tone, 0.9 cc. of 95% ethyl alcohol and 4 drops of diethylamine; to this was added 0.31 g. of quinoline-2-aldehyde hydrate. The reaction mixture was allowed to remain at room temperature for two days, and at the end of that time crystals of VIII had separated: soluble in benzene, xylene, butyl alcohol; recrystallized from xylene; m. p. 208–210°; yield 32%.

Anal. Calcd. for $C_{28}H_{20}O_3N_2$: N, 7.53. Found: N, 7.60.

Summary

Lepidine and 6-methoxylepidine are oxidized by selenium dioxide to quinoline-4-aldehyde and 6-methoxyquinoline-4-aldehyde, respectively. Quinoline-4-aldehyde condenses with nitromethane to give a product of the aldol type, while acetophenone yields diacetophenonyl-lepidine. Quinoline-2-aldehyde, previously described, forms aldol-like products with acetone, acetophenone and nitromethane.

UNIVERSITY HEIGHTS
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[CONTRIBUTION FROM PRIVATE LABORATORIES, AND RESEARCH LABORATORIES, MERCK AND COMPANY, INC.]

Studies of Crystalline Vitamin B_1. XV. C-Methylated 6-Amino- and 6-Oxypyrimidines

BY ROBERT R. WILLIAMS, A. E. RUEHLE AND JACOB FINKELSTEIN

For purposes of comparison with the pyrimidine cleavage products of vitamin B_1 (Aneurin), we have prepared 6-amino- and 6-oxypyrimidine and all of the possible mono- and di-C-methyl derivatives and have observed the ultraviolet absorption of each. 5-ethyl-6-aminopyrimidine is also included. The literature references indicate the methods of preparation of certain known compounds.[1] Some known compounds were obtained by new methods: *e. g.*, 4-methyl-6-oxy- and 5-methyl-6-oxypyrimidines were obtained by oxidizing the 2-thio derivatives with hydrogen peroxide.[2] 2,5-Dimethyl-6-oxypyrimidine resulted from condensing sodioformylpropionic ester with acetamidine and the oxy derivative was converted in the conventional way to the amino. The preparative operations were not carried out repeatedly so the reported yields in general are probably not optimal.

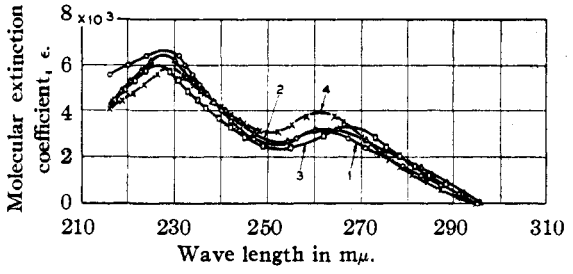


Fig. 1.—1. 6-Oxypyrimidine. 2. 4-Methyl-6-oxypyrimidine. 3. 2-Methyl-6-oxypyrimidine. 4. 5-Methyl-6-oxypyrimidine.

Discussion of Results

Curves of ultraviolet absorption in water solution for the series of 6-oxypyrimidines are shown in Figs. 1, 2 and 3. A great similarity of character is observed throughout, all the compounds exhibiting absorption in two bands which vary somewhat in frequency and slightly in intensity according to the number and position of substituent alkyls. Curves of this character are observed also in other oxy compounds in which one hydrogen of a substituent methyl group is replaced by ethoxy, halogen or sulfonic group as will develop in later papers.

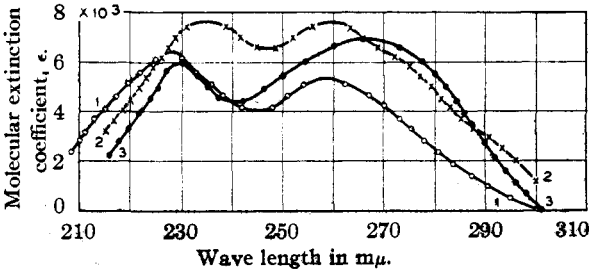


Fig. 2.—1. 2,4-Dimethyl-6-oxypyrimidine. 2. 4,5-Dimethyl-6-oxypyrimidine. 3. 2,5-Dimethyl-6-oxypyrimidine.

The 6-amino series shows a kindred absorption in water solution (Figs. 4 and 5), but the curves are less homogeneous in type than in the oxy series. The amino compounds show a striking

(1) (a) 6-Oxypyrimidine—Wheeler, *J. Biol. Chem.*, **3**, 287 (1907); (b) 6-aminopyrimidine—Buttner, *Ber.*, **36**, 2232 (1903); (c) 2-methyl-6-oxy- and 2-methyl-6-aminopyrimidine—Gabriel, *ibid.*, **37**, 3638 (1904); (d) 5-ethyl-6-aminopyrimidine—v. Merkatz, *ibid.*, **52**, 871 (1919); (e) 2,4-dimethyl-6-oxypyrimidine—Wollner, *J. prakt. Chem.*, [2] **29**, 132 (1884); (f) 2,4-dimethyl-6-aminopyrimidine—v. Meyer, *ibid.*, [2] **27**, 152 (1883).

(2) Wheeler and McFarland, *Am. Chem. J.*, **42**, 105 (1909).

influence of added acid and alkali as well as a lesser influence of the number and position of substituent alkyl groups. Comparison of each amino derivative with the corresponding oxy compound reveals that the marked acid–alkali contrasts are associated with the presence of the basic amino group (*cf.* Fig. 3 with Figs. 4 and 5).

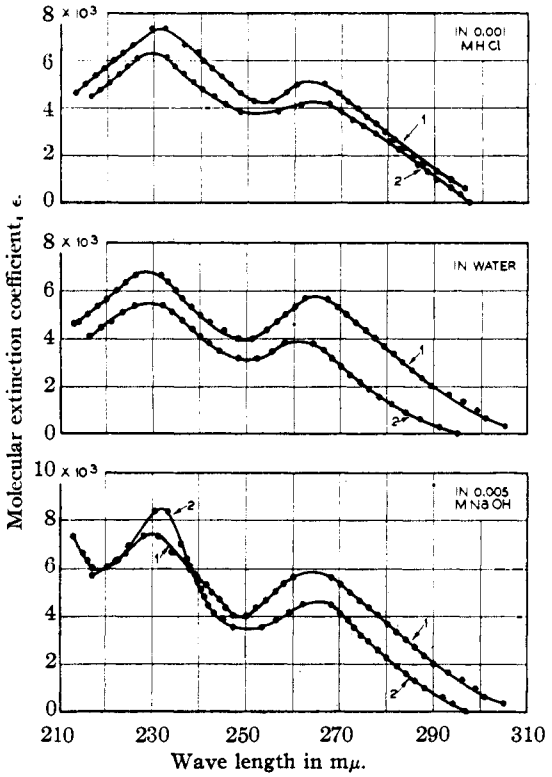


Fig. 3.—(a) In 0.001 *M* HCl, (b) in water, (c) in 0.005 *M* NaOH. 1. 2,5-Dimethyl-6-oxypyrimidine. 2. 5-Methyl-6-oxypyrimidine.

It will be noted that in the absence of added acid or alkali the 6-amino series shows a considerable range in respect to the relative prominence of the two absorption bands and that the addition of alkali in each case tends in the direction of equal prominence of the bands, that of acid toward submerging both bands under a more intense one at about 250 mμ. This fact suggested that the variation exhibited by the series in the absence of added base or acid might be a reflection of the basic strength of the several members of the series. Colorimetric determinations of the *p*H of concentrated water solutions of the compounds (see Table I), however, do not bear out this view in a quantitative sense.

The phenomenon which we have observed may well be intimately related in some fashion to the effect of acid and alkali on guanine,[3] as well as to the effect of acid on the vitamin itself.[4]

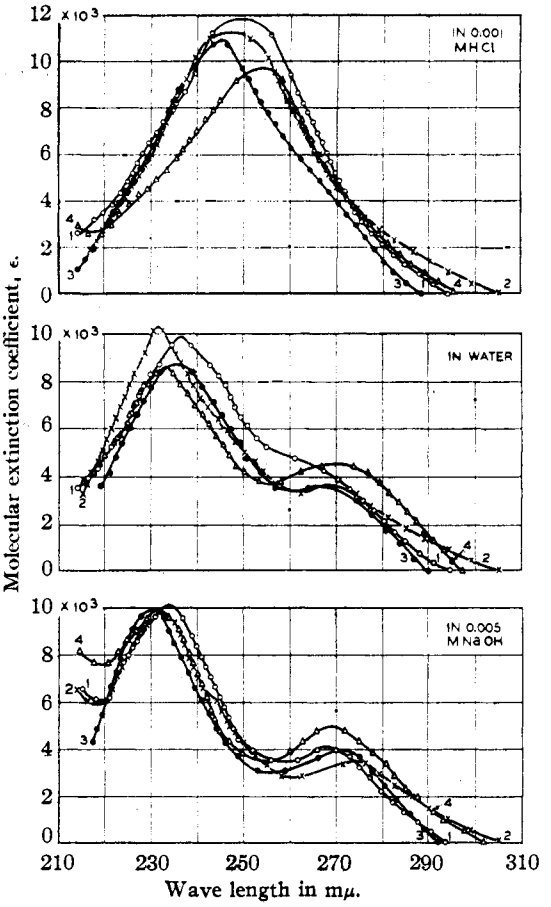


Fig. 4.—(a) In 0.001 *M* HCl, (b) in water, (c) in 0.005 *M* NaOH. 1. 4-Methyl-6-aminopyrimidine. 2. 6-Aminopyrimidine. 3. 2-Methyl-6-aminopyrimidine. 4. 5-Methyl-6-aminopyrimidine.

In the amino as in the oxy series, there is evidence which will be presented at a later time that substituents in side chains do not in general affect absorption profoundly.

It is of interest to note that the amino sulfonic acid, derived from the vitamin, exhibits only an inconspicuous band at 268 mμ in water solution (Fig. 6) which, however, becomes prominent in alkali and then more resembles that of the oxy-sulfonic acid. This behavior appears quite consistent with that of the 6-aminopyrimidines when one reflects that the acidic character of the sulfonic group would be expected to modify the absorption in the direction of submerging the band at the longer wave length. The weight

(3) F. F. Heyroth and J. R. Loofbourow, THIS JOURNAL, **56**, 1728 (1934); Holiday, *Biochem. J.*, **24**, 619 (1930).

(4) Holiday, *ibid.*, **29**, 718 (1935).

of the sulfonic group on the other hand presumably tends to shift this band toward longer wave lengths.

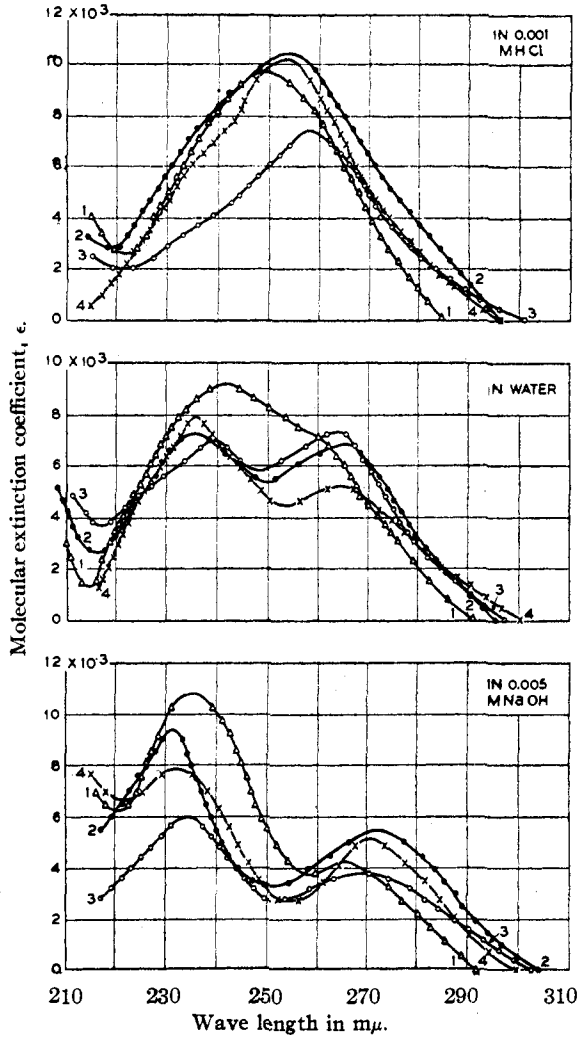


Fig. 5.—(a) In 0.001 *M* HCl, (b) in water, (c) in 0.005 *M* NaOH. 1. 2,4-Dimethyl-6-aminopyrimidine. 2. 2,5-Dimethyl-6-aminopyrimidine. 3. 4,5-Dimethyl-6-aminopyrimidine. 4. 5-Ethyl-6-aminopyrimidine.

Neither in the oxy nor the amino series is the effect of the number and position of substituent alkyls upon absorption sufficiently well differentiated to justify a broad generalization, although a considerable degree of consistency can be observed.

Experimental

4-Methyl-6-oxypyrimidine.—Two hundred and sixty-five cc. of 12% hydrogen peroxide was warmed to 70° to start reaction and 20 g. of 4-methyl-2-thio uracil[2] was added with stirring in small portions to avoid rise of temperature above 90°. The solution was evaporated to small volume, made alkaline with sodium carbonate and brought to dryness on the steam-bath. The residue was extracted with benzene, the benzene evaporated and the residue sublimed in high vacuum at 100–110°. Sublimate was recrystallized from ethyl acetate, m. p. 148–149°; yield 8 g.

Anal. Calcd. for $C_5H_6N_2O$: C, 54.54; H, 5.45; N, 25.45. Found: C, 54.93, 54.98; H, 5.28, 5.39; N, 25.71.

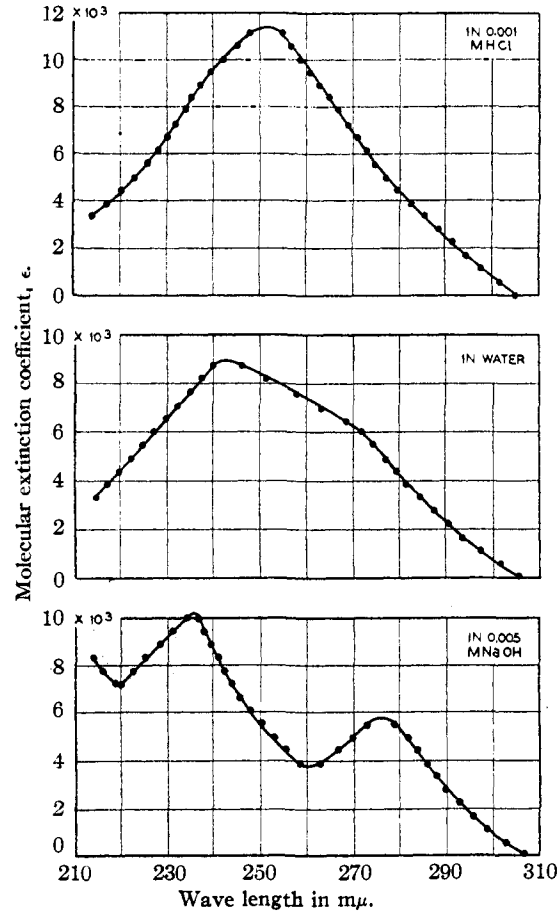


Fig. 6.—(a) In 0.001 *M* HCl, (b) in water, (c) in 0.005 *M* NaOH. Amino sulfonic acid.

This compound was prepared by Gabriel and Colman[5] from 4-methyl-2,6-dichloropyrimidine who found the m. p. 149–150°.

4-Methyl-6-aminopyrimidine.—One gram of 4-methyl-6-chloropyrimidine was heated with alcoholic ammonia at 110° for ten hours, the solution evaporated to dryness, the residue taken up in a minimum amount of water, potassium hydroxide added while cooling with ice and the crystalline product filtered off, recrystallized from hot water and sublimed in high vacuum at 85–90°; yield 0.4 g.; m. p. 194–195°.

Anal. Calcd. for $C_5H_7N_3$: N, 38.53. Found: N, 38.52, 38.52.

The same substance, prepared by Gabriel and Colman[5] from 2-chloro-4-methyl-6-aminopyrimidine, melted at 194–195°.

(5) Gabriel and Colman, *Ber.*, **32**, 2929 (1899).

5-Methyl-6-oxypyrimidine.—Seven and one-half grams of thiothymine[6] was oxidized with hydrogen peroxide as described above for methyl thiouracil. The solution was made alkaline with ammonia and evaporated to dryness. The residue was extracted with chloroform, the chloroform solution dried and evaporated and the residue sublimed in high vacuum at 110°, yielding 4.14 g. of a compound melting at 153–154°.

Anal. Calcd. for $C_5H_6N_2O$: C, 54.54; H, 5.45; N, 25.45. Found: C, 54.90, 54.81; H, 5.10, 5.32; N, 25.05, 25.17.

5-Methyl-6-aminopyrimidine.—One gram of the above compound was warmed gently in a water-bath with 4 cc. of phosphorus oxychloride until completely in solution. The solution was evaporated *in vacuo* to one-half volume and added to crushed ice, made alkaline with sodium hydroxide and extracted with chloroform. The extract was dried and evaporated *in vacuo* at 20° leaving an oil which did not crystallize. This was heated at 120° for eight hours with excess alcoholic ammonia. The product was isolated as in the case of 4-methyl-6-aminopyrimidine and sublimed in high vacuum at 110°, yielding a white sublimate of m. p. 175–176°.

Anal. Calcd. for $C_5H_7N_3$: C, 55.05; H, 6.42; N, 38.53. Found: C, 55.48, 55.71; H, 6.47, 6.46; N, 38.88, 38.60.

This compound was prepared from 4-iodo-5-methyl-6-aminopyrimidine by Gerngross[7] who recorded its melting point as 176°.

2 - Thio - 4,5 - dimethyl - 6 - oxypyrimidine.—Seventy-two grams of thiourea and 132 g. of ethyl α-methylacetoacetate were refluxed with stirring on a steam-bath with 21 g. of sodium dissolved in 500 cc. of absolute alcohol for three hours. The reaction mixture was evaporated on the steam-bath nearly to dryness, cooled and acidified with acetic acid. After standing in an ice-box overnight the crystalline precipitate was collected and recrystallized first from 95% alcohol and then from absolute. The substance does not melt up to 255°; yield 88 g.

Anal. Calcd. for $C_6H_8N_2OS$: C, 46.15; H, 5.13; N, 17.95. Found: C, 46.17; H, 5.20; N, 18.19.

4,5-Dimethyl-6-oxypyrimidine.—Ten grams of the above thiopyrimidine was oxidized with hydrogen peroxide and the product was isolated as in the case of 5-methyl-6-oxypyrimidine. Instead of subliming it was recrystallized from hot ethyl acetate in which it is sparingly soluble; m. p. 202–203°; yield 4.22 g.

Anal. Calcd. for $C_6H_8N_2O$: C, 58.06; H, 6.45; N, 22.58. Found: C, 58.23, 58.13; H, 6.42, 6.37.

Schlenker[8] prepared this substance, m. p. 204°, by reducing the 2,6-dichloro-4,5-dimethylpyrimidine.

4,5-Dimethyl-6-chloropyrimidine.—2.2 grams of the 6-oxy compound was converted to the 6-chloro derivative as in the case of the 5-methyl-6-oxy analog. The solid product was sublimed in high vacuum at 50°; m. p. 52°; yield 1.57 g.

Anal. Calcd. for $C_6H_7N_2Cl$: C, 50.53; H, 4.91; N, 19.65. Found: C, 50.93, 50.79; H, 4.93, 4.82; N, 19.55, 19.46.

(6) Wheeler and McFarland, *Am. Chem. J.*, **43**, 19 (1910).
(7) Gerngross, *Ber.*, **38**, 3403 (1905).
(8) Schlenker, *ibid.*, **34**, 2823 (1901).

Schlenker[8] has reported this compound melting at 51°.

4,5-Dimethyl-6-aminopyrimidine.—One gram of the above chloro derivative was converted to the corresponding amino by heating for fifteen hours at 110° with alcoholic ammonia. Isolation was effected as in the case of the previous aminopyrimidines; purification by recrystallization first from hot water and then from benzene; m. p. 229–231°; yield 0.35 g.

Anal. Calcd. for $C_6H_9N_3$: C, 58.54; H, 7.32; N, 34.15. Found: C, 58.32; H, 7.39; N, 34.37.

Schlenker[8] prepared this compound from 2-chloro-4,5-dimethyl-6-aminopyrimidine and recorded the m. p. as 230°.

2,5-Dimethyl-6-oxypyrimidine.—Forty-two grams of ethyl sodioformylpropionate[9] and 26 g. of acetamidine hydrochloride were dissolved in 200 cc. of water and allowed to stand for two days. The solution was evaporated on the steam-bath until crystallization started, made alkaline with ammonia and repeatedly extracted with chloroform. The extract was dried over sodium sulfate and evaporated to dryness *in vacuo*. The residue was sublimed in high vacuum at 125°, and the sublimate was recrystallized twice from acetone; m. p. 174°; yield, approximately 6 g.

Anal. Calcd. for $C_6H_8N_2O$: C, 58.06; H, 6.45; N, 22.58. Found: C, 58.22, 57.96; H, 6.36, 6.30; N, 23.23, 23.29.

2,5-Dimethyl-6-chloropyrimidine.—2.58 grams of 2,5-dimethyl-6-oxypyrimidine was converted to the chloro derivative in the usual way. The crude product was distilled at 40 mm. yielding a colorless oil which, due to its volatility, could not be weighed readily for satisfactory micro-analyses.

2,5-Dimethyl-6-aminopyrimidine.—One gram of the above 2,5-dimethyl-6-chloro pyrimidine was heated with excess alcoholic ammonia in a sealed tube at 125° for seven hours. Isolation of the product proceeded as in the case of other 6-aminopyrimidines. The product was purified by sublimation at 80°; m. p. 201–202°; yield 0.35 g.

Anal. Calcd. for $C_6H_9N_3$: C, 58.54; H, 7.31; N, 34.15. Found: C, 58.95, 59.02; H, 6.94, 7.20; N, 33.74, 33.63.

Picrate from water solution with aqueous picric acid, m. p. 222°.

Anal. Calcd. for $C_{12}H_{12}N_6O_7$: N, 23.87. Found: N, 24.10.

Approximate colorimetric determinations of the *p*H of water solutions of each of the aminopyrimidines were made using meta cresol purple and thymol blue as indicators. The solutions were approximately 0.01 *M* except in the case of 5-ethyl-6-amino which was insufficiently soluble and was, therefore, tested as a saturated solution.

TABLE I

ALKALINITY OF 6-AMINOPYRIMIDINE SOLUTIONS

Base	Solubility	*p*H
6-Amino	Moderate	8.3
2-Methyl-6-amino	Free	8.6–8.7
4-Methyl-6-amino	Moderate	8.3
5-Methyl-6-amino	Moderate	8.3
5-Ethyl-6-amino	Very sparing	7.9–8.0
2,4-Dimethyl-6-amino	Free	8.6–8.7
2,5-Dimethyl-6-amino	Sparing	8.5–8.6
4,5-Dimethyl-6-amino	Sparing	8.2–8.3

The ultraviolet absorption of all of the compounds was measured with a Hilger "Spekker" Photometer in conjunction with a Hilger E316 spectrograph. The source was a tungsten steel spark in some cases and a wide aperture hydrogen discharge tube in others. Either source was satisfactory. The Spekker photometer gave much more satisfactory results than the rotating sector used for earlier work.[9] Eastman d. c. Ortho and Cramer Contrast plates were both used with success. Solutions were in distilled water unless otherwise indicated.

We wish to express our gratitude to Mr. D. F. Hayman and Mr. S. Adler for the micro-analyses, to Dr. Joseph K. Cline for the preparation of 2-methyl-6-oxy- and 2-methyl-6-aminopyrimidines, to Mr. R. E. Waterman for the *p*H determinations and to Dr. H. T. Clarke for the use of facilities in preparing some of the spectrograms.

(9) Wintersteiner, Williams and Ruehle, THIS JOURNAL, **57**, 517 (1935).

Summary

1. Methods of preparation are given for all of the previously unknown mono- and di-C-methyl derivatives of 6-oxy and 6-aminopyrimidine.

2. The ultraviolet absorption is given of both complete series including the non-alkylated members.

3. Addition of acid modifies the absorption of the 6-amino pyrimidines by reducing the prominence of the longer wave length band; alkali tends to equalize the prominence of the two bands.

4. The absorption of aminosulfonic acid from the vitamin reveals the influence of the acidity of the sulfonic group and is affected by alkali in a similar manner as non-sulfonated 6-aminopyrimidines.

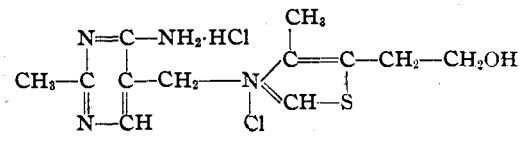
NEW YORK, N. Y.
RAHWAY, N. J. RECEIVED DECEMBER 30, 1936

[CONTRIBUTION FROM RESEARCH LABORATORY, MERCK AND COMPANY, AND PRIVATE LABORATORIES]

Studies of Crystalline Vitamin B_1. XVI. Identification of the Pyrimidine Portion

BY JOSEPH K. CLINE, ROBERT R. WILLIAMS, A. E. RUEHLE AND ROBERT E. WATERMAN

In a recent communication[1] the structure



was assigned to vitamin B_1 (aneurin) upon the basis of evidence which was indicated briefly at that time. Since then this structure has been confirmed by our synthesis[2] of the vitamin, by Grewe's synthesis[3] of two cleavage products, $C_6H_{10}N_4$, obtained from the vitamin by Windaus and collaborators,[4] and the amino sulfonic acid described previously[5] by our own group of associates, and finally by Bergel and Todd's synthesis of thiochrome.[6] Except for some questions which have not yet been fully resolved about possible stereoisomerism of the vitamin, the structure of the substance is established. The purpose of this paper is therefore merely to relate in greater detail the experimental evidence upon which our conclusions were based.

(1) THIS JOURNAL, **58**, 1063 (1936).
(2) *Ibid.*, **58**, 1504 (1936).
(3) R. Grewe, *Z. physiol. Chem.*, **242**, 89 (1936).
(4) A. Windaus, T. Tschesche and R. Grewe, *ibid.*, **237**, 98 (1935).
(5) THIS JOURNAL, **57**, 1093 (1935).
(6) Bergel and Todd, *Nature*, **138**, 406 (1936).

The earliest clear evidence of a bridge between the two nuclei in the vitamin came to us through the discovery that the vitamin is split at room temperature in liquid ammonia solution yielding a base, $C_6H_{10}N_4$, whose picrate melts at 225° and is evidently identical with the picrate of Windaus.[4] The base exhibits absorption in the ultraviolet characteristic of a C-alkylated 6-aminopyrimidine rather than a diaminopyrimidine. In Fig. 1 the absorption curve of this base is compared with those of 2,5-dimethyl-6-aminopyrimidine,[7] 4-methyl-5,6-diaminopyrimidine,[8] 4,5-dimethyl-2,6-diaminopyrimidine[9] and 5-ethyl-4,6-diaminopyrimidine.[10] These curves for diaminopyrimidines representing the several possible positions on the ring of the second amino group all differ radically from that for the base obtained from the vitamin. The latter substance, however, resembles in absorption the mono- and dialkylated 6-aminopyrimidines as displayed in the preceding paper[7] as well as the sodium salt of the amino sulfonic acid and the free oxysulfonic acid previously de-

(7) Williams, Ruehle and Finkelstein, THIS JOURNAL, **59**, 526 (1937).
(8) Gabriel and Colman, *Ber.*, **34**, 1245 (1901).
(9) Schlenker, *ibid.*, **34**, 2826 (1901).
(10) A. v. Merkatz, *ibid.*, **52**, 874 (1919).