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.

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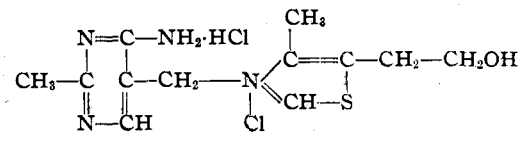
[CONTRIBUTION FROM RESEARCH LABORATORY, MERCK AND COMPANY, AND PRIVATE LABORATORIES]

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).

rived from the vitamin. The resemblance to 2,5-dimethyl-6-aminopyrimidine is particularly close. Bearing in mind the influence of alkali upon the absorption of 6-aminopyrimidines and upon that of the aminosulfonic acid, the added basicity of the second amino group in the base $C_6H_{10}N_4$ would be expected to operate in the same direction. Since the absorption of the diamino base closely resembles those of the monoaminopyrimidines when the latter are observed in alkaline solution, we concluded that the second amino group is in a side chain. This side chain is common also to the aminosulfonic acid and therefore to the vitamin itself. This conclusion was fortified by the observation that groups other than amino in side chains influence the absorption only to a minor degree. Thus as we shall show in a later paper, ethoxymethyl, bromomethyl, carbethoxymethyl, or sulfomethyl groups on the pyrimidine ring behave in general somewhat as unsubstituted methyl groups in their influence on ultraviolet absorption of 6-amino and 6-oxy derivatives.

While the attachment of the second amino group to a side chain was clear, one could not be certain of the position of the side chain from absorption evidence alone. Now that the position of this side chain has been fixed by other independent evidence, the better correlation in absorption of the base $C_6H_{10}N_4$ with 2,5-dimethyl-6-amino-pyrimidine than with the other dimethylated 6-amino compounds is seen to be significant.

Satisfactory comparison of absorption curves cannot be made in the form of the picrates on account of the strong absorption of picric acid. Hydrochlorides are also unsatisfactory, as these on account of their acidity display only single banded and therefore less distinctive absorption.

In the light of this evidence, the aminosulfonic acid was reduced with metallic sodium in liquid ammonia solution. The larger part of the pyrimidine appeared as unidentifiable amorphous, insoluble and unsublimable products, possibly resulting from coupling of two rings.[11] We were, however, able to isolate a small yield of a base, m. p. 202°, which formed a picrate, m. p. 221–222°, identical by mixed melting point with that of 2,5-dimethyl-6-aminopyrimidine.[7] This provided the first unequivocal evidence of the positions of alkylation as well as final confirmation of the presence of the 6-aminopyrimidine nucleus in the vitamin.

Accepting the above evidence for 2,5-methylation, it was still necessary to determine whether the thiazole of the vitamin was located on the 2- or the 5-side chain. Consideration of the evidence as to the structure of thiochrome[4,12] led us to prefer the 5-position, our process of reasoning following the general lines upon which Makino and Imai[13] based their suggestion of the possibility of a methylene bridge. We had prepared synthetically, as will appear in a later paper, several 6-oxypyrimidines[1] with an ethoxymethyl group in various other positions. By treating 2-methyl-6-oxy-5-ethoxymethylpyrimidine with sodium sulfite, we were able to reproduce the oxy-sulfonic acid[5] previously derived from the vitamin and thus fixed the final detail of the structure of the latter.

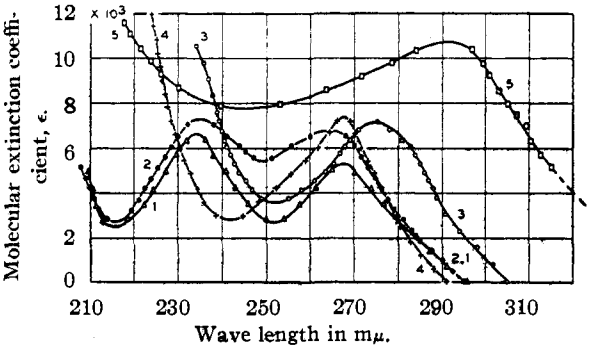


Fig. 1.—
1. Liquid ammonia cleavage product of vitamin.
2. 2,5-Dimethyl-6-aminopyrimidine.
3. 4,5-Dimethyl-2,6-diaminopyrimidine.
4. 5-Ethyl-4,6-diaminopyrimidine.
5. 4-Methyl-5,6-diaminopyrimidine.

Experimental Part

Liquid Ammonia Cleavage of Vitamin.—212 mg. of vitamin hydrochloride was dried for two hours at 80° *in vacuo* in a fragile glass bulb which was sealed off at the end of the drying period and transferred to a heavy-walled bomb tube. The latter was then connected to a system consisting of liquid ammonia cylinder, soda lime tube and a bomb tube with side-arm containing metallic sodium. The glass system was then evacuated and dried, liquid ammonia was admitted and condensed upon the sodium by packing the appropriate tube in solid carbon dioxide. Thence the ammonia was distilled off into the bomb tube containing the vitamin within the small tube. This bomb tube was sealed off and the bulb within was broken by shaking. The vitamin dissolved with a light brown color

(11) Kircher, *Ann.*, **385**, 295 (1911).

(12) Barger, Bergel and Todd, *Ber.*, **68**, 2257 (1935); Kinnersley O'Brien and Peters, *Biochem. J.*, **29**, 2369 (1935).

(13) K. Makino and T. Imai, *Z. physiol. Chem.*, **239**,1 (1936).

which, as the ammonia approached room temperature, turned to a deep green which grew in intensity for a half hour then slowly faded over a period of forty-two hours at room temperature. The tube was then cooled and opened and the ammonia distilled off under anhydrous conditions and finally *in vacuo*. A light colored gummy mass containing much crystalline matter remained. This was extracted successively with 8-, 5- and 3-cc. portions of chloroform. On evaporation of the chloroform, 129.8 mg. of oil remained which on analysis contained 3% sulfur.

This oil after standing for a week was no longer fully soluble in chloroform. On extracting successively with 3-, 2- and 2-cc. portions of chloroform, the combined extracts left a partly crystalline residue of 88.3 mg. This was treated with 4 drops of water and the insoluble residue washed successively with 2, 2 and 1 drops of water. The aqueous solution was evaporated to dryness leaving a yellow crystalline mass which was washed with 0.5 cc. of an equal volume mixture of chloroform–alcohol and finally recrystallized three times from hot absolute alcohol; yield 13.9 mg. of dense colorless columnar crystals growing in rosets; m. p. 211–215°. The ultraviolet absorption in water solution is shown in Fig. 1.

Anal. Calcd. for $C_6H_{10}N_4$: C, 52.17; H, 7.25; N, 40.58. Found: C, 51.35; H, 6.35; N, 38.53.[14]

Dumas nitrogen determinations in these pyrimidines are troublesome.[15] The method here used was one devised by Hayman[16] and previously tested on a series of synthetic pyrimidines of similar structure.

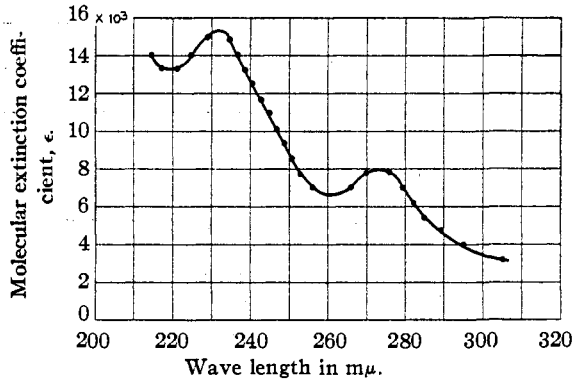


Fig. 2.—4-Methyl-5-formylamino-6-aminopyrimidine.

A portion of the base was converted to the hydrochloride and treated in aqueous solution with picric acid to incipient precipitation. On standing overnight rosets of yellow needles appeared; m. p. 225°.

Anal. Calcd. for $C_6H_{10}N_4[C_6H_2OH(NO_2)_3]_2$: C, 36.24; H, 2.68; N, 23.49. Found: C, 36.94; H, 2.56; N, 23.80.

The fate of the thiazole portion of the molecule was not determined. Considerable evolution of hydrogen sulfide was noted on distilling one of the by-products *in vacuo* and it seems probable that an extensive degradation occurs. The thiazole by itself does not give the green color in liquid ammonia.

Preparation of Diaminopyrimidines.—The preparation of 4-methyl-5,6-diaminopyrimidine,[8] 4,5-dimethyl-2,6-diaminopyrimidine[9] and 5-ethyl-4,6-diaminopyrimidine[10] followed the literature. It should be added that in preparing the last named from 4-iodo-5-ethyl-6-aminopyrimidine it was necessary to heat with saturated alcoholic ammonia at 220° for fifteen hours. The product melted at 235–237° compared with the literature figure of 233–235° but on further purification by sublimation or recrystallization from ethyl acetate, melted at 245°. It formed a dipicrate, m. p. 165–167°.

Anal. Calcd. for $C_6H_{10}N_4[C_6H_2OH(NO_2)_3]_2$: N, 23.49. Found: N, 23.68.

4-Ethyl-5,6-diaminopyrimidine[17] was also prepared by Dr. E. R. Buchman in a manner analogous to that of the cited literature[8] for 4-methyl-5,6-diamino. Dr. Buchman kindly supplied a sample for ultraviolet examination. Its absorption is almost identical with that of the 4-methyl analog. The formyl derivatives of the 5,6-diamino compounds show a striking modification of absorption (see Fig. 2).

Reduction of Amino Sulfonic Acid with Sodium.—27 mg. of the aminosulfonic acid was suspended in 5 cc. of anhydrous liquid ammonia (distilled from sodium) and 10 mg. of metallic sodium was added in portions with shaking. After standing a half hour, the ammonia was evaporated by passing in a stream of dry air. A few drops of alcohol were added to destroy any excess of sodium and the residue sublimed *in vacuo* at about 120° at 0.1 mm., and then at atmospheric pressure at 180–200°. The final sublimate melted sharply at 202°.

Anal. Calcd. for $C_6H_9N_3$: C, 58.54; H, 7.32; N, 34.15. Found: C, 58.34; H, 7.40; N, 33.67.

An aqueous solution of the sublimate was treated with aqueous picric acid solution to faint opalescence. Upon standing overnight needle crystals of a picrate separated which melted at 221–222°. When mixed with the picrate of 2,5-dimethyl-6-aminopyrimidine, the melting point was not depressed.

Anal. Calcd. for $C_6H_9N_3C_6H_2OH(NO_2)_3$: N, 23.86. Found: N, 23.41.

Synthesis of Oxysulfonic Acid.—300 mg. of 2-methyl-5-ethoxymethyl-6-oxypyrimidine prepared according to a method to be described in a later paper was dissolved in 5 cc. of water containing 1 g. of sodium sulfite. The mixture was saturated with sulfur dioxide and heated for eighteen hours at 144° in a sealed tube. To the pale yellow solution concentrated hydrochloric acid was added and the solution was saturated with dry hydrogen chloride to precipitate sodium chloride. The filtrate from the sodium chloride was concentrated to a small volume, again saturated with hydrochloric acid to remove further sodium chloride. After removing sodium chloride as completely as possible, the oily residue was dissolved in 3 cc. of water and 8 cc. of alcohol was added. On standing white crystals separated. These were purified by dissolving in 3 cc. of concentrated hydrochloric acid, centrifuging off a trace

(14) This analysis was made after heating *in vacuo* to 100° for one hour during which a small loss of weight occurred. Previous to this heating values for C and H of 50.66 and 5.98%, respectively, were obtained. The results suggested that the base absorbs carbon dioxide from the air.

(15) Milner and Sherman, *Ind. Eng. Chem., Anal. Ed.*, **8**, 331 (1936).

(16) D. F. Hayman and S. Adler, *ibid.*, in press.

(17) Robinson and Tomlinson, *J. Chem. Soc.*, II, 1283 (1935).

of insoluble matter, evaporating to dryness and recrystallizing from water and alcohol as before. The crystals did not melt up to 360°.

Anal. Calcd. for $C_6H_8N_2SO_4$: C, 35.27; H, 3.95; N, 13.73; S, 15.69. Found: C, 35.23, 35.53; H, 3.78, 3.89; N, 14.06, 13.78; S, 15.30, 15.33.

Through the kindness of Mr. H. W. Hermance the crystals were compared crystallographically with the natural oxysulfonic acid obtained from the vitamin. He reported: "The bulk of the crystals of the synthetic sulfonic acid occurred in the form of slightly tapering rods, 50–100 microns in length, with a sharp terminal angle (not measured because of the smallness of the crystal). Some twinning was observed but individually developed crystals were more general. The extinction angle, measured from the long edge, was fairly constant through about twenty observations. It was found to be 59 ± 0.5°. The crystals appeared to belong either to the monoclinic or triclinic system. As oriented on the slide, two indices of refraction were evident. The higher, observed at the extinction position, was slightly greater than 1.632, the lower, slightly less than 1.623.

"The natural oxysulfonic acid corresponded in the above crystallographic constants with the synthetic within the limits of experimental error. It showed a somewhat greater tendency to twinning which, however, cannot be regarded as evidence of a difference in molecular structure."

The synthetic sulfonic acid was also compared with the natural with respect to ultraviolet absorption. Close agreement was observed (Fig. 3). Since no melting point was available as a criterion of identity, and since experience with alkylated oxypyrimidines[7] had indicated that ultraviolet absorption is not a definitive index of the position of alkyl substituents, it still appeared possible that the synthetic oxysulfonic acid was merely isomeric and not identical with the natural. This was especially true since all such sulfonic acids might be expected also to resemble one another in other physical properties, such as solubilities. Under these circumstances resort was had to a special form of solubility comparison. Portions of absolute ethyl alcohol were saturated with (a) the natural sulfonic acid, (b) the synthetic and (c) both synthetic and natural. The intensities of ultraviolet absorption of these three solutions were then compared and found to be identical throughout the range of frequency. If the substances were not identical, solution (c) should have absorbed with an intensity approximating that of (a) + (b). In making such comparisons, it was found essential to clarify the solutions thoroughly. This was done by centrifuging in a horizontal position in the optical cells in which solution had been effected so that any sediment deposited on the cylindrical walls. It was of course necessary to check carefully the equality of intensity of illumination produced by the two parallel beams of light.

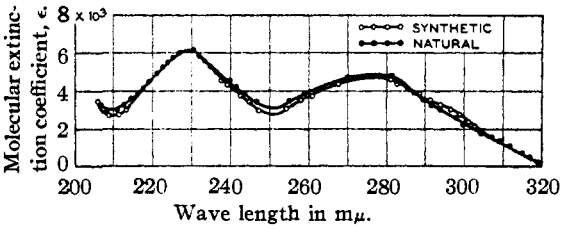


Fig. 3.—2-Methyl-6-oxy-5-methylsulfonic acid.

We gratefully acknowledge our indebtedness to Mr. D. F. Hayman and Mr. S. Adler for the microanalyses, to Mr. J. Finkelstein for the synthesis of 2,5-dimethyl-6-amino- and several diaminopyrimidines, as well as to Dr. E. R. Buchman and Mr. H. W. Hermance for the courtesies noted in the text. Thanks are also due to Dr. R. T. Major for advice and for facilities put at our disposal.

Summary

1. The vitamin undergoes cleavage with liquid ammonia producing a base, $C_6H_{10}N_4$, which exhibits ultraviolet absorption akin to that of C-methylated-6-aminopyrimidines but unlike that of 6-aminopyrimidines having another amino group elsewhere on the ring.

2. Reduction by means of sodium in liquid ammonia of the aminosulfonic acid obtained by sulfite cleavage of the vitamin yields 2,5-dimethyl-6-aminopyrimidine.

3. Reaction of sulfite with 2-methyl-5-ethoxymethyl-6-oxypyrimidine yields 2-methyl-6-oxypyrimidine-5-methylsulfonic acid which is identical with the oxy-sulfonic acid previously derived from the vitamin.

4. The structure of the vitamin is thereby established.

NEW YORK, N. Y.
RAHWAY, N. Y.

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