Dihydrocerin (Cerinol).—One and one-half grams of cerin in 100 ml. of *n*-amyl alcohol was reduced by adding 3 g. of sodium to the hot solution over a period of fifteen minutes. After the sodium had dissolved completely, the alcohol was removed by steam distillation and the product recrystallized from dioxane. The dicarbinol, for which we propose the name *cerinol*, crystallized from dioxane in narrow white laths, which melted at 293–295°; $[\alpha]_{5461}^{25}$ +9.4 (C = 0.48).

Anal. Calcd. for $C_{30}H_{52}O_2$: C, 81.01; H, 11.75. Found: C, 81.05, 80.86; H, 11.63, 11.77.

Cerinol Diacetate.—Cerinol is smoothly converted by acetic anhydride alone into a diacetate. This product crystallizes from ethyl acetate–ethyl alcohol in narrow almost needle-like laths which melt at 267–269°.

Anal. Calcd. for $C_{34}H_{56}O_4$: C, 77.22; H, 10.67. Found: C, 77.04, 77.05; H, 10.35, 10.40.

The Purification of Cerin.—Several recrystallizations of crude material from chloroform are necessary to obtain pure cerin. A properly purified sample should melt at 250–256°; at least six recrystallizations are necessary starting with crude extract. A sample so prepared yielded the following analytical results. Calcd. for $C_{30}H_{50}O_2$: C, 81.38; H, 11.39. Found: C, 81.37; H, 11.35. Less extended recrystallizations result in products with a broader melting range and a considerably lower carbon content. Cerin is not at all affected by refluxing with alcoholic potassium hydroxide, but is very sensitive to mineral acids.

Cerin Acetate.—The treatment of cerin with boiling acetic anhydride alone or with boiling acetic anhydride containing a trace of sulfuric acid results in the formation of a mixture of products from which it is not possible to obtain a pure substance. The reaction products, even after many recrystallizations, possess carbon and hydrogen contents which do not correspond to any of those demanded by cerin monoacetate, cerin diacetate or the acetate of a dehydration product, $C_{30}H_{48}(OCOCH_3)$. Under much milder conditions, however, by the use of pyridine and acetic anhydride at room temperature, it is possible to obtain a monoacetate of cerin; 1 g. of cerin was dissolved in 80 ml. of dry pyridine and 10 ml. of acetic anhydride was added over a period of about five minutes. The mixture was allowed to stand at room temperature overnight (*ca.* 25°). The product was isolated by dilution of the pyridine with water, and recrystallized in succession from benzene–ethyl acetate, ethyl acetate, benzene, ethyl acetate, glacial acetic acid and ethyl acetate. The substance melted at 256–259° with decomposition.

Anal. Calcd. for $C_{30}H_{52}O_3$: C, 79.28; H, 10.82. Found: C, 79.05, 79.19; H, 10.82, 10.88.

This product was further crystallized from glacial acetic acid, and finally from ethyl acetate, from which it separated in narrow almost needle-like laths which melted at 259–261°.

Summary

1. Friedelin oxime, 2,4-dinitrophenylhydrazone and *p*-nitrophenylhydrazone have been prepared.

2. Friedelin oxime undergoes the Beckmann rearrangement and yields a product which so far has defied all attempts to cleave it by hydrolysis.

3. Cerin methyl ether, its oxime and 2,4-dinitrophenylhydrazone have been prepared.

4. Cerin has been reduced to a dihydroxy compound which was characterized by the formation of a diacetate.

5. Cerin has been characterized by the formation of an oxime and a 2,4-dinitrophenylhydrazone.

6. The monoacetate of cerin has been prepared by acetylation in pyridine at room temperature.

COLLEGE PARK, MD. RECEIVED JULY 24, 1935

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, TEACHERS COLLEGE, COLUMBIA UNIVERSITY]

Studies of Crystalline Vitamin B_1. XI. Presence of Quaternary Nitrogen[1]

BY ROBERT R. WILLIAMS AND A. E. RUEHLE

The necessity for reconciling the chemical characteristics of the basic cleavage product[2] of vitamin B_1 with the undoubted binuclear character of the vitamin led our associate, Dr. E. R. Buchman, to suggest the presence of quaternary nitrogen in the latter. If present, the tetrasubstituted nitrogen should be recognizable by its strong basicity. On titrating the vitamin hydrochloride with sodium hydroxide the presence of a moderately strong basic nitrogen was revealed, too strong for a tertiary base though not strong enough for a true quaternary base. The basic cleavage product behaved as a typical tertiary base but its methiodide[2b] closely resembled the vitamin not only in basic strength but also in exhibiting an unusual pseudo basic behavior. Both the vitamin and the methiodide of its basic cleavage product required an additional molecular equivalent of alkali for com-

(1) Presented before the Division of Organic Chemistry at the New York Meeting of the American Chemical Society, April 22, 1935.

(2) (a) R. R. Williams, R. E. Waterman, J. C. Keresztesy and E. R. Buchman, THIS JOURNAL, **57**, 536 (1935); (b) Paper X of this series, E. R. Buchman, R. R. Williams and J. C. Keresztesy, *ibid.*, **57**, 1849 (1935).

plete liberation of the base. This parallelism of behavior constituted strong evidence of a close structural resemblance between these compounds, associated with a quaternary nitrogen in each. In view of Dr. H. T. Clarke's suggestion[3] of the presence of a thiazole nucleus in the vitamin, it was of interest to secure similar data regarding synthetic thiazolium compounds. 4-Methylthiazole ethiodide behaved similarly to the methiodide of the basic cleavage product while 2,4-dimethylthiazole ethiodide exhibited the characteristics of a salt of a true quaternary base. These observations furnish strong confirmation of this feature of the structural formula of the vitamin recently announced by Williams.[3]

Birch and Harris[4] and Moggridge and Ogston[5] have recently described titrations of vitamin B_1 similar to our own. These authors, however, are neither in complete agreement with each other nor with us as to the interpretation to be placed on such curves. For that reason we feel the necessity of discussing our results somewhat in detail.

Experimental

Apparatus

The indicator electrode used was the MacInnes and Dole membrane type of glass electrode,[6] made from Corning 015 glass and supported by lime glass tubing. The reference electrode was a normal calomel half-cell. The potential measurements were made with a Leeds and Northrup type K potentiometer and 2500-e galvanometer used in conjunction with a vacuum tube electrometer.[7] The titrations were carried out in a small cell through which a slow stream of nitrogen was passed to exclude carbon dioxide and to stir the solution. All measurements were made in an air thermostat kept at 25.0°.

No attempt was made to maintain a constant salt concentration because of the difficulty which would thereby be introduced in recovering the vitamin from the solution for other uses. Neither was any attempt made to evaluate liquid junction potentials except in so far as correcting for the "asymmetry potential" of the glass electrode, using calibrated buffers would eliminate this error. It is also recognized that the change in volume during titration introduces a small error in the values of the dissociation constants obtained.

Materials

NaOH and HCl.—0.1 *N* solutions were used in all titrations.

Vitamin B_1 was obtained by the method of Williams, Waterman and Keresztesy;[8] 50 mg. of the crystalline vitamin dissolved in 10 cc. of water served as the sample for titration.

The basic cleavage product (hydrochloride) was prepared by cleaving the vitamin with sodium sulfite.[2a]

Methiodide of Basic Cleavage Product.—Prepared by addition of methyl iodide to the cleavage product.[2b]

4-Methylthiazole Ethiodide.—Prepared as described elsewhere.[9]

2,4-Dimethylthiazole Ethiodide.—Prepared as described elsewhere.[9]

Experimental Results

The results obtained are shown graphically in Figs. 1–4. The pK_B values given in Table I represent the half neutralization points on the curves

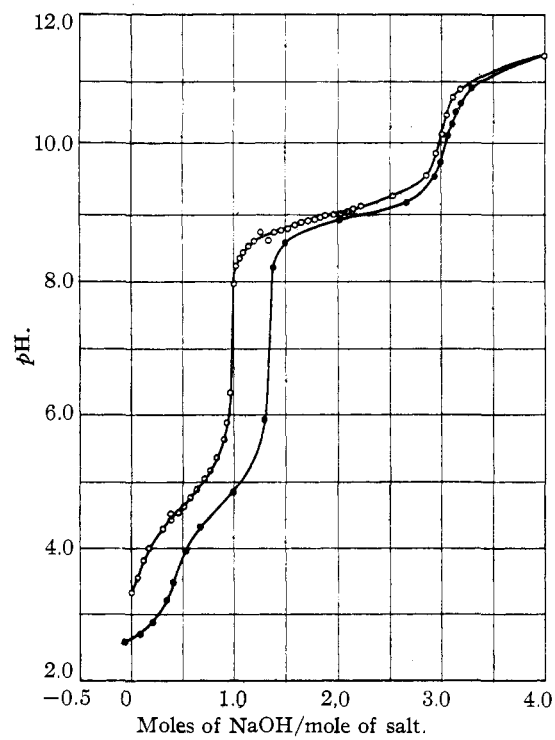

Fig. 1.—Titration of vitamin B_1: O—O, titration with NaOH, ●—●, back titration with HCl.

and are calculated from the relation $p\mathrm{H} + p\mathrm{OH} = 14$ for aqueous solution at 25° and the approximation[10] $p\mathrm{OH} = pK_B$ at half the neutralization point of a weak base.

The titrations demonstrate seven important points, six of which are illustrated directly by the figures.

(3) R. R. Williams, THIS JOURNAL, **57**, 229 (1935).

(4) T. W. Birch and L. J. Harris, *Nature*, **135**, 654 (1935).

(5) R. C. G. Moggridge and A. G. Ogston, *Biochem. J.*, **29**, 866 (1935).

(6) D. A. MacInnes and M. Dole, *Ind. Eng. Chem., Anal. Ed.*, **1**, 57 (1929).

(7) K. G. Compton and H. E. Haring, *Trans. Am. Electrochem. Soc.*, **62**, 345 (1932).

(8) R. R. Williams, R. E. Waterman and J. C. Keresztesy, THIS JOURNAL, **56**, 1187 (1934).

(9) Paper XII of this series, H. T. Clarke and S. Gurin, THIS JOURNAL, **57**, 1876 (1935).

(10) This approximation holds strictly only for weak bases. Hence the absolute values given in Table I for stronger bases are somewhat in error.

TABLE I

Base	pK_B
Vitamin B_1 (stronger base)	5.0
Vitamin B_1 (weaker base)	9.5
Basic cleavage product (quaternary)	4.1
Basic cleavage product (tertiary)	10.6
4 Methylthiazole (quaternary)	4.5
4 Methylthiazole (tertiary) (curve not shown)	10.4
2,4-Dimethylthiazole (quaternary)	2.8

1. Two bases are liberated, in titrating the vitamin with sodium hydroxide, one of which is considerably stronger than the other. The stronger base is somewhat weaker than would be expected for a quaternary base, but the strength of the base liberated from the methiodide of the basic cleavage product is of the same order of strength as the stronger base in the vitamin.

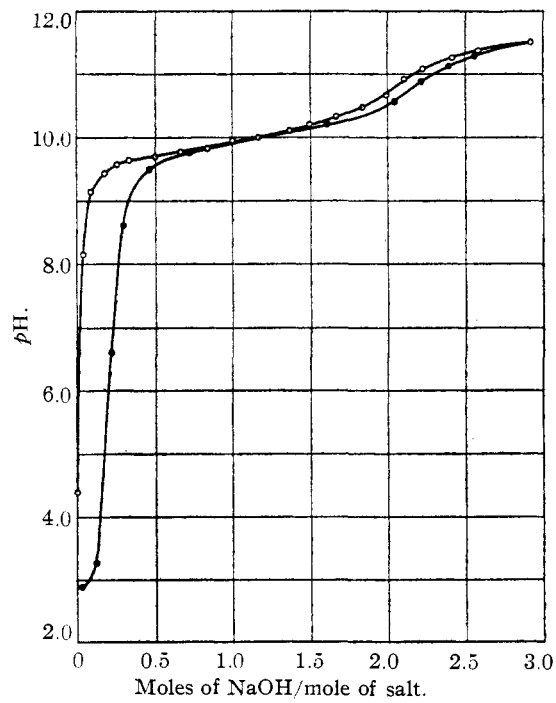

Fig. 2.—Titration of methiodide of basic cleavage product: ○—○, titration with NaOH; ●—●, back titration with HCl.

2. Both the vitamin and the methiodide of its basic cleavage product require an additional equivalent of alkali to liberate completely the strong base present in each.

3. During the portion of the titration referred to in (2) there is a marked slowness in coming to equilibrium. With each addition of reagent a large change in potential occurs, but after ten to fifteen minutes there is only a small net change. The behavior suggests the liberation of a very strong base with a slow rearrangement to a much weaker one. The additional equivalent of alkali required in this portion of the titration suggests that this "weaker base" gives rise to an acid which is neutralized as it is formed.

4. The back titrations of both the stronger base of the vitamin (pK_B = 5.0) and the methiodide of the basic cleavage product show a loss of material amounting to 10–20% of the original amount present. The slowness in attainment of equilibrium is equally evident in the back titration. The weaker base in the vitamin (pK_B = 9.5) is reneutralized by exactly one equivalent of acid, showing no alteration of the portion of the molecule associated with it.

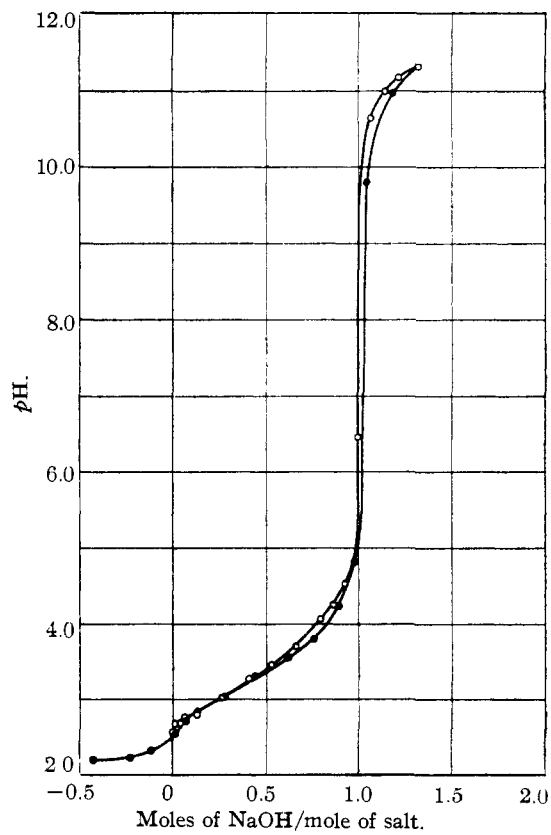

Fig. 3.—Titration of hydrochloride of basic cleavage product: ○—○, titration with NaOH; ●—●, back titration with HCl.

5. The hydrochloride of the basic cleavage product is demonstrated to be the salt of a very weak base which requires only one equivalent of reagent to liberate it. Equilibrium is attained almost instantaneously after each addition of sodium hydroxide. There is no loss of material on back titration.

6. The curve of 4-methylthiazole ethiodide is entirely analogous in form to that of the methiodide of the basic cleavage product. The same phenomenon of slowness in coming to equilibrium is manifest in both the forward and back titrations. Two equivalents of alkali are required to liberate the base and less than two of acid to reneutralize it.

7. The curve of 2,4-dimethylthiazole ethiodide, on the other hand, is the curve of a typical quaternary base, as is evident by comparison with the water curve. Evidently the methyl group in position 2 modifies the behavior of thiazolium salts in a marked way. A mauve color developed in this case on addition of the first drops of alkali (thiazolo-cyanine dye formation).[11] The back titration of this substance showed no significant points of inflection, apparently due to the relative stability of the colored substance, but afforded additional assurance of an entirely different behavior when position 2 is occupied by a methyl group.

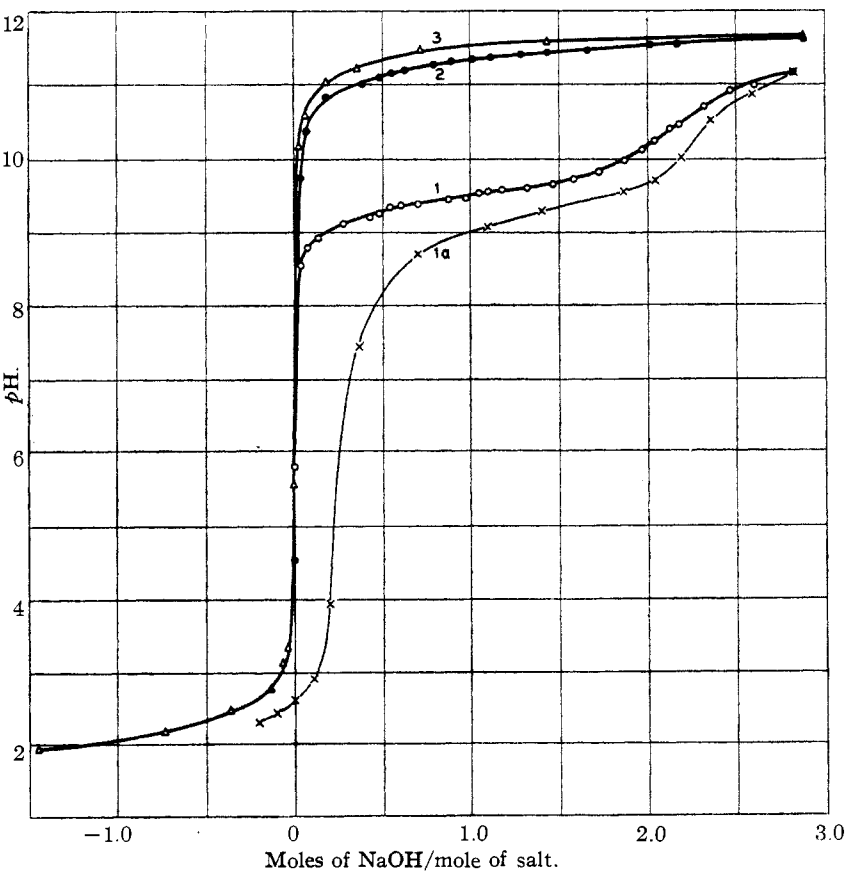

Fig. 4.—(1), Titration of 4-methylthiazole ethiodide with NaOH; (1a), back titration with HCl; (2), titration of 2,4-dimethylthiazole ethiodide with NaOH; (3), water curve.

Discussion of Results

There can be no doubt that the vitamin reacts exactly like the quaternary salt of its basic cleavage product toward sodium hydroxide, whereas its behavior is markedly different from that of the salt of the tertiary base. Furthermore, this behavior is not associated with the amino group contained in the vitamin, contrary to the suggestion of Moggridge and Ogston, since this group is not present in this cleavage product.[2b] Moggridge and Ogston have partially avoided the effect of the slow drift mentioned above by performing a rapid titration, which gives an indication of the actual strength of the stronger base. The curve is similar to that of the salt of a very strong base, although they conclude that there is no quaternary base present. It is unfortunate that their titration curve with equilibrium values is not shown above *p*H 7, as this should also reveal the presence of the quaternary base.

A study of the mechanism associated with the formation and neutralization of the pseudo base is in progress and will appear shortly in a paper by Dr. H. T. Clarke and associates. For the present it is sufficient to point out that the anomalous behavior of the vitamin closely resembles that of the quaternary salt of the basic cleavage product but not that of the salt of the basic cleavage product itself.

We are indebted to Dr. H. T. Clarke for advice and for furnishing us with samples of the synthetic thiazolium salts used in this study. We also wish to acknowledge financial aid by the Carnegie Corporation of New York through the Carnegie Institution of Washington.

(11) L. G. S. Brooker, F. M. Hamer and C. E. K. Mees, *J. Opt. Soc. Am.*, **23**, 216 (1933).

Summary

1. Vitamin B_1 contains two basic groups, one of which is of the same order of strength as the nitrogen in the quaternary salt of its basic cleavage product and in 4-methylthiazole ethiodide.

2. The vitamin and the methiodide of its basic cleavage product, like 4-methylthiazole ethiodide, form pseudo bases in alkaline solutions, but this phenomenon is not exhibited by a 2-methyl substituted thiazolium base or by simple thiazoles.

NEW YORK CITY RECEIVED JULY 6, 1935

[CONTRIBUTION FROM THE RESEARCH LABORATORY OF ORGANIC CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, No. 130]

The Dehydrogenation of Nicotine in Toluene as a Solvent

BY AVERY A. MORTON AND DAVID HORVITZ[1]

The experiments reported in this paper show that nicotine can be dehydrogenated by sulfur in boiling toluene as a solvent. This temperature is the lowest at which a dehydrogenation by sulfur or selenium has been carried out and is one of the few instances of the application of such a reaction to an alkaloid.[2] The object of this work was to perfect the exceedingly important method of dehydrogenation by lowering the temperature, controlling the concentrations, and changing the solvent so that the correctness of conclusions relative to the carbon framework of organic compounds might be subject to less doubt.[3] It was also desired to extend the application of dehydrogenation to nitrogen-containing compounds. Nicotine was chosen as suitable for study because the product of the proposed reaction is already known, having been prepared from nicotine by oxidation with potassium ferricyanide[4] or silver oxide[5] or by dehydrogenation over platinized asbestos at 320°.[6] Fusion of nicotine with sulfur has also been carried out by Cahours and Étard.[4]

Under the mild conditions attending the use of toluene as a solvent the amount of hydrogen sulfide evolved corresponds to 69% of the theoretical as given in equation 1 for the formation of nicotyrine, $C_{10}H_{10}N_2$. Actually nicotyrine was

$$C_{10}H_{14}N_2 + 2S = C_{10}H_{10}N_2 + 2H_2S \qquad (1)$$

obtained in only about 2.5% yield, the larger amount of material being thiodinicotyrine[7] $C_{20}H_{18}N_4S$ (equation 2), from which nicotyrine can be obtained by distillation over copper.[4] The yield of this substance when purified was about 18%.

$$2C_{10}H_{14}N_2 + 6S = C_{20}H_{18}N_4S + 5H_2S \qquad (2)$$

No appreciable quantities of tar were formed. The remainder of the material appeared to be unchanged nicotine and possibly products not completely dehydrogenated. Cahours and Étard[4] obtained this sulfur compound by the action of sulfur on nicotine in the absence of a solvent but failed to observe any nicotyrine.

Under a wide variety of conditions such as were possible by changing the concentrations and quantities of reagents the amount of hydrogen sulfide liberated could not be increased. Addition of acid at the end of the experiment failed to reveal any combined hydrogen sulfide. Iron, which has been claimed by Cheung[8] to break up sulfur addition compounds at higher temperatures, had no effect on the velocity or extent of hydrogen sulfide evolution. The addition of a small quantity of diphenylguanidine in the hope that it would act catalytically was found to inhibit greatly the rate and the completion of the reaction. Other solvents, boiling within the range of 99–121°, which were tried were epichlorohydrin, tetrachloroethylene, glacial acetic acid, and a gasoline fraction (99–102° b. p.). These solvents were far inferior to toluene both in the quantity of hydrogen sulfide formed and in the freedom from tarry products. Even the addition of acetic acid to toluene retarded greatly the rate of gas evolution although the total amount finally ap-

(1) From the thesis of David Horvitz submitted in partial fulfilment of the requirements for the degree of Bachelor of Science, 1934.

(2) See Blount [*J. Chem. Soc.*, 124 (1935)] for leading references to the application of dehydrogenation of alkaloids by selenium.

(3) See Vocke, *Ann.*, **497**, 248 (1932); Cook and Hewett, *J. Chem. Soc.*, 1103 (1933); 365 (1934); Clemo and Ormston, *ibid.*, 352 (1933), for criticisms of the present method of fusion.

(4) Cahours and Étard, *Bull. soc. chim.*, **34**, 449 (1880).

(5) Blau, *Ber.*, **27**, 2535 (1894).

(6) Wibaut and Overhoff, *Rec. trav. chim.*, **47**, 935 (1928).

(7) This compound was named thiotetrapyridine by Cahours and Étard on the basis of the erroneous belief that nicotyrine contained two pyridine rings. A more correct name would be thiodinicotyrine.

(8) Cheung, *Bull. inst. pin.* 108 (1929); *C. A.*, **23**, 3467 (1929).