was obtained. When purified by recrystallization from dilute alcohol, **1,3-dipropionylphenobarbital** melts at 108–109°. This derivative behaves similarly to the monopropionyl compound toward boiling water or alkaline solution.

Anal. Calcd. for $C_{18}H_{20}N_2O_5$: N, 8.14. Found: N, 8.23.

1-Bromodiethylacetylphenobarbital.—Twenty-one grams of silver phenobarbital and 75 cc. of anhydrous toluene were mixed, 16 g. of bromodiethylacetyl bromide[11] was added and the well-stirred mixture was maintained at boiling temperature for eight hours. The material was allowed to stand at room temperature for three days before the precipitate was filtered and washed twice with 25-cc. portions of benzene. The solid was washed four times by decantation with ether, then was filtered and washed with ether. The residual silver bromide weighed 11.4 g. (11.64 g. theoretically obtainable). The ether washings yielded phenobarbital of which, after recrystallization from benzene, 5.25 g. was recovered.

The filtrate from the reaction mixture was evaporated, under diminished pressure, to a gum which dissolved in 60 cc. of dry ether and was diluted with 150 cc. of petroleum ether. Spontaneous evaporation of the solvents during three days caused crystallization. The solid was filtered, and boiled with four separate 100-cc. portions of petroleum ether, permitting recovery of 1.25 g. of phenobarbital. The petroleum ether extracts yielded 9.27 g. (net yield 67%) of product melting at 132–136°. After recrystallization from dilute alcohol, **1-bromodiethylacetylphenobarbital** melts at 141–142° (cor.). This compound could be dissolved in *N* alkali and be reprecipitated unchanged by acidification after an hour.

Anal. Calcd. for $C_{18}H_{21}BrN_2O_4$: Br, 19.53; N, 6.85. Found: Br, 19.50; N, 6.97.

Through the courtesy of Eli Lilly and Company, eight of these derivatives of phenobarbital have received preliminary testing for anticonvulsant activity and the results are tabulated.

(11) Auwers, *Ann.*, **439**, 141 (1924).

Compound	Approximate M.H.D. in rats by mouth[a]	Approximate comparative anti-convulsant value,[b] %
[Diphenylhydantoin]	No action	100
[5-Ethyl-5-phenylhydantoin]	200	100
1-Propionylphenobarbital	...	125
1,3-Dipropionylphenobarbital	...	102
1-β-Hydroxyethylphenobarbital	1000	51
1-β-Chloroethylphenobarbital	200	90
1-β-Bromoethylphenobarbital	400	34
1-*p*-Phenylphenacylphenobarbital	No action	22
1-Phenacylphenobarbital	No action	None
1-*p*-Bromophenacylphenobarbital	No action	None

[a] 100–1000 mg. per kg.

[b] Equivalent doses of 50 mg. per kg.

Parke, Davis and Company have tested 1-bromodiethylacetylphenobarbital and found it to be toxic in doses of 260 mg. per kg. by stomach tube in rats; the compound exhibits definite activity as an anticonvulsant.

Summary

1. By interaction of the mono-sodium or silver salt of phenobarbital with an appropriate halogen compound, N-derivatives of phenobarbital have been prepared containing an hydroxyalkyl, haloalkyl, ketonyl, acyl, or haloacyl group.

2. In addition to ten mono-substituted derivatives, in three instances, 1,3-disubstituted phenobarbitals were obtained.

3. None of the new phenobarbital derivatives possess hypnotic power, but several exhibit strong anticonvulsant activity.

AUSTIN, TEXAS RECEIVED AUGUST 25, 1941

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

Chemistry of Vitamin B_6. IV. Reactions in Solutions at Elevated Temperatures

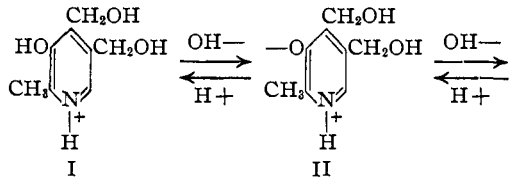
BY STANTON A. HARRIS

In testing the preparation of sterile solutions of vitamin B_6 base, Dr. J. Rosin and Mr. H. Mack[1] observed the precipitation of insoluble material when 10% aqueous solutions were heated at 120° for thirty minutes. Investigation has shown that this partially crystalline product is the result of a polymerization reaction of vitamin B_6 with the loss of water, and the steps in the determination of the reaction mechanism are now described. Short periods of heating produced crystalline material which could be recrystallized for purification. The longer periods of heating produced amorphous gelatinous material which was quite insoluble. Analyses were in agreement with the formula $C_{16}H_{20}N_2O_5$ for the crystalline product. This formula corresponds to the product of the interaction of two molecules of vitamin B_6 with the elimination on one molecule of water.

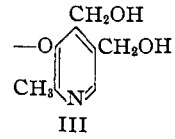
Harris, Webb and Folkers[2] have shown that vitamin B_6 exhibits the following tautomerism in acid (I), neutral (II), and basic (III) water solutions. The polymerization reaction has been

(1) Control Division, Merck & Co. Inc.

(2) Harris, Webb and Folkers, THIS JOURNAL, **62**, 3198 (1940).

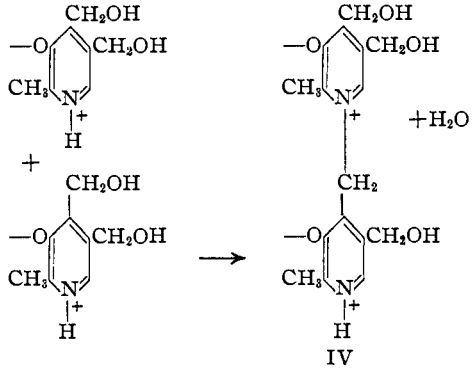


I II



III

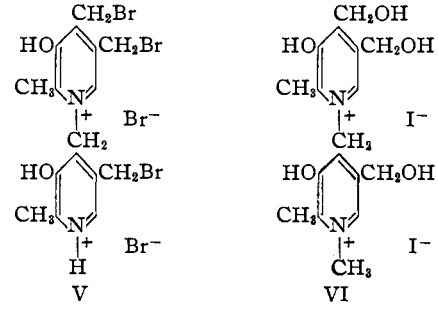
found to occur only in neutral water solutions in which the zwitterion (II) was shown to predominate.[2] Vitamin B_6 is stable in the presence of an excess of acid or alkali or in a solution buffered with sodium borate.[3] These solutions have been shown not to contain the zwitterion modification[2,3] of vitamin B_6. Thus, it was thought probable that the zwitterion reacted with the active 4-hydroxymethyl group to give a dimer as illustrated by formula IV. A substance of



IV

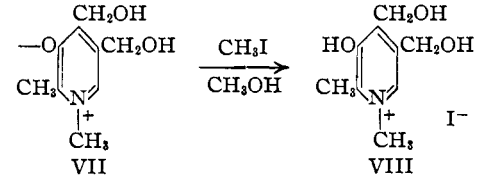
structure IV would be capable of further condensation reactions to give polymeric derivatives, a mechanism which seems to describe the amorphous and gelatinous material produced by longer heating periods. When the heating period was confined to one-half hour, a crystalline precipitate usually was obtained. The analyses and molecular weight[4] showed that the dimer was composed of two molecules of vitamin B_6 minus one molecule of water.

The carbon–nitrogen linkage of the vitamin B_6 dimer (IV) was indicated by its reaction with methyl iodide and with hydrobromic acid. If the dimer were an O-ether, heating with 48% hydrobromic acid would convert it to the known vitamin B_6 dibromide hydrobromide (XI). The actual product of this reaction had an analysis corresponding to structure V. Methyl iodide in methyl alcoholic solution gave a crystalline monomethiodide-iodide (VI) which indicated that,



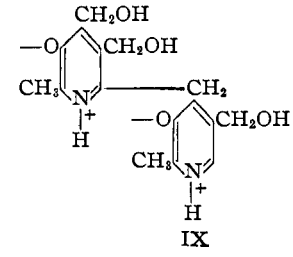
V VI

of the two nitrogens, one was incapable of adding methyl iodide. This was presumably due to the fact that one nitrogen was already made quaternary by the complex B_6 radical. The dimer reacted with methyl iodide alone or with methyl iodide in dioxane to give a product which has not as yet been crystallized. The reaction of methyl iodide with methyl alcohol in the presence of an organic base accounts for the hydrogen iodide which formed a salt with the substituted betaine portion of the molecule. When N-methyl-vitamin B_6-betaine (VII) was treated with methyl iodide in methyl alcohol, the methiodide (VIII) was obtained, whereas the addition of methyl iodide in the absence of methyl alcohol gave the methoxy[5] derivative.



VII VIII

The only alternative structure possible is represented by IX.



IX

The arguments for structure IV instead of structure IX are: (1) the dimer forms a mono-methiodide instead of a dimethiodide; (2) the poly-

(3) Scudi, Bastedo and Webb, *J. Biol. Chem.*, **136**, 399 (1940).

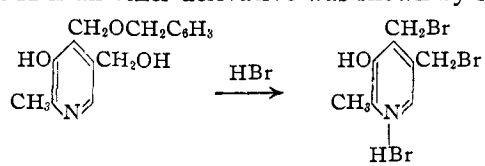
(4) The molecular weight was kindly determined by Dr. Niederl and Mr. Levy of New York University by the method which was reported by Schmitt and Niederl before the Microchemical Division of the American Chemical Society at Detroit on September 11, 1940. Niederl and Levy, *Science*, **92**, 225 (1940).

(5) Harris, THIS JOURNAL, **62**, 3203 (1940).

merization takes place only in neutral solutions in which it has been shown previously[2] that vitamin B_6 exists in the zwitterion form (II). It is difficult to visualize structure IX as being derived from the zwitterion; (3) N-methylvitamin B_6 betaine did not undergo polymerization under conditions which gave the insoluble products with vitamin B_6. This is further indication that the nitrogen atom of vitamin B_6 is involved in the polymerization reaction.

In view of these facts it is highly probable that formula IV represents the structure of vitamin B_6 dimer.

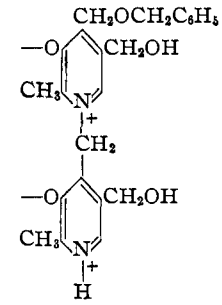
The involvement of the 4-hydroxymethyl group in the polymerization reaction was indicated by its unusual reactivity toward hydrogenation and heating with methyl alcohol as previously described.[5] It has now been found that heating vitamin B_6 with benzyl alcohol produces a monobenzyl ether of vitamin B_6 (X). The fact that X is an ether derivative was shown by reac-



X XI

tion with 48% hydrobromic acid to give vitamin B_6 dibromide hydrobromide XI. The presence of a phenolic hydroxyl group was shown by a positive ferric chloride test, and by a positive dichloroquinone chlorimide reaction in presence of a borate buffer. Scudi, Bastedo and Webb[3] have shown that vitamin B_6 forms a complex with boric acid which involves the 3-hydroxyl and 4-hydroxymethyl groups and thus prevents the color reaction with dichloroquinone chlorimide. Vitamin B_6 derivatives which have the 4-hydroxyl group substituted have been shown to give the color test in the presence of borate buffer. Thus, it is concluded that this compound (X) is 2-methyl-3-hydroxy-4-benzyloxymethyl-5-hydroxymethylpyridine. It is also interesting to note that only the 4-hydroxymethyl group of vitamin B_6 reacts with benzyl alcohol.

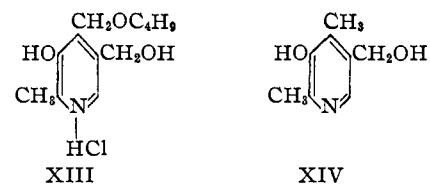
An interesting by-product was obtained in about 10% yield from benzylation of vitamin B_6. Its analysis showed the presence of one benzyl group for every two vitamin B_6 nuclei. From other evidence given in this paper, its structure is best formulated as the monobenzyl ether of vitamin B_6 dimer (XII). This indicates that the



XII

polymerization reaction may also take place in non-aqueous solutions. An identical product was obtained by heating vitamin B_6 dimer with benzyl alcohol.

Dr. Scudi[6] of this Laboratory found that refluxing vitamin B_6 in butyl alcohol caused an increase in the color test with dichloroquinone chlorimide in borate buffer. He postulated the formation of a butyl ether in the 4-hydroxymethyl position. This has been verified by the isolation and identification of 2-methyl-3-hydroxy-4-butoxymethyl-5-hydroxymethylpyridine hydrochloride (XIII).



XIII XIV

Other reactions which indicated that the 4-hydroxymethyl group was involved in the polymerization are as follows: (1) when a 10% water solution of the 4-methoxyl derivative of vitamin B_6[5] was heated at 120° for one and one-half hours, a gelatinous product was obtained. Analysis showed that this compound contained only one-third of the methoxyl content which would have been present if polymerization had involved the 5-hydroxymethyl group. Therefore, this condensation took place with the loss of methyl alcohol; (2) when the 4-desoxy derivative (XIV)[5] of vitamin B_6 was treated in the same manner, no insoluble precpitate was obtained which further indicates that the 4-hydroxymethyl group is essential to the polymerization; (3) vitamin B_6 dimer when heated with benzyl alcohol gave a monobenzyl ether of the dimer, indicating that only one reactive hydroxy group was present.

Vitamin B_6 dimer gave a slightly positive dichloroquinone chlorimide test in the presence of a

(6) Scudi, *J. Biol. Chem.*, **139**, 707 (1941).

borate buffer,[3] as kindly determined by Dr. N. R. Trenner of this Laboratory. In the presence of veronal buffer[7] one mole of vitamin B_6 dimer gave a color about equal to that given by one mole of vitamin B_6 under the same conditions. The methiodide of the dimer gave no color test with this reagent. It was shown previously[2] that substitution of the nitrogen of vitamin B_6 with a methyl group prevents the color reaction with the above reagent. Since the dimer gave only half the color intensity of that given by vitamin B_6 it would indicate that one nitrogen probably is substituted while in the dimer methiodide (VI) both nitrogens must be substituted.

The biological activity of each of the following compounds was determined in the Merck Institute for Therapeutic Research by Dr. Klaus Unna using a single dose curative assay[8] on vitamin B_6 depleted rats. Comparable curative effects with the vitamin B_6 dimer were obtained at a dose level 40 times greater than that of vitamin B_6 hydrochloride. In the case of the vitamin B_6 benzyl ether, it required a dose level approximately 20 times greater than that of the vitamin to obtain a similar effect; thus, the benzyl ether showed somewhat less activity than did the corresponding methyl and ethyl ethers as previously reported by Unna.[9] The butyl ether of vitamin B_6 was found to be as active as the methyl and ethyl ethers.

Experimental Part

Vitamin B_6 Dimer (IV).—A suspension of 8.6 g. of vitamin B_6 hydrochloride in a little water was neutralized to *p*H 6 with 2.5 *N* sodium hydroxide. The solution was diluted to 69 cc. and then subjected to repeated autoclaving at 15 lb. pressure for one-half hour periods, the liquor being cooled and the solid removed between each treatment. The product was usually crystalline although occasionally a gelatinous product was obtained. The more crystalline material was recrystallized from water; yield 0.43 g. (6.4%); m. p. 205–209°. The material readily absorbed small amounts of water which caused slightly varying analyses. However, when the sample was dried by the Hayman[10] micro pig method for three hours at 100°, the following carbon and hydrogen analyses were obtained.

Anal. Calcd. for $C_{16}H_{20}O_5N_2$: C, 59.99; H, 6.29; N, 8.75; mol. wt., 320. Found: C, 60.30, 60.19; H, 6.42, 6.06; N, 8.45, 8.68; mol. wt., 303.[4]

Vitamin B_6 Polymer.—Ten cc. of a solution containing 0.85 g. of vitamin B_6 base and 7 cc. of *N* sodium hydroxide was heated for three hours at 15 lb. steam pressure. After cooling, the solution was neutralized to *p*H 7 with 7 cc. of *N* hydrochloric acid. There was no immediate precipitation and only a trace formed after several days standing. A control experiment with vitamin B_6 base in 10 cc. of water with no sodium hydroxide when heated as above, gave a heavy gelatinous precipitate. It weighed 0.24 g. or 28.3% of the original weight.

The water insoluble gelatinous precipitate was treated with boiling alcohol which left behind a slight residue. On cooling the alcoholic solution a gelatinous precipitate separated and was removed by centrifuging. It was washed with acetone and dried by spreading on a porous plate. Its analysis, after drying in a Hayman micro pig at 100° for three hours, agreed with the formula for a compound formed from three moles of vitamin B_6 with the loss of two moles of water.

Anal. Calcd. for $C_{24}H_{32}N_2O_7$: C, 61.13; H, 6.20; N, 8.91. Found: C, 61.03; H, 6.37; N, 8.48, 8.35.

Methiodide-iodide of Vitamin B_6 Dimer, VI.—A solution of 0.2 g. of the vitamin B_6 dimer in 10 cc. of methanol, to which was added 10 cc. of methyl iodide and 20 cc. of benzene, was refluxed for sixteen hours, after which the methyl alcohol and methyl iodide were removed by distillation. An oil separated from the benzene which soon crystallized and was recrystallized from absolute alcohol plus a little ether. It was washed with alcohol and acetone and dried; m. p. 197–198°.

Anal. Calcd. for $C_{17}H_{24}O_3N_2I_2$: C, 34.59; H, 4.10; N, 4.75; (N) CH_3, 2.55; OCH_3, 0; I, 43.01. Found: C, 34.77; H, 4.40; N, 4.64; (N) CH_3, 2.10; OCH_3, 0.73: I, 42.73.

The small amount of OCH_3 may be accounted for by a trace of OCH_3 in the compound or as a fraction of the NCH_3 which came over during the determination. If calculated as NCH_3, the total would be 2.45%, which is in excellent agreement with the theoretical of 2.53%. This analysis indicated that only one nitrogen was methylated, and that the crystalline vitamin B_6 dimer was composed of two vitamin B_6 units minus a molecule of water. Methylation of the dimer in the absence of alcohol gave a non-crystalline product.

Bromo Derivative of Vitamin B_6 Dimer (V).—A solution of 0.1 g. of the vitamin B_6 dimer dissolved in 5 cc. of 48% hydrobromic acid was concentrated by distillation at atmospheric pressure to a volume of 1–2 cc. After centrifuging, the material commenced to crystallize. After recrystallization from hydrobromic acid, it was removed by centrifuging and washed with acetone; yield 0.095 g. It sublimed and decomposed above 230° without melting. A mixed melting point with vitamin B_6 dibromide hydrobromide showed a depression of 10°.

Anal. Calcd. for $C_{16}H_{19}N_2O_2Br_5$: C, 28.64; H, 2.86; N, 4.18; Br, 59.56. Found: C, 28.71; H, 3.02; N, 4.11; Br, 59.48.

The compound showed signs of slight decomposition on drying at 100° in a vacuum for two hours.

Polymerization of 2-Methyl-3-hydroxy-4-methoxymethyl-5-hydroxymethylpyridine.—A solution containing (0.01 mole) 1.83 g. of the methoxy vitamin was made by dissolving 2.19 g. of the hydrochloride in 10 cc. of 1 *N* sodium hydroxide and diluting the solution to 18 cc. with

(7) Scudi, Koones and Keresztesy, *Proc. Soc. Exp. Biol. Med.*, **43**, 118 (1940).

(8) Reedman, Sampson and Unna, *ibid.*, **43**, 112 (1940).

(9) Unna, *ibid.*, **43**, 122 (1940).

(10) Hayman, *Ind. Eng. Chem., Anal. Ed.*, **10**, 55 (1938).

water. The *p*H of the solution was about 6. There was no precipitate formed on autoclaving the solution for one-half hour at 15 lb. pressure. However, two additional heating periods of one hour each gave gelatinous precipitates which on redissolving in hot water came out again in a gelatinous form. It did not melt below 300°. The analysis corresponded to a trimer which had lost two molecules of methyl alcohol.

Anal. Calcd. for $C_{25}H_{32}N_3O_7$: N, 8.44; OCH_3, 6.24. Found: N, 8.43, 8.20; OCH_3, 6.98.

Attempted Polymerization of 2,4-Dimethyl-3-hydroxy-5-hydroxymethylpyridine (4-Desoxyvitamin B_6).—A solution containing 1.9 g. of 2,4-dimethyl-3-hydroxy-5-hydroxymethylpyridine hydrochloride[5] was neutralized to *p*H 6 with 10 cc. of *N* sodium hydroxide. The compound dissolved to give a yellow solution. It was diluted to 18 cc. with distilled water and autoclaved at 15 lb. steam pressure for two one-hour periods. No solid appeared on cooling and standing overnight in the refrigerator.

Attempted Polymerization of N-Methylvitamin B_6 Betaine.—Two grams of N-methylvitamin B_6 betaine was dissolved in 10 cc. of water and was heated in an autoclave at 15 lb. pressure for two and one-half hours. There was no separation of insoluble material even on standing overnight. The solution was evaporated to dryness and the residue recrystallized from alcohol; yield 1.77 g., 88.5%. The melting point and mixed melting point with starting material was 196–199°. There was no evidence of a polymeric product.

Benzylation of Vitamin B_6.—Two grams of vitamin B_6 base was refluxed in 20 cc. of benzyl alcohol for two hours and then the benzyl alcohol was removed by vacuum distillation. The oily residue was extracted with ether when it crystallized. This residue (1.4 g.) was dissolved in ethanol and treated with ether when 0.2 g. of fine crystals separated slowly. After recrystallization from alcohol, it melted at 217–218°. It analyzed for a benzyl ether of vitamin B_6 dimer (XII). This product was also obtained by heating vitamin B_6 dimer with benzyl alcohol.

Anal. Calcd. for $C_{23}H_{26}N_2O_5$: C, 67.30; H, 6.39; N, 6.83. Found: C, 67.13, 67.46; H, 6.39, 6.46; N, 6.76.

The mother liquor was treated with dry hydrogen chloride when a salt separated that melted at 144–145° after recrystallization from alcohol. This analyzed for the hydrochloride of vitamin B_6 monobenzyl ether.

Anal. Calcd. for $C_{16}H_{18}NO_3Cl$: C, 60.91; H, 6.13; N, 4.74. Found: C, 61.05; H, 5.85; N, 4.74.

The original ether solution was evaporated when crystals melting at 160–162° were obtained. After two recrystallizations from alcohol, the melting point was constant at 166.5°. It analyzed for a monobenzyl ether of vitamin B_6 (X).

Anal. Calcd. for $C_{15}H_{17}NO_3$: C, 69.47; H, 6.61; N, 5.40. Found: C, 69.61, 69.65; H, 6.79, 6.89; N, 5.37.

The hydrochloride from this base proved to be identical with the hydrochloride described above, having a melting point and mixed m. p. of 144–145°. One-tenth gram of the benzyl ether was refluxed with 2–3 cc. of 48% hydrobromic acid in a test-tube for two or three minutes when an oil steam distilled away which was presumably benzyl bromide. On cooling, crystallization took place. The melting point and mixed m. p. with 2-methyl-3-hydroxy-4,5-*bis*-(bromomethyl)-pyridine hydrobromide was 224°. The benzyl ether gave a positive ferric chloride test and a positive dichloroquinone chlorimide color test in the presence of a borate buffer. From these reactions, and for reasons given in the introduction, this compound was shown to be 2-methyl-3-hydroxy-4-benzyloxymethyl-5-hydroxymethylpyridine (IX).

2-Methyl-3-hydroxy-4-butoxymethyl-5-hydroxymethylpyridine Hydrochloride (XIII).—One gram of vitamin B_6 base was refluxed in 150 cc. of *n*-butanol for 25 hours. The alcohol was removed and the residue extracted thoroughly with ether. The extract was concentrated, taken up in ether and filtered. After repeating this process, the solution was treated with alcoholic hydrogen chloride when a white fluffy precipitate was obtained, yield 0.52 g. The material apparently contained a little vitamin B_6 hydrochloride which was removed on extraction with acetone from which the butyl ether of B_6 crystallized; m. p. 127–128°.

Anal. Calcd. for $C_{12}H_{20}O_3NCl$: C, 55.06; H, 7.60; N, 5.53. Found: C, 55.12; H, 7.72; N, 5.41.

Acknowledgments.—The author wishes to thank Drs. R. T. Major and K. A. Folkers for their suggestions and coöperation. Appreciation is also expressed to Messrs. D. F. Hayman, W. R. Reiss, H. S. Clark and R. N. Boos for their cooperation in performing the microanalyses and to Mr. A. N. Wilson for assistance in the preparations.

Summary

Neutral aqueous solutions of vitamin B_6 when heated to 120° for sterilization purposes are shown to undergo polymerization involving the splitting of water from the 4-hydroxymethyl group of one vitamin B_6 molecule, and the N-hydrogen atom of the zwitterion modification of another molecule. This reaction does not occur in acid or alkaline solutions in which the zwitterion does not exist.

Vitamin B_6 base in boiling benzyl alcohol reacts with the solvent to form mainly monobenzyl ether of the 4-hydroxymethyl group. Polymerization in this solvent occurs to a less extent with the formation of a monobenzyl ether of vitamin B_6 dimer. Likewise, butyl alcohol gave the corresponding monobutyl ether of B_6.

The 4-methoxymethyl derivative of vitamin B_6 undergoes polymerization in aqueous solution at 120° with the loss of methyl alcohol while the 4-desoxy derivative failed to undergo this reaction. N-Methyl vitamin B_6 betaine also failed to undergo the polymerization reaction.

RAHWAY, NEW JERSEY RECEIVED SEPTEMBER 18, 1941