hours from the dropping funnel. Alcohol was removed simultaneously from the head of the column at such a rate that it did not accumulate in the reaction mixture. When no more alcohol was obtained as distillate, the reaction mixture was cooled and poured on a mixture of ice and hydrochloric acid. The organic layer was separated, washed with water containing a little salt, dried over calcium chloride and fractionated until all the diethyl carbonate had been removed. The residue was distilled from a Claisen flask at reduced prssure: yield 152.5 g. (86%), m. p. 16–17°, b. p. 129–30° at 2 mm.

Summary

A procedure has been developed for condensing alkyl carbonates with organic esters by metal alcoholates for the convenient production of malonic esters. This has been applied to numerous aliphatic and aryl substituted aliphatic esters. In certain cases alkyl carbonates exhibit an alkylating action.

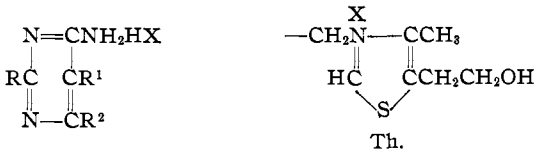
ST. LOUIS, MISSOURI RECEIVED MAY 8, 1941

[CONTRIBUTION FROM THE RESEARCH LABORATORY OF MERCK & CO., INC., AND THE MERCK INSTITUTE FOR THERAPEUTIC RESEARCH]

Some Analogs of Thiamin and their Physiological Activity[1]

BY GUSTAV A. STEIN, W. L. SAMPSON, J. K. CLINE AND JOSEPH R. STEVENS

The following analogs of thiamin, some of which have been credited with physiological activity, were prepared



Th.

I, R = H; R^1 = Th; R^2 = CH_3; X = Br (Makino vitamin); 6-methyl-5-thiazolium isomer (activity claimed)[2]

II, R = H; R^1 = CH_3; R^2 = Th; X = Br (reversed Makino); 5-methyl-6-thiazolium isomer

III, R = CH_3; R^1 = H; R^2 = Th; X = Br, 2-methyl-6-thiazolium isomer (activity claimed)[3]

IV, R = C_2H_5; R^1 = Th; R^2 = H; X = Br, 2-ethyl-5-thiazolium homolog

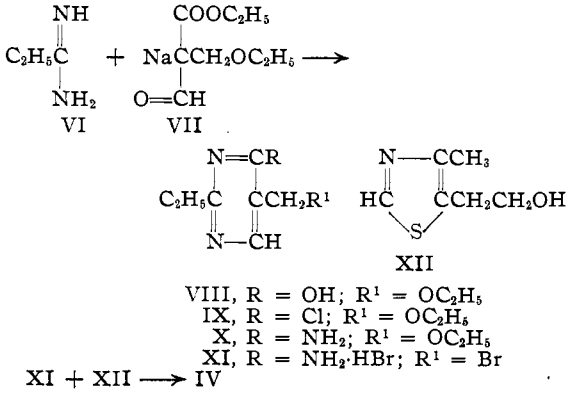
V, R = CH_3; R^1 = Th; R^2 = H; X = Cl, (thiamin)

The biological activity of these compounds was studied and compared with that of thiamin hydrochloride, V.

Discussion

Whereas compounds I, the so-called Makino vitamin, and III were prepared according to known methods,[2,3] the ethyl homolog IV was made by adapting the Williams and Cline[4] synthesis for the 2-methyl homolog, by condensing 2-ethyl-4-amino-5-bromomethylpyrimidine dihydrobromide, XI, with 4-methyl-5-(β-hydroxyethyl)-thiazole, XII. The synthesis of XI starts with the condensation of propionamidine, VI, with the sodium formyl derivative of γ-ethoxyacetoacetic ester, VII, to yield 2-ethyl-4-hydroxy-5-ethoxymethylpyrimidine, VIII, m. p. 146–146.5°. Upon chlorination with phosphorus oxychloride, III gave 2-ethyl-4-chloro-5-ethoxymethylpyrimidine, IX, which without further purification was aminated to yield 2-ethyl-4-amino-5-ethoxymethylpyrimidine, X, m. p. 64.5–65.5°. Treatment of X with glacial acetic hydrobromic acid yielded 2-ethyl-4-amino-5-bromomethylpyrimidine dihydrobromide, XI, m. p. (crude) 175–178°.

The above series of reactions may be represented by the following formulas



VIII, R = OH; R^1 = OC_2H_5
IX, R = Cl; R^1 = OC_2H_5
X, R = NH_2; R^1 = OC_2H_5
XI, R = NH_2·HBr; R^1 = Br

XI + XII ⟶ IV

In a similar way the synthesis of the 2-methyl-6-thiazolium isomer, III, was tried as follows:

Condensing acetamidine, XIII, with ethyl γ-ethoxyacetoacetate, XIV, in the usual way, gave 2-methyl-5-hydroxy-6-ethoxymethylpyrimidine,[3] XV, which, on treatment with phosphorus oxy-

(1) This paper is No. XX in the R. R. Williams Vitamin B_1 Series (XIX, Joseph R. Stevens and Gustav A. Stein, THIS JOURNAL, **62**, 1045–1048 (1940)).

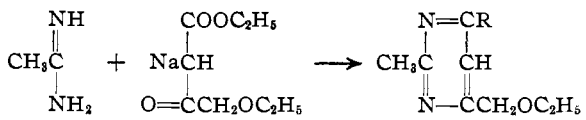
(2) H. Andersag and K. Westphal, "Über die Synthese des antineuritischen Vitamins," *Ber.*, **70**, 2035 (1937).

(3) British Patent 471,416 to I. G. Farbenindustrie A. G., accepted Aug. 30, 1937; French Patent 816,432 to I. G. Farbenindustrie A. G.; published August 7 (1937).

(4) R. R. Williams and J. K. Cline, THIS JOURNAL, **58**, 1504–1505 (1936).

chloride gave 2-methyl-5-chloro-6-ethoxymethylpyrimidine, XVI, which was aminated in the same way in the crude form to give 2-methyl-4-amino-6-ethoxymethylpyrimidine, XVIII, m. p. 96°.

The above set of reactions is represented by the formulas



XV, R = OH
XVI, R = Cl
XVII, R = NH_2

Attempts to split the ether linkage in XVII, using existing methods, failed and the other method[3] of preparation, already mentioned above, was used.

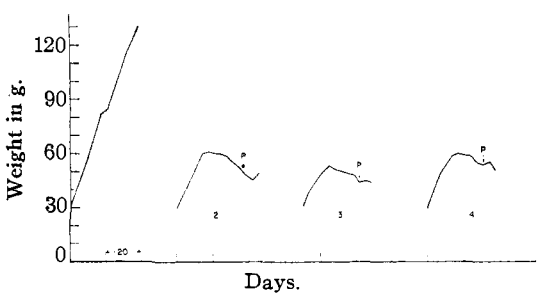
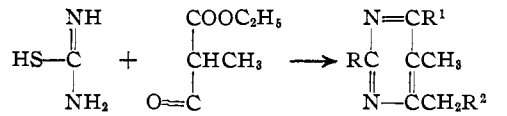


Fig. 1.—Average growth response of rats on a vitamin B_1 free diet to daily feeding with Makino vitamin and the 4 thiazolium isomer. Seven rats in each group: Curve 1, 5 micrograms thiamin hydrochloride; Curve 2, 100 micrograms Makino vitamin; Curve 3, 500 micrograms 4 thiazolium isomer; Curve 4, negative control; P, polyneuritis.

The isomer II, the reversed Makino vitamin, with the thiazole linkage attached to the 6 position of the pyrimidine ring and the methyl group in position 5, was prepared by condensing 4-amino-5-methyl-6-bromo-methylpyrimidine hydrobromide, XXIV, with the corresponding thiazole alcohol, XII. The synthesis of XXIV starts with the condensation of thiourea, XVIII, with α-methyl-γ-ethoxyacetoacetic ester, XIX, to give 2-thio-4-hydroxy-5-methyl-6-ethoxymethylpyrimidine, XX[5] which was oxidized with hydrogen peroxide in a way similar to that used by Andersag and Westphal for related pyrimidines to give 4-hydroxy-5-methyl-6-ethoxymethylpyrimidine, XXI, m. p. 118°. Chlorination of XXI with phosphorus oxychloride gave XXII which on amination yielded 4-amino-5-methyl-6-ethoxymethylpyrimidine, XXIII, m. p. 136–137°.

(5) Johnson and Chernoff, THIS JOURNAL, **35**, 585–597 (1913).

Treatment of XXIII with hydrogen bromide in glacial acetic acid gave 4-amino-5-methyl-6-bromomethylpyrimidine hydrobromide XXIV. The above procedure is expressed by the following formulas:



XX, R = SH; R^1 = OH; R^2 = OC_2H_5
XXI, R = H; R^1 = OH; R^2 = OC_2H_5
XXII, R = H; R^1 = Cl; R^2 = OC_2H_5
XXIII, R = H; R^1 = NH_2; R^2 = OC_2H_5
XXIV, R = H; R^1 = NH_2·HBr; R^2 = Br

XXIV + XII ⟶ II

Discussion of the Pharmacological Results

The compounds mentioned in this report were tested for their vitamin B_1 activity by both rat curative and rat prophylactic methods. When compounds I, II and III were fed in single doses up to 1000 micrograms to vitamin B_1 depleted rats, no evidence of a curative effect was observed. The rats so treated continued to lose weight and to exhibit marked symptoms of polyneuritis. Furthermore, daily doses of 100 micrograms of I and 500 micrograms of II administered to weanling rats maintained on a vitamin B_1 free diet failed to support growth in the animals. All animals receiving these compounds began to lose weight after fifteen days on the test and soon evidenced typical polyneuritic symptoms followed by death (Fig. 1). The Makino vitamin, I, was also tested for vitamin B_1 activity by the yeast fermentation method of Schultz, Atkin and Frey.[6] In this test, quantities of I up to 1 mg. exerted little or no effect on the rate of fermentation, whereas, under the same conditions, 1 to 2 micrograms of thiamin hydrochloride markedly increased the gas formation.

The 2-ethyl vitamin IV, however, was found to be highly effective (see also Fritz Schultz).[7] By the curative method on a molecular basis it was found to be equally as effective as thiamin hydrochloride and, as can be seen from the attached curves (Fig. 2), no difference in growth response between the 2-ethyl analog and the thiamin hydrochloride reference standard was shown when allowance was made for the difference in molecular weight. Both groups of rats grew at approximately the same rate throughout the experiment and in both groups the leveling off in the weight

(6) Schultz, Atkin and Frey, *ibid.*, **59**, 2457 (1937).
(7) Schultz, *Z. physiol. Chem.*, **265**, 113–128 (1940).

curves occurred at the same time whereas, in the negative control group, all developed polyneuritis and died before completion of the experiment. The similarity of response in both curative and prophylactic tests shows that the 2-ethyl analog, IV, is equivalent in vitamin B_1 activity to thiamin hydrochloride, V.

The above findings again demonstrate the specificity of the thiamin molecule which makes it "a unique or nearly unique molecule which cannot undergoalte ration without impairment of physiological utility."[8,9]

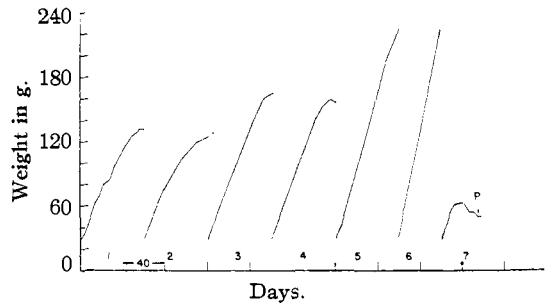


Fig. 2.—Average growth response of rats on a vitamin B_1 free diet to daily administration of thiamin hydrochloride and its 2 ethyl isomer. Ten rats in each group: Curve 1, 3 micrograms thiamin hydrochloride; Curve 2, 2 ethyl isomer equivalent to 3 micrograms thiamin hydrochloride; Curve 3, 5 micrograms thiamin hydrochloride; Curve 4, 2 ethyl isomer equivalent to 5 micrograms thiamin hydrochloride; Curve 5, 2 ethyl isomer equivalent to 25 micrograms thiamin hydrochloride; Curve 6, regular stock diet (positive control); Curve 7, vitamin B_1 free diet only (negative control); P, polyneuritis.

Experimental

2-Ethyl-4-hydroxy-5-ethoxymethylpyrimidine, VIII.—To a suspension of sodium dust in petrolatum (5.75 g. sodium in about 350 cc. light petrolatum) was added gradually over a period of an hour with constant stirring, a mixture of 36.5 g. (0.25 mole) of β-ethoxypropionic acid ethyl ester, and 18.5 g. (0.25 mole) of ethyl formate while keeping the temperature of the reaction between 30–35°. Stirring was continued for eight hours after the addition, after which, the flask was externally cooled to −5° and a solution of 31.3 g. of propionamidine hydrochloride[10] in 20 cc. of water and 10 g. fine ice was added with good stirring. After an hour, a second solution of 10.4 g. of propionamidine hydrochloride in 5 cc. of water and 5 g. of ice was added, followed by 25 cc. of 33% sodium hydroxide. After good stirring, the reaction was allowed to run to completion by standing overnight at 0°.

After separation of the aqueous layer, the reaction mixture was extracted once with ether, then acidified to *p*H 6.5 with glacial acetic and repeatedly extracted with chloroform. The chloroform extract was dried with sodium sulfate, evaporated *in vacuo* and yielded a residue weighing about 25 g. (55% crude yield).

On recrystallization from amyl ether or acetone, a white powder melting at 146–146.5° was obtained. A sample, purified by sublimation at 120° (0.5 mm.) was analyzed.

Anal. Calcd. for $C_9H_{14}O_2N_2$: C, 59.30; H, 7.75; N, 15.38. Found: C, 59.23, 59.60; H, 7.74, 7.87; N, 15.46.

2-Ethyl-4-chlor-5-ethoxymethylpyrimidine, IX.—Twelve grams of the oxypyrimidine, VIII, was heated at 80° for three hours with 43 cc. of phosphorus oxychloride. The phosphorus oxychloride was then distilled off *in vacuo;* the brown viscous residue was taken up with a little chloroform and poured onto ice with good stirring. The reaction mixture was made alkaline with ammonia while cooling well, and the aqueous layer was then repeatedly extracted with chloroform. The extracts were dried over sodium sulfate and the chloroform evaporated in a good vacuum producing 8 g. of crude chloropyrimidine (56% yield) which was aminated immediately without further purification.

2-Ethyl-4-amino-5-ethoxymethylpyrimidine, X.—Eight grams of the crude chloropyrimidine, IX, was dissolved in 64 cc. of cold saturated ammoniacal ethanol and heated in bomb tubes at 120° for four hours. After evaporating the alcohol *in vacuo*, the residue was taken up in water. The solution was clarified with charcoal, then extracted repeatedly with chloroform and the extract was dried over sodium sulfate. The solvent was evaporated *in vacuo* and the gummy residue was extracted several times with ligroin (b. p. 94–96°). The needle-like crystals, which had a tendency to form a yellow oil were dried over sulfuric acid yielding about 3 g. of material, m. p. 64.5–65.5°. This was further purified for analysis by sublimation in vacuum (oil pump).

Anal. Calcd. for $C_9H_{15}ON_3$: C, 59.62; H, 8.35; N, 23.20. Found: C, 59.61; H, 8.21; N, 22.70.

2-Ethyl-4-amino-5-bromomethylpyrimidine Hydrobromide, XI.—One gram of above crude, X, was heated on the steam-bath for about three hours with 25 cc. of glacial acetic–hydrobromic acid. After cooling, the crystals (1.5 g.) were filtered or centrifuged, washed with glacial acetic acid and anhydrous ether and then dried over sulfuric acid giving a crude product, m. p. 175–178°.

2-Ethyl-4-amino-5-(4-methyl-5-β-hydroxyethyl-thiazolium Bromide) Methylpyrimidine Hydrobromide, IV.—The above crude (1.8 g.) 2-ethyl-4-amino-5-bromomethylpyrimidine hydrobromide was well mixed with 4 cc. of light petrolatum and 2.0 cc. of freshly distilled 4-methyl-5-β-hydroxyethylthiazole, XII, b. p. 106–107° (1.2 mm.), after which it was heated at 117°, over butanol, for about fifteen minutes. The solidified cold melt was well washed with anhydrous ether until it had disintegrated to a fine powder which was dissolved in a minimum of hot water. On the addition of about 10 volumes of boiling absolute ethanol, the vitamin crystallized in the form of colorless crystals in very much the same way as thiamin, giving about 1.8 g. of material, m. p. 235.5–236°[11] (decompn.).

(8) R. R. Williams and Tom D. Spies, "Vitamin B_1 and Its Use in Medicine."

(9) R. R. Williams, "The Chemistry of Thiamin (Vit. B_1)," *J. Am. Med. Assoc.*, **110**, 727–732 (1938).

(10) Pinner and Klein, *Ber.*, **11**, 1484 (1878).

(11) British Patent 471,416 to I. G. Farbenindustrie A. G. accepted August 30, 1937, page 7, line 27, gives 236°.

Anal. Calcd. for $C_{13}H_{20}N_4OBr_2S$: C, 35.20; H, 4.58; N, 12.73; Br, 36.32. Found: C, 35.35; H, 4.58; N, 12.31; Br, 36.54.

2-Methyl-4-chloro-6-ethoxymethylpyrimidine, XVI.—Eight grams of 2-methyl-4-hydroxy-6-ethoxymethylpyrimidine, XV, m. p. 158°, was heated at 80° for three hours with 25 cc. of phosphorus oxychloride, after which the chlorinating agent was distilled off in vacuum. The sirupy residue was dissolved in chloroform and the solution carefully poured onto ice. The reaction mixture was then made ammoniacal, keeping the temperature below 10° during neutralization. Extraction of the water layer with chloroform, drying of the extract with sodium sulfate and concentration *in vacuo* yielded 8.6 g. of a brown oil which was aminated without purification.

2-Methyl-4-amino-6-ethoxymethylpyrimidine, XVII.—The above crude chloropyrimidine (8.6 g.) was dissolved in about 70 cc. of methanol saturated at 0° with ammonia and the resulting solution was heated at 125° for ten hours; the solvent was evaporated off *in vacuo* and the brown crystalline residue was extracted repeatedly with skellysolve D (boiling range 77–115°). The crystals (3.5 g.) thus obtained melted at 95.5–96°.

Anal. Calcd. for $C_8H_{13}ON_3$: C, 57.44; H, 7.84; N, 25.14. Found: C, 57.61; H, 7.63; N, 25.19.

4-Hydroxy-5-methyl-6-ethoxymethylpyrimidine, XXI.—Two grams of 2-thio-5-methyl-6-ethoxymethylpyrimidine, XX, was added in small portions to 30 cc. of 8% hydrogen peroxide solution, the temperature being kept at 75–80° during the addition. After the reaction was over, the solution was heated to 100° for about five minutes. The solution was then made ammoniacal and concentrated *in vacuo* until an oily layer separated, which was extracted with chloroform. The extract was dried with sodium sulfate; the solvent was evaporated *in vacuo* and the residue repeatedly extracted with hot petroleum ether (b. p. 100°). The crystals (0.81 g.) thus obtained melted at 118° and gave a negative test for sulfur.

Anal. Calcd. for $C_8H_{12}N_2O_2$: C, 57.10; H, 7.20; N, 16.67. Found: C, 56.95; H, 7.22; N, 16.86.

4-Chloro-5-methyl-6-ethoxymethylpyrimidine, XXII.—Five grams of the oxy compound, XXI, was refluxed with phosphorus oxychloride (10 cc.) for about one-half hour. The phosphorus oxychloride was then removed *in vacuo*, and the residue taken up with a little chloroform. The solution was slowly poured onto ice, rendered ammoniacal and extracted with chloroform. The product distilled at 90° in an oil pump vacuum, yield 80%.

4-Amino-5-methyl-6-ethoxymethylpyrimidine, XXIII.—Five grams of the chloropyrimidine, XXII, was heated with 6 mols of alcoholic ammonia at 125° for three hours. After evaporation of the alcohol, the residue was extracted with chloroform. The product crystallized from ether; yield 2.2 g., m. p. 137–138°.

Anal. Calcd. for $C_8H_{13}N_3O$: C, 57.44; H, 7.84; N, 25.14. Found: C, 57.54; H, 7.80; N, 25.45.

4-Amino-5-methyl-6-bromomethylpyrimidine Hydrobromide, XXIV.—By passing dry hydrogen bromide gas through a hot (80°) solution of above aminopyrimidine (2.7 g.) in glacial acetic acid (about 30 cc.) for several hours, 2.8 g. of crude 4-amino-5-methyl-6-bromomethylpyrimidine hydrobromide was obtained by precipitation with anhydrous ether.

4-Amino-5-methyl-6-(4-methyl-5-β-hydroxyethyl-thiazolium Bromide) Methylpyrimidine Hydrobromide, II.—One mol of the crude bromopyrimidine hydrobromide, XXIV, was heated with one mol of thiazole, XII, at 115–120° for about fifteen to twenty minutes. After washing the cold melt with anhydrous ether, it was dissolved in a minimum amount of hot water acidulated with a few drops of hydrobromic acid and the solution was clarified with charcoal and diluted with ten volumes of hot absolute ethanol. The vitamin crystallized slowly in thick rosets yielding about 25% of material, m. p. 233–234° (decomposition).

Anal. Calcd. for $C_{12}H_{18}ON_4SBr_2$: C, 33.80; H, 4.26; N, 13.15. Found: C, 33.77; H, 4.38; N, 12.81.

Acknowledgment.—The authors are grateful to Drs. Randolph T. Major, and R. R. Williams for their many helpful suggestions and to Mr. T. Perrine, Mr. S. G. Stearns and Mr. W. J. Moran, Jr., who assisted with the experimental work. The bioassays were made by Miss Saramae Woodford and the microanalyses by Mr. D. Hayman, Mr. W. Reiss and Mr. H. S. Clark.

Summary

Change of functionally important groups in the thiamin molecule results in complete inactivity, as shown by physiological tests of the three isomers, 6-methyl-5-thiazolium isomer, I (Makino vitamin); 5-methyl-6-thiazolium isomer, II (reversed Makino); and the 2-methyl-6-thiazolium isomer, III. The 2-ethyl-5-thiazolium homolog IV was found to be as active as thiamin itself.

RAHWAY, NEW JERSEY RECEIVED APRIL 26, 1941