starting material, are in good agreement both qualitatively and quantitatively with those obtained by Peterson and Sober[6] in these solvents.

Spectra obtained in 0.1 *N* hydrochloric acid are presented in Fig. 1. With the hydrazone, maxima are observed at 314 mμ (ϵ = 11,920) and 337 mμ (ϵ = 14,160). The 337 mμ maximum should be useful for spectrophotometric determinations, as the absorption of pyridoxal phosphate and pyridoxamine phosphate is minimal in this region.[6] The spectrum obtained with the azine in 0.1 *N* hydrochloric acid was essentially coincident with that resulting from the addition of the spectra of pyridoxal-5-phosphate and the hydrazone. Thus hydrolysis of the azine is essentially complete at low concentrations in acidic medium.

Spectra obtained in 0.1 *M* potassium phosphate at *p*H 7 are presented in Fig. 2. Upon formation of the hydrazone the maximum at 388 mμ which is specific for the –CHO group of pyridoxal phosphate[6] disappears as expected and maxima are observed at 284 mμ (ϵ = 10,800) and 320 mμ (ϵ = 8,630). Extinction values at the maxima were reproducible during the time required to examine the spectrum and exhibited no significant change after standing 14 hours in the buffer. The spectrum is indicative of the homogeneity of the hydrazone.

In addition to maxima observed at 288 and 316 mμ, the azine has a maximum at 379 mμ in the buffer. Similar azine structures show a maximum in this general region, e.g. 2,2′-dihydroxybenzalazine has a maximum at 355 mμ.[7] Immediately following solution of the azine in phosphate buffer *p*H 7 an initial decrease in absorption at both the 288 and 379 mμ maxima was observed. Reproducible values were obtained after approximately one hour and decreased less than 2% in 12 hours. The azine spectrum in Fig. 2 was obtained after 90 minutes. The spectrum of a mixture of equimolecular quantities of pyridoxal phosphate and its hydrazone in 0.1 *M* potassium phosphate buffer *p*H 7 was essentially coincident with that of the azine. This was interpreted as evidence for the existence of an equilibrium among azine, hydrazone, and pyridoxal phosphate in the solutions of the azine itself. Further support for this conjecture was obtained when additions of the separate spectra of the hydrazone and pyridoxal phosphate produced a curve which was not coincident with that of the azine. In the latter case the maximum at 379 mμ was about 25% lower than that for the azine itself. Available information does not permit a more complete explanation of this observation.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF FAIRFIELD UNIVERSITY, THE DEPARTMENT OF CHEMISTRY AND CHILDREN'S HOSPITAL, UNIVERSITY OF BUFFALO, AND ROSWELL PARK MEMORIAL INSTITUTE]

A 2-Trifluoromethyl Analog of Thiamin[1]

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Two synthetic routes for thiamin, adapted from classical methods, were found satisfactory for the preparation of 3-[(4-amino-2-trifluoromethyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium chloride hydrochloride [(VII) "trifluorothiamin"]. Solvolysis of intermediates occurred under certain conditions. "Trifluorothiamin" is biologically active as a thiamin antagonist in microorganisms and in mice where paralysis and opisthotonus occur. Growth of a transplanted carcinoma and a leukemia was suppressed in mice on a thiamin-deficient diet.

Although many analogs of thiamin have been prepared,[3a-d] the synthesis of a trifluoromethyl analog seemed pertinent because the presence of such a group could have a significant electronic effect on the molecule, but should not have a significant steric effect. In a preceding paper,[4] the syntheses of 4-amino-2-trifluoromethyl-5-hydroxymethylpyrimidine (I) and 4-amino-5-aminomethyl-2-trifluoromethylpyrimidine (IV) from trifluoroacetamidine were described. Their biological activity[5] contributed to our further interest in 2-trifluoromethylpyrimidines.

This paper is concerned with the use of compounds I and IV as starting materials for the preparation of 3-[(4-amino-2-trifluoromethyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methylthiazo-

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lium chloride hydrochloride (VII, "trifluorothiamin"). Information is presented on two alternate synthetic routes, and the two independent preparations constitute a proof of structure for the compound.

Okuda and Price[3d] found that Dornow and Petsch[6] had actually prepared 2-ethylthio-4-amino-5-isopropoxymethylpyrimidine hydrobromide, a solvolysis product, rather than the 2-ethylthiothiamin reported. In this research, alcoholysis of 4-amino-5-chloromethyl-2-trifluoromethylpyrimidine (II), prepared from I using thionyl chloride, was encountered during an attempt at recrystallization from ethanol-benzene and a preparative experiment for 4-amino-5-ethoxymethyl-2-trifluoromethylpyrimidine (III) is included. When the synthesis of VII, "trifluorothiamin," from compound II was attempted in nonhydrolytic solvents like acetone, 2-butanone, and dioxan, either the yields were not as satisfactory as described in method A (56%) or indications were that the reaction did not occur until the solvent was evaporated. Method A involved fusing compound II with 5-(2-hydroxyethyl)-4-methylthiazole to give "trifluorothiamin" (VII) as suggested by the work of Williams and Cline[7] in the synthesis of thiamin. In our preparation, it was possible to isolate the free base of VII, 3-[(4-amino-2-trifluoromethyl-5-pyrimidinyl)methyl]-5-(2-hydroxylethyl)-4-methylthiazolium chloride.

The synthesis of VII, "trifluorothiamin," from compound IV was accomplished *via* the reaction of potassium dithioformate with compound IV below 15° in dilute aqueous ethanol to give 4-amino-2-trifluoromethyl-5-thioformylaminomethylpyrimidine (V) in 86% yield. When the reaction mixture in aqueous ethanol solution was heated, 4-amino-2-trifluoromethyl-5-formylaminomethylpyrimidine (VI) was the product. The reaction of compound V with γ-bromo-γ-acetopropyl acetate (method B) according to the general procedure of Todd and Bergel,[8] gave VII, "trifluorothiamin," in 34% yield. When the method of Huber[3b] for the 2-amino analog was used, considerable decomposition was noted, and difficulty was encountered in the isolation of VII, "trifluorothiamin," because of the presence of the hydrochloride of IV, which was identified by comparison with an authentic sample.[4]

Although the procedure involving method A seems like the better one for the preparation of VII, "trifluorothiamin," from the data presented here, an examination of the previous paper[4] will show that compound IV, the precursor for method B, is easier to prepare than compound I, the precursor for method A.

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It was previously noted that certain 4-amino-5-hydroxymethyl-2-substituted analogs of the thiamin pyrimidine inhibited the growth of *Bacillus subtilis* and that the inhibition was completely reversed by the normal pyrimidine.[9] "Trifluorothiamin" (VII) was tested for possible activity as an antagonist of thiamin or its pyrimidine moiety (2-methyl-4-amino-5-hydroxymethylpyrimidine) or its thiazole moiety, 5-(2-hydroxyethyl)-4-methylthiazole, in bacteria by placing filter paper disks impregnated with 0.01 cc. of $10^{-2}M$ or $10^{-3}M$ solutions upon the surface of solid agar layers of medium seeded with the test organism. Simultaneous tests on *Bacillus subtilis* were carried out with the appropriate minimal synthetic culture medium (incubated at 30°) and with the medium supplemented by the pyrimidine or thiazole moiety of thiamin as well as by thiamin itself. "Trifluorothiamin" (VII) was found to be a more potent thiamin antagonist than either pyrithiamin (neo) or oxythiamin. The thiazole moiety reversed pyrithiamin inhibition and the pyrimidine moiety reversed oxythiamin inhibition. In contrast, inhibition by VII, "trifluorothiamin," was enhanced by the pyrimidine and thiazole moieties and reversed only by thiamin.

When administered in doses of 100 mg./kg. daily to mice on a thiamin-deficient diet, "trifluorothiamin" (VII) induced weight loss, extremity paresis and paralysis, opisthotonic convulsions, and inhibition of transplanted Leukemia L-1210 and Krebs 2 carcinoma. These effects have not been seen when the same doses have been given to mice with Leukemia L-1210 on a regular diet.

Detailed results of metabolic, pharmacologic, and tumor inhibition studies with these compounds will be published elsewhere.

EXPERIMENTAL[10]

4-Amino-2-trifluoromethyl-5-hydroxymethylpyrimidine (I). This compound was prepared[11] according to the method previously described.[4]

4-Amino-5-chloromethyl-2-trifluoromethylpyrimidine (II). Four g. (0.021 mol.) of I and 40 ml. of thionyl chloride in 160 ml. of dry chloroform were refluxed for 7 hr. The liquid was evaporated and the resulting solid was recrystallized from acetone-benzene to give 3.85 g. (88%) of crude II, m.p. 185–189°. The analytical sample, m.p. 191–192°, was obtained by recrystallizing from acetone–benzene.

Anal. Calcd. for $C_6H_5ClF_3N_3$: C, 34.05; H, 2.38. Found: C, 34.26; H, 2.35.

4-Amino-5-ethoxymethyl-2-trifluoromethylpyrimidine (III). One g. (0.0052 mol.) of I and 60 ml. of thionyl chloride in 40 ml. of chloroform were refluxed for 10 hr. After removal of the liquid phase, the solid was refluxed with 25 ml. of absolute ethanol for 2 hr. Most of the ethanol was evaporated and the residue was crystallized from ethanol-water

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(10) Microanalyses by Galbraith Laboratories, Knoxville, Tenn. Melting points are uncorrected.

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to give 0.71 g. (62%) of crude III, m.p. 123–125°. The analytical sample, m.p. 126–127°, was obtained by recrystallizing from ethanol-water.

Anal. Calcd. for $C_8H_{10}F_3N_3O$: C, 43.44; H, 4.56. Found: C, 43.32; H, 4.65.

4-Amino-5-aminomethyl-2-trifluoromethylpyrimidine (IV). This starting material was also prepared according to the method previously described.[4]

Potassium dithioformate. The compound was prepared from potassium sulfide and chloroform in ethanol according to the method of Levi.[12]

4-Amino-2-trifluoromethyl-5-thioformylaminomethylpyrimidine (V). A solution of 2.93 g. (0.025 mol.) of potassium dithioformate in 15 ml. of water was added with stirring to 4.42 g. (0.023 mol.) of IV in 27 ml. of ethanol and 20 ml. of water keeping the temperature below 15°. After stirring in the cold for an additional 1 hr., 62 ml. of water was added and the mixture was placed in the refrigerator for 2 hr. The product was filtered to give 4.65 g. (86%) of crude V, m.p. 178–180°. The analytical sample, m.p. 184–185°, was obtained by recrystallizing from ethanol-benzene.

Anal. Calcd. for $C_7H_7F_3N_4S$: C, 35.59; H, 2.99. Found: C, 35.31; H, 3.08.

4-Amino-2-trifluoromethyl-5-formylaminomethylpyrimidine (VI). A solution of 5.18 g. (0.044 mol.) of potassium dithioformate in 15 ml. of water was added, with stirring, to 7.68 g. (0.040 mol.) of crude IV in 50 ml. of ethanol and 25 ml. of water at room temperature. After the addition, the stirring was continued for 2 hr. and then 50 ml. of solvent was evaporated at atmospheric pressure. After cooling the crystals were filtered. The product was dissolved in hot 50% acetic acid, decolorized with charcoal, neutralized with concentrated ammonium hydroxide while hot, cooled, and filtered. This treatment was repeated to give 6.4 g. (73%) of VI, m.p. 204–205°. The analytical sample, m.p. 204.5–205.5°, was obtained by recrystallizing from water. A small-scale experiment indicated that the solvolysis of the thioformyl to the formyl group, to a great extent, had taken place prior to recrystallization from 50% acetic acid.

Anal. Calcd. for $C_7H_7F_3N_4O$: C, 38.17; H, 3.20. Found: C, 38.12; H, 3.27.

γ-Bromo-γ-acetopropyl acetate.[13] 5-Hydroxy-2-pentanone[14] was acetylated with acetic anhydride and then brominated according to the method of Huber.[3b] It was noted that the product could be kept for at least 1 week without decomposition (coloration) if left in ether solution (over calcium chloride) in the refrigerator.

3-[(4-Amino-2-trifluoromethyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium chloride hydrochloride (VII). *Method A.* One g. (0.0047 mol.) of II and 1.0 g. (0.0070 mol.) of 5-(2-hydroxyethyl)-4-methylthiazole[15] were heated to a temperature of 145–150° inside the reaction flask by using an oil bath. The temperature was maintained for 10–15 min. The melt was allowed to cool and was dissolved in hot ethanol. The addition of ethanol–hydrogen chloride followed by concentration and cooling gave a white precipitate which was heated with ethanol–hydrogen chloride, cooled, filtered, and washed with ethanol–hydrogen chloride. After drying in a vacuum desiccator, there remained 1.07 g. (56%) of VII, m.p. 187–189° (dec.), as its monohydrate. The analytical sample, m.p. 192.5–194.5° (dec.) was obtained by recrystallizing twice from ethanol-hydrogen chloride.

Anal. Calcd. for $C_{12}H_{15}Cl_2F_3N_4OS \cdot H_2O$: C, 35.21; H, 4.19; S, 7.82. Found: C, 35.60; H, 4.13; S, 7.56.

When the fusion product prior to treatment with ethanol–hydrogen chloride was dissolved in hot 1-butanol, cooled, and precipitated with ether, the free base, 3-[(4-amino-2-trifluoromethyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium chloride, m.p. 196–197.5° (dec.) was obtained. However, formation of the hydrochloride led to a better recovery of product from the reaction mixture. 5-(2-Hydroxyethyl)-4-methylthiazole, II and III were very soluble in ethanol–hydrogen chloride whereas VII was not; hence the use of ethanol–hydrogen chloride in isolating the desired product.

Anal. Calcd. for $C_{12}H_{14}ClF_3N_4OS$: C, 40.61; H, 3.98. Found: C, 40.79; H, 4.12.

An attempt to use crude II which had only been washed with ether and dried, but not recrystallized, yielded, from acetone solution, a product which was purified by dissolving in 1-butanol and by precipitating with ether. This solid, m.p. 96–97°, evidently was 5-(2-hydroxyethyl)-4-methylthiazole hydrochloride[16] because it gave no melting point depression when mixed with a sample prepared by adding ether to a solution of 5-(2-hydroxyethyl)-4-methylthiazole in ethanol–hydrogen chloride. It also gave a picrate, m.p. 164–165° (lit.[17] 162–163°).

Method B. A 2.4-g. sample (0.010 mol.) of V was heated with 3.6 g. (0.015 mol.) of γ-bromo-γ-acetopropyl acetate with vigorous stirring. First a solution formed and then, at a temperature of 85–90°, an increase in viscosity was noted. The temperature was maintained with stirring for 15 min. The product was triturated with ether until a finely divided solid was obtained. This was dissolved in ethanol and precipitated with ethanol–hydrogen chloride. The mixture was cooled and filtered to give two crops, 1.88 g., of a solid which was recrystallized by dissolving in ethanol and adding ethanol–hydrogen chloride. The product, which was filtered after cooling, yielded 1.17 g. of crude VII, m.p. 186–188.5°, and gave a negative test for bromide ion (chlorine water and chloroform). An additional 0.23 g. was obtained by concentration of the mother liquor and recrystallization of the material which precipitated for a total yield of 34%. A sample which was recrystallized twice from ethanol–hydrogen chloride was identical with the analytical sample from Method A as evidenced by the melting points and mixed melting point. Both samples of VII gave the same picrate, m.p. 186–188°, from dilute aqueous ethanol.

Crude VII was also obtained by the method of Huber[3b] for the 2-amino analog of thiamin, but yields were lower due to decomposition and often extensive contamination with the hydrochloride of IV, most of which was separated by fractional crystallization. This was identified by comparison with an authentic sample.[4] Each specimen had the same melting point, there was no depression on mixing, and each gave the same picrate, m.p. 211–213°, from water solution.

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