of boiling methanol and the combined methanol extracts were cooled and filtered to recover the crude amide, which was recrystallized twice from methanol; 4.2 g. (28%) m.p. 234–36°.

Anal. Calcd.: C, 55.25; H, 5.30; N, 18.41. Found: C, 55.45; H, 5.07; N, 18.05.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF BUFFALO]

2-Trifluoromethylpyrimidines[1]

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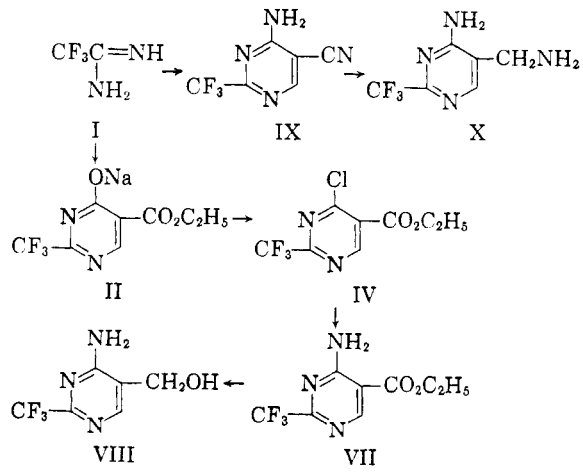
A series of 2-trifluoromethylpyrimidines has been synthesized from trifluoroacetamidine. Included was 4-amino-2-trifluoromethyl-5-hydroxymethylpyrimidine which showed biological activity.

The discovery of 5-hydroxymethylcytosine in the deoxyribonucleic acid of the T-even bacteriophages of *Escherichia coli*[3a,b] is largely responsible for recent interest in related pyrimidines. The subsequent synthesis of this[4a,b] and other 5-hydroxymethylpyrimidines has led to compounds with interesting biological activity. Ulbricht and Price,[5] for example, have synthesized 4-amino-5-hydroxymethyl-2-methylthiopyrimidine (Methioprim) which has received some attention.[6]

It has been noted[7] that amethopterin-resistant mutants of *Bacillus subtilis* are collaterally sensitive to certain 2-substituted-4-amino-5-(substituted methyl)pyrimidines. The reversal of this inhibition by 4-amino-5-hydroxymethyl-2-methylpyrimidine suggested that these compounds are thiamine pyrimidine antagonists in this organism. Furthermore, since amethopterin is used clinically in cancer chemotherapy it was of interest to investigate further those pyrimidines related to the thiamin pyrimidine.

It is well known that there is a large difference in the electronic effect of the trifluoromethyl group and the methyl group but not a large difference in size. Since this type of substitution can lead to different biological activity, some 2-trifluoromethyl analogs of the thiamin pyrimidine were synthesized. Among the compounds prepared were 4-amino-2-trifluoromethyl-5-hydroxymethylpyrimidine (VIII) and 4-amino-5-aminomethyl-2-trifluoromethylpyrimidine (X). Because the reaction of acetamidine with (1) diethyl ethoxymethylenemalonate,[8] (2) ethoxymethylenemalononitrile,[9] and (3) ethyl ethoxymethylenecyanoacetate[8,10] had led to pyrimidines, these three routes were investigated with trifluoroacetamidine (I)[11,12] as starting material.

Diethyl ethoxymethylenemalonate was condensed with I in the presence of sodium ethoxide to form 5-carbethoxy-2-trifluoromethyl-4-hydroxypyrimidine (III). This was converted to 5-carbethoxy-4-chloro-2-trifluoromethylpyrimidine (IV). Low yields of IV were obtained from III but its sodium salt (II), obtained directly from the condensation, gave IV in 56% yield when treated with phosphorus oxychloride. This procedure is similar



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to one found satisfactory for the preparation of 5-carbethoxy-4-chloro-2-methylthiopyrimidine.[13]

The reactivity of the 4-chloro atom in IV seemed comparable to that of the chloro in 2-alkylthio-5-carbethoxy-4-chloropyrimidines[13] in the reactions studied. The treatment of IV with ammonia and with *o*-chloroaniline gave the corresponding 4-amino (VII) and 4-(*o*-chloroanilino)pyrimidine (V) in good yield. Sodium phenoxide reacted with IV to give the expected 4-phenoxypyrimidine (VI). The reduction of VII with lithium aluminum hydride to give VIII was easily accomplished.

Ethoxymethylenemalonitrile and I gave 4-amino-5-cyano-2-trifluoromethylpyrimidine (IX), which was readily reduced with hydrogen and Raney nickel using a procedure analogous to that of Huber[14] for 2,4-diamino-5-aminomethylpyrimidine. Both X and its hydrochloride were prepared. The treatment of IX with 10% sodium hydroxide followed by acidification resulted in the preparation of the corresponding acid (XI). This compound was identical with the acid obtained by the saponification of VII. An amide(XII) was isolated when IX was heated with 0.1*N* sodium hydroxide in a manner similar to that used for 4-amino-5-cyano-2-methylthiopyrimidine.[15]

Although ethyl ethoxymethylenecyanoacetate has been condensed with amidines, ureas, and thioureas to form both 4-amino-5-carbethoxypyrimidines and 5-cyano-4-pyrimidones, depending upon which groups enter into the reaction, it apparently did not condense with I. In several experiments employing a variety of conditions the only substance identified was 1,3-dicarbethoxy-1,3-dicyano-1-propene (XIII).[16]

Although the synthetic intermediates were inactive, VIII was a potent inhibitor of *Bacillus subtilis*.[17] This inhibition, however, was distinctly different from that produced by certain other analogs of the thiamin pyrimidine; for example, inhibition by the other analogs was completely reversed by the metabolite, 4-amino-5-hydroxymethyl-2-methylpyrimidine,[7] whereas it caused only partial reversal of the inhibition by VIII. Further biological testing of the compounds prepared is in progress. The results will be reported elsewhere.

EXPERIMENTAL

Trifluoroacetamidine (I). This compound was prepared by the method of Husted[11] as modified by Reilly and Brown[12] in comparable yields. The compound gave off an ammonia odor and became viscous on standing.[11] The best yields of II and IX were obtained by using freshly prepared material.

5-Carbethoxy-2-trifluoromethyl-4-hydroxypyrimidine (III). Four tenths of a mole of sodium ethoxide was prepared by adding sodium to 400 ml. of absolute ethanol. Then, 44.8 g. (0.40 mole) of freshly prepared I dissolved in 40 ml. of absolute ethanol was added in one portion followed by 86.4 g. (0.40 mole) of diethyl ethoxymethylenemalonate during 15 min. with stirring. After dissipation of the heat of reaction, the mixture was refluxed for 3 hr. and allowed to stand overnight. The alcohol was evaporated at reduced pressure and 120 ml. of water was added. The voluminous solid which formed was filtered and washed with water and then with ether. The solid was recrystallized from acetone to give 75 g. (73%) of the crude sodium salt of III (II), m.p. 315–317°, which after recrystallization melted at 325–326°.

Five grams of crude II was dissolved in 100 ml. of water with heating, filtered, and made strongly acid by the addition of 7 ml. of 10% hydrochloric acid. A white solid formed. The mixture was extracted twice with 50 ml. of chloroform and the chloroform was allowed to evaporate. The residue was recrystallized from ligroine yielding 3.1 g. (68% from II) of III, m.p. 81–82°, which was again recrystallized from ligroine to obtain an analytical sample.

Anal. Calcd. for $C_5H_7F_3N_2O_3$: C, 40.69; H, 2.99; N, 11.86. Found:[18] C, 40.81; H, 3.42; N, 11.86.

Larger quantities of III were not prepared because a preliminary experiment using 4.4 g. of III indicated that only a 24% overall yield of VII from II could be obtained using a method analogous to that of Todd and Bergel[8] without isolation of IV. This was compared to 53% of VII from II as given in the following experiments.

5-Carbethoxy-4-chloro-2-trifluoromethylpyrimidine (IV). Twenty-seven milliliters of phosphorus oxychloride was added dropwise to 9.0 g. (0.035 mole) of II, which had been dried in a vacuum desiccator. Initially the rate of addition was slow to avoid overheating. The mixture was refluxed for 3 hr. and then the excess phosphorus oxychloride distilled at reduced pressure. Ice was added to the resulting product. The mass was extracted with ether and the ether extract was washed with water followed by potassium carbonate solution until the washings were no longer acid. Evaporation of the ether gave an oil which solidified after treatment with aqueous potassium carbonate. The crystalline mass was filtered, washed with water, and dried to give 5.2 g. (56%) of crude product, m.p. 38–39°, which was completely soluble in chloroform and was used directly for the preparation of VII. The analytical sample was obtained by recrystallizing twice from ethanol-water to give white needles, m.p. 41–42°.

Anal. Calcd. for $C_8H_6ClF_3N_2O_2$: C, 37.75; H, 2.38. Found: C, 37.74; H, 2.44.

5-Carbethoxy-4-(o-chloroanilino)-2-trifluoromethylpyrimidine (V). A solution of 1.02 g. (0.0040 mole) of IV and 1.02 g. (0.0080 mole) of *o*-chloroaniline in 10 ml. of ethanol was allowed to stand overnight. The solid formed was filtered and recrystallized from ethanol-water to give 1.03 g. (74%) of crude 5-carbethoxy-4-(*o*-chloroanilino)-2-trifluoromethylpyrimidine. The analytical sample, m.p. 138–139°, was obtained by recrystallizing twice from ethanol-water.

Anal. Calcd. for $C_{14}H_{11}ClF_3N_3O_2$: C, 48.73; H, 3.20. Found: C, 48.80; H, 3.13.

5-Carbethoxy-2-trifluoromethyl-4-phenoxypyrimidine (VI). A solution of 0.0050 mole of IV in 10 ml. of ethanol was added to a solution of 0.0050 mole of sodium phenoxide (prepared from phenol and sodium ethoxide) in 15 ml. of ethanol. The mixture was stirred for 0.5 hr. at room temperature and 0.5 hr. under reflux. The sodium chloride was filtered and 0.82 g. (52%) crude 5-carbethoxy-2-trifluoromethyl-4-phenoxypyrimidine was obtained by crystallization after the addition of water. The analytical sample, m.p. 65–66°, was obtained by recrystallizing twice from ethanol-water.

Anal. Calcd. for $C_{14}H_{11}F_3N_2O_3$: C, 53.95; H, 3.54. Found: C, 53.78; H, 3.53.

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4-Amino-5-carbethoxy-2-trifluoromethylpyrimidine (VII). Anhydrous ammonia was bubbled into a solution of 10.2 g. (0.040 mole) of crude IV in 60 ml. of absolute ethanol at 5°. After a short time crystal formation occurred. The mixture was kept cold for 1 hr. and then an equal volume of water was added. The product was cooled in an ice bath and the crystals were filtered, washed with water, and dried giving a yield of 8.9 g. (94%) of crude VII, m.p. 148–150°. The analytical sample, m.p. 150–151°, was obtained by recrystallizing twice from ethanol-water. A qualitative experiment indicated that VII can also be prepared from IV using concentrated ammonium hydroxide and 95% ethanol.

Anal. Calcd. for $C_8H_8F_3N_3O_2$: C, 40.85; H, 3.43. Found: C, 40.76; H, 3.62.

4-Amino-2-trifluoromethyl-5-hydroxymethylpyrimidine (VIII). A solution of 7.05 g. (0.030 mole) of VII in 150 ml. anhydrous ether was added dropwise to 1.98 g. (0.051 mole) of lithium aluminum hydride in 300 ml. of ether during 1 hr. The mixture was refluxed for 80 min., cooled in an ice bath and hydrolyzed with water. After filtration, the filter cake was washed with ether. The ether solutions were evaporated (Soxhlet extraction of the filter cake gave negligible results). The residue was recrystallized from ethanol-benzene to give 3.4 g. (59%) of VIII, m.p. 179–180°. The analytical sample, m.p. 180.5–181.5°, was obtained by a second recrystallization.

Anal. Calcd. for $C_6H_6F_3N_3O$: C, 37.32; H, 3.14; F, 29.52. Found: C, 37.22; H, 3.10; F, 29.25.

Ethoxymethylenemalononitrile. This starting material, purchased from Kay-Fries Chemicals, Inc., was distilled at reduced pressure and recrystallized from absolute ethanol.

4-Amino-5-cyano-2-trifluoromethylpyrimidine (IX). A solution of 44.8 g. (0.40 mole) of freshly prepared I in 100 ml. of absolute ethanol was cooled in an ice bath and then 48.8 g. (0.40 mole) of ethoxymethylenemalononitrile in 300 ml. of absolute ethanol was added dropwise during 45 min. with stirring. Shortly after initiation of the addition, a white solid began to appear. Stirring was continued for 1 hr. after the addition was complete. The product was filtered and washed with cold ethanol. The filtrate was concentrated to 75 ml. at reduced pressure. The resulting solid was also filtered and washed. The total yield, m.p. 244–246°, was 54.2 g. (72%). The analytical sample, m.p. 245–246° (with sublimation), was obtained by recrystallizing twice from methanol.

Anal. Calcd. for $C_6H_3F_3N_4$: C, 38.31; H, 1.61. Found: C, 38.23; H, 1.73.

4-Amino-5-aminomethyl-2-trifluoromethylpyrimidine (X). Eight grams of Raney nickel[19] was added to a suspension of 9.7 g. (0.052 mole) of IX in 130 ml. of methanol containing 13 g. of dry ammonia. Hydrogenation[20] at about 60 pounds pressure resulted in the required amount of hydrogen being absorbed in about 1 hr. Shaking was continued for an additional hour. The Raney nickel was filtered and the filtrate was evaporated to dryness at reduced pressure. The resulting solid was treated with hot benzene and filtered to remove insoluble matter. The filtrate yielded 7.3 g. (74%) of crude X, m.p. 146–148°, which was orange colored. Several decolorizations with activated charcoal in benzene followed by recrystallization gave a white solid, m.p. 147–148.5°.

Anal. Calcd. for $C_6H_7F_3N_4$: C, 37.52; H, 3.67. Found: C, 37.60; H, 3.79.

The procedure for X was repeated except that after evaporation of the methanol, the mixture was acidified with concentrated hydrochloric acid and evaporated to dryness. Recrystallization from absolute ethanol yielded 8.1 g. (69%) of the hydrochloride of X. The analytical sample, m.p. 279–280°, was obtained by recrystallization from absolute ethanol.

Anal. Calcd. for $C_6H_8ClF_3N_4$: N, 24.51. Found: N, 24.24.

Treatment of the crude hydrochloride with *N* sodium hydroxide gave, after purification, a colorless sample with the same melting point as X. A mixed melting point with X showed no depression.

4-Amino-2-trifluoromethyl-5-pyrimidinecarboxylic acid (XI). One gram of IX was hydrolyzed with 25 ml. of 10% sodium hydroxide. Acidification with hydrochloric acid, followed by filtration of the precipitate and ether extraction of the filtrate, yielded 0.85 g. (77%) of the corresponding acid (crude). The analytical sample, m.p. 312–314°, (dec.), was obtained by recrystallizing twice from ethanol-water. A compound obtained from VII by saponification and acidification gave the same melting point (dec.). A mixed melting point gave no depression and the two samples had similar ultraviolet absorption curves.

Anal. Calcd. for $C_6H_4F_3N_3O_2$: C, 34.77; H, 1.99. Found: C, 34.95; H, 2.11.

4-Amino-2-trifluoromethyl-5-pyrimidinecarboxamide (XII). One gram of IX was heated with 10 ml. of 0.1*N* sodium hydroxide for 15 min., cooled, and filtered to give 0.80 g. (73%) of the corresponding amide (crude). The analytical sample, m.p. 292–293° (with sublimation), was obtained by recrystallizing twice from ethanol-water. Acidification of the alkaline filtrate from the preparation of XII gave 0.1 g. of a compound which was probably XI.

Anal. Calcd. for $C_6H_5F_3N_4O$: C, 34.96; H, 2.45; N, 27.17. Found: C, 34.69; H, 2.40; N, 27.34.

1,3-dicarbethoxy-1,3-dicyano-1-propene (XIII).[21] One-tenth mole each of I, ethyl ethoxymethylenecyanoacetate, and sodium ethoxide, dissolved in absolute ethanol, were refluxed for 2 hr. The alcohol was removed at reduced pressure and the residue was dissolved in hot water. On cooling, 5.2 g. of yellow needles formed. Recrystallization from water gave the sodium salt (dihydrate) of XIII, m.p. 270–272° (Lit. 265°)[22] which on acidification gave XIII (one-half hydrate), m.p. 188–189° (Lit. 187–188°).[16]

Anal. Calcd. for $C_{11}H_{12}N_2O_4 \cdot {}^1/_2H_2O$: C, 54.05; H, 5.36. Found: C, 53.98; H, 5.47.

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BUFFALO 14, N. Y.

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