TABLE V

THE EFFECT OF COMBINATIONS OF SELENOGUANINE AND AZASERINE ON THE SURVIVAL TIME OF MICE BEARING EHRLICH ASCITES CELLS

Treatment	Daily dosage, mg./kg.[a]	Av. survival, days	Av. Δ body wt., g.[b]
Control	...	10.0	+2.7
Selenoguanine	3.9	12.0	+4.4
	6.5	10.6	+4.2
	13.0	11.0	−3.3
Azaserine	0.2	19.8	+4.2
Azaserine[b] + SeG	0.2 + 3.9	23.8	−0.6
	0.2 + 6.5	23.4	−1.3

[a] Therapy was initiated 24 hr. after implantation of tumor cells, with the indicated daily dosage given for 6 consecutive days. Ten mice were used in each experimental group. [b] Average weight change from onset to termination of therapy.

Anal. Calcd. for $C_5H_6N_2Se_2$: C, 23.82; H, 2.30; N, 11.11. Found: C, 24.10; H, 2.58; N, 11.06.

4-Amino-5-methyl-2-selenopyrimidine (5-Methylselenocytosine).—A solution of 3.0 g. (0.012 mole) of 2,4-diseleno-5-methylpyrimidine in 30 ml. of concd. ammonium hydroxide was heated in a pressure bottle over a steam bath for 17 hr. The product was isolated in a fashion analogous to the preparation of selenocytosine. A yield of 1.2 g. (53.2%) of white needles melting at 227–229° dec. was obtained. Ultraviolet Spectrum: pH 1, λ_{max} 308 mμ; ϵ_{max}. 15,000.

Anal. Calcd. for $C_5H_7N_3Se$: C, 31.92; H, 3.75; N, 22.34. Found: C, 32.04; H, 4.00; N, 22.34.

Antitumor Testing.—These transplantable tumors were used in the studies: Sarcoma 180 and Ehrlich carcinoma in Ha/ICR Swiss mice, 6C3HED lymphosarcoma in C3H mice (A. R. Schmidt Co., Madison, Wis.), L-1210 lymphoma and L-5178Y lymphoblastoma in BDF_1 mice (Cumberland View Farms, Clinton, Tenn.). Tumor transplantations were carried out by withdrawing ascites fluid from donor mice bearing 7-day tumor growths. The fluid was centrifuged for 2 min. (at 1,600 *g*), the supernatant fluid decanted and the cells diluted ten-fold with an isotonic solution of sodium chloride. Into each mouse 0.1 ml. of this suspension was inoculated, either subcutaneously or intraperitoneally. Mice were maintained on a diet of Rockland rat chow pellets; water was available *ad libitum*.

Thioguanine II and selenocytosine were dissolved in 1.6% sodium carbonate; the solutions were adjusted to pH 7 with hydrochloric acid. Fresh solutions of II and 2-selenocytosine were prepared daily. Therapy was initiated 24 hr. after tumor implantation and was continued daily for 6 consecutive days; tumor-bearing animals receiving injections of isotonic saline served as controls. Animals were weighed daily, with weight-changes being used as an indication of drug toxicity. Both prolongation of life and reduction of tumor weight were studied.

Trifluoromethyl Compounds Related to Nucleic Acid Bases[1,2]

JOHN A. BARONE

Department of Chemistry, Fairfield University, Fairfield, Connecticut

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In a continuing program designed to prepare potential antimetabolites, a number of trifluoromethylpyrimidines were synthesized by conventional means and used to obtain trifluoromethyl analogs of a *v*-triazolo[d]-pyrimidine, a pyrazolo[3,4-d]pyrimidine, and a pyrimido[4,5-d]pyrimidine. The rearrangement of a 4-(N-nitroamino)-2-trifluoromethylpyrimidine to a 4-amino-5-nitro-2-trifluoromethylpyrimidine and ring closure of 4-amino-2-trifluoromethyl-5-pyrimidinecarboxamide to a purine under conditions for a Hofmann reaction are reported. Some of the compounds prepared were evaluated and found to be inactive as tumor inhibitors.

In previous work,[3a,b,c] certain 2-trifluoromethylpyrimidines related to the thiamine pyrimidine were prepared and found to inhibit the growth of *Bacillus subtilis* and transplanted Leukemia L-1210. This activity was enhanced when the pyrimidine was incorporated into a thiamine analog, and it has since been found[4] that 40 mg./kg. daily doses of "trifluorothiamine" to mice on a thiamine-deficient diet gave up to 64% inhibition of transplanted Leukemia L-1210 without toxicity. The biological activity with *B. subtilis* and Leukemia L-1210 was reversed by thiamine and was decreased substantially[4,5] when pentafluoroethyl and heptafluoro-*n*-propyl groups were introduced in place of the trifluoromethyl group.

In view of these results, it seemed desirable to continue a program aimed at substituting the trifluoromethyl group for groups of similar size in natural products. It was hoped (but not subsequently realized) that the synthesis of 2-trifluoromethylpyrimidines and other trifluoromethyl compounds related to the nucleic acid bases would yield agents that would be active as tumor inhibitors. Moreover, the possible electronic effect of the trifluoromethyl group on other groups and positions could be further observed.[3c,6]

The fact that trifluoroacetamidine (I) would undergo conventional pyrimidine syntheses already has been described.[3a,7] Related cyclization reactions were carried out in the preparation of four new 2-trifluoromethylpyrimidines from I and the appropriate reagents. These pyrimidines were 4-hydroxy-6-dimethoxymethyl - 2 - trifluoromethylpyrimidine (II), 4,6-diamino - 5 - phenylazo - 2 - trifluoromethylpyrimidine (IV), 4 - amino - 2,6 - bis(trifluoromethyl) - 5 - pyrimidinecarbonitrile (VI), and 4-amino-6-hydroxy-5-nitroso-2-trifluoromethylpyrimidine (IX).

The hydrolysis of II and oxidation with chromic acid[8]

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(2) Presented in part before the Division of Medicinal Chemistry, 140th Meeting of the American Chemical Society, Chicago, Ill., September 1961, Abstracts of Papers, p. 25-O.

(3) (a) J. A. Barone, E. Peters, and H. Tieckelmann, *J. Org. Chem.*, **24**, 198 (1959); (b) J. A. Barone, H. Tieckelmann, R. Guthrie, and J. F. Holland, *J. Org. Chem.*, **25**, 211 (1960); (c) J. A. Barone and H. Tieckelmann, *J. Org. Chem.*, **26**, 598 (1961).

(4) J. F. Holland, Roswell Park Memorial Institute, Buffalo, N. Y., unpublished results. For the methods used, see J. F. Holland, R. Guthrie, P. Sheehe, and H. Tieckelmann, *Cancer Research*, **18**, 776 (1958).

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(8) C. Heidelberger and R. B. Hurlbert, *J. Am. Chem. Soc.*, **72**, 4704 (1950).

gave 4 - hydroxy - 2 - trifluoromethyl - 6 - pyrimidinecarboxylic acid (III). The reduction of IV gave 4,5,6-triamino - 2 - trifluoromethylpyrimidine[2,7] which was used to prepare 5-amino-7-trifluoromethyl-*v*-triazolo[d]pyrimidine (V) by the addition of sodium nitrite in aqueous acetic acid.[9] Nitration of 4-amino-6-hydroxy-2-trifluoromethylpyrimidine[2,7] under mild conditions led to attack on the 4-amino group with the formation of 6 - hydroxy - 4 - (N - nitroamino) - 2 - trifluoromethylpyrimidine (VII). Under more vigorous conditions, oxidation and 5-nitration to crude 4,6-dihydroxy-5-nitro-2-trifluoromethyl-pyrimidine[2,7] was observed in varying yields. Compound VII was a valuable intermediate because, when dissolved in concentrated sulfuric acid, it rearranged to 4-amino-6-hydroxy-5-nitro-2-trifluoromethylpyrimidine (VIII). This type of intramolecular rearrangement has been reported for N-nitroaniline and derivatives,[10] but apparently no such rearrangement has been observed previously for pyrimidines. However, a pyrimidine Claisen rearrangement has been reported recently.[11] Reduction of VIII with sodium hydrosulfite gave 4,5-diamino-6-hydroxy - 2 - trifluoromethylpyrimidine (X), which was also prepared by the hydrosulfite reduction of crude IX. This was obtained from I, ethyl hydroxyiminocyanoacetate,[12] and sodium ethoxide.

In an examination of the effect of the 2-trifluoromethyl group on other positions of the pyrimidine ring, nitrosation of 4,6-dihydroxy-2-trifluoromethylpyrimidine[2,7] with nitrous acid was found to proceed readily yielding 4,6 - dihydroxy - 5 - nitroso - 2 - trifluoromethylpyrimidine (XI). By comparison, attempts to nitrosate the isomeric 2,4-dihydroxy-6-trifluoromethylpyrimidine[6] by Giner-Sorolla and Bendich[6] and in this laboratory were unsuccessful, although its nitration has been reported.[13] The nitrosation of 4,6-dihydroxy-2-trifluoromethylpyrimidine, as well as the preparation of VIII from VII, indicates that the electron-withdrawing effect of the 2-trifluoromethyl group on the 5-position of a pyrimidine can be sufficiently counteracted by the presence of *o,p*- orientating groups in the 4- and 6-positions so that routine aromatic substitution can occur. It was also shown that 4-chloro-2-trifluoromethylpyrimidine[2,7] was sufficiently active to react with aniline yielding 4-anilino-2-trifluoromethylpyrimidine (XII).

8-Hydroxy-2-trifluoromethylpurine (XIII) was obtained in good yield from 4-amino-2-trifluoromethyl-5-pyrimidinecarboxamide[3a] by treatment with potassium hypobromite and potassium hydroxide. The only other compound isolated was 4-amino-2-trifluoromethyl-5-pyrimidinecarboxylic acid,[3a] and the amount could be minimized. In contrast, Taylor[14] stated that 6-aminopyrazolono[3,4-d]pyrimidine, as well as a purine, were obtained using 2,4-diamino-5-pyrimidinecarboxamide under similar conditions. That the main product in this case was XIII and not its isomer, 6-trifluoromethylpyrazolono[3,4-d]pyrimidine (XV), was indicated by the synthesis of XV from ethyl 4-chloro-2-trifluoromethyl-5-pyrimidinecarboxylate[3a] *via* ethyl 4-hydrazino-2-trifluoromethyl-5-pyrimidinecarboxylate (XIV). Chlorination of XIII was accomplished with phosphorus oxychloride and N,N-diethylaniline but in low yield.

4 - Amino - 2 - trifluoromethyl - 5 - pyrimidinecarboxamide[3a] gave 4 - hydroxy - 7 - trifluoromethylpyrimido[4,5-d]pyrimidine by an adaptation of the method of Dymicky and Caldwell[15] for the 7-methylmercapto analog.

Ultraviolet spectrophotometric studies, now in progress, show that the 2-trifluoromethyl group strongly increases the acidity and decreases the basicity of the 4-hydroxy and 4-aminopyrimidines, respectively, as expected. For example, the pK_a of 4-amino-2-trifluoromethylpyrimidine[2,7] was 1.39 as compared to 5.71 for 4-aminopyrimidine.[16] However, unexpected results with other compounds have made it desirable to plan on reporting spectrophotometric data after further investigation. Also by way of preliminary observation, the infrared spectrogram[17] for 4-hydroxy-2-trifluoromethylpyrimidine[2,7] shows very strong hydrogen bonding in a very broad band in the region 2400–3000 cm.$^{-1}$. Moreover, there is no strong absorption in the spectrogram above 1600 cm.$^{-1}$ which could be attributed to a carbonyl stretch. This necessitates further spectrophotometric study to see whether the presence of a 2-trifluoromethyl group can cause a "4-hydroxypyrimidine" to exist in the pyrimidinol (lactim) structure in contrast to the pyrimidone (lactam) structure expected from observations[18] with other "4-hydroxypyrimidines." The data of Bergmann, Cohen, and Shahak[19] indicate that 4-hydroxy-2-methyl-6-trifluoromethylpyrimidine also exists as a pyrimidone.

It is important to note that, even though the electron density of the 4-amino group of a pyrimidine was reduced by a 2-trifluoromethyl group, this did not prevent participation of the amino group in the closure of a second ring. Proof for this was the synthesis of a triazolopyrimidine (V), a pyrazolopyrimidine (XV), a pyrimidopyrimidine (XVII), and several purines which included XIII as well as 6-amino-2-trifluoromethylpurine and 6-hydroxy-2-trifluoromethylpurine, previously prepared[6] using a different approach.

Compounds III, V, XIII, XIV, XV, and XVII have been evaluated as tumor inhibitors. Using Krebs 2 carcinomas transplanted subcutaneously in Swiss mice, preliminary results[4] obtained with daily dosages of 200 mg./kg. indicated that the tumor-inhibitory properties of the compounds were not significant.

Experimental[20]

4-Hydroxy-6-dimethoxymethyl-2-trifluoromethylpyrimidine (II).—Freshly prepared trifluoroacetamidine (I)[21a,b] (0.20 mole)

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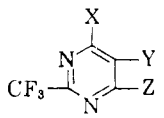
(20) Microanalyses by Galbraith Laboratories, Knoxville, Tenn. Melting points are uncorrected but were determined using a Mel-Temp apparatus with a thermometer calibrated for exposed stem. Analytical results, melting points, and yields are included in Tables I and II.

(21) (a) D. Husted, U. S. Patent 2,676,985, April 27, 1954. (b) W. L. Reilly and Henry C. Brown, *J. Am. Chem. Soc.*, **78**, 6032 (1956).

TABLE I

2-TRIFLUOROMETHYLPYRIMIDINES



No.	X	Y	Z	Yield, %	M.p., °C.[a]	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
II	OH	H	$CH(OCH_3)_2$	36	93–94	$C_8H_9F_3N_2O_3$	40.51	3.82	11.81	40.47	3.90	11.80
III	OH	H	COOH	59	232–234	$C_6H_3F_3N_2O_3$	34.63	1.45	13.46	34.82	1.22	13.37
IV	NH_2	$N{=}NC_6H_5$	NH_2	68	326–329	$C_{11}H_9F_3N_6$	46.79	3.21	—	46.45	3.21	—
VI	NH_2	CN	CF_3	17	176–178	$C_7H_2F_6N_4$	32.83	0.78	21.88	33.03	0.77	21.95
VII	$NHNO_2$	H	OH	82	171–172	$C_5H_3F_3N_4O_3$	26.80	1.35	25.00	27.06	1.41	24.76
VIII	NH_2	NO_2	OH	70	238–240	$C_5H_3F_3N_4O_3$	26.80	1.35	25.00	26.64	1.29	24.84
X	NH_2	NH_2	OH	79[b]	284–285	$C_5H_5F_3N_4O$	30.94	2.64	28.87	30.89	2.81	28.54
XI	OH	NO	OH	83	157–159	$C_5H_2F_3N_3O_3$	28.72	0.96	20.11	28.65	0.98	20.02
XII	NHC_6H_5	H	H	91	126–128	$C_{11}H_8F_3N_3$	55.23	3.37	—	55.09	3.41	—
XIV	$NHNH_2$	$CO_2C_2H_5$	H	69	80–82	$C_8H_9F_3N_4O_2$	38.40	3.63	—	38.57	3.55	—

[a] Values are for analytical samples. Unless otherwise indicated in the experimental, two recrystallizations were carried out on the material listed as product. [b] Yield for method B was 76%.

TABLE II

COMPOUNDS RELATED TO 2-TRIFLUOROMETHYLPURINE

No.	Yield, %	M.p., °C.[a]	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
V	56	314–315	$C_5H_3F_3N_6$	29.42	1.48	41.18	29.33	1.69	40.93
XIII	72	310–311	$C_6H_3F_3N_4O$	35.31	1.48	27.45	35.27	1.32	27.25
XV	74	341–344	$C_6H_3F_3N_4O$	35.31	1.48	27.45	35.35	1.66	27.42
XVI	9	141–143	$C_6H_2ClF_3N_4$	32.37	0.91	25.17	32.10	0.98	24.98
XVII	52	264–266	$C_7H_3F_3N_4O$	38.91	1.40	25.93	38.80	1.71	25.63

[a] See *a* in Table I.

was added in one portion to a solution of 0.20 mole of sodium ethoxide in 200 ml. of absolute ethanol. Then, 0.20 mole of crude methyl γ,γ-dimethoxyacetoacetate, b.p. 90–94° (6 mm.), was added during 30 min. with stirring. The reaction mixture was refluxed for 3 hr., but stirring was continued overnight at room temperature. After the solvent was evaporated at reduced pressure, the residue was dissolved in water. The solution was then acidified with 10% hydrochloric acid. The mixture was extracted with several portions of ether and the ether solution was dried over magnesium sulfate. After the ether was removed by distillation, the residue partially solidified. It was allowed to stand overnight and the crude solid was purified by two crystallizations from ether–ligroin. Decolorization, as well as three more recrystallizations, were carried out to obtain the analytical sample.

4-Hydroxy-2-trifluoromethyl-6-pyrimidinecarboxylic Acid (III).—Compound III was prepared from II by an adaptation of the method of Heidelberger and Hurlbert[8] for orotic acid. The solid, removed by filtration from the oxidation mixture, was recrystallized from acetone–benzene to give the product. III was also obtained by chromic acid oxidation of 4-hydroxy-6-methyl-2-trifluoromethylpyrimidine.[2,7] However, the yield of product has thus far been unsatisfactory.

4,6-Diamino-5-phenylazo-2-trifluoromethylpyrimidine (IV).—Phenylazomalononitrile[22] (0.10 mole) was added in portions to 0.10 mole of I in 100 ml. of 1-butanol, stirred for 30 min., and refluxed for 1 hr. During the reflux period, a red solid appeared. After the mixture was left in the refrigerator overnight, the red crystals were removed by filtration and washed with ether. A portion of these crystals was used as the analytical sample without further purification. Reduction of IV with zinc and glacial acetic acid in ethyl acetate[23] gave 4,5,6-triamino-2-trifluoromethylpyrimidine.[2,7]

5-Amino-7-trifluoromethyl-*v*-triazolo[*d*]pyrimidine (V).—This compound was prepared from 0.024 mole of 4,5,6-triamino-2-trifluoromethylpyrimidine by the adaptation of a general method[9] for triazolopyrimidines. The product from the acetic acid solution was recrystallized from ethanol–water without the type of acid-base treatment described.

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4-Amino-2,6-bis(trifluoromethyl)-5-pyrimidinecarbonitrile (VI) was prepared from I in a manner analogous to that used for the corresponding 2,6-dimethylpyrimidine[22] and recrystallized from acetone-benzene.

6-Hydroxy-4-(N-nitroamino)-2-trifluoromethylpyrimidine (VII).—A mixture of 18 ml. of concd. sulfuric acid and 18 ml. of concd. nitric acid was added to 0.034 mole of 4-amino-6-hydroxy-2-trifluoromethylpyrimidine[2,7] at 0–5°. After stirring for 15 min. at that temperature and 1 hr. at room temperature, the solution was poured onto ice-water. The solid product was collected by filtration and recrystallized from water. Fuming nitric acid was also satisfactory in place of the mixed acid, but the solid which formed at 0° had to be removed by filtration (fritted glass funnel) while the mixture was cold. The fact that nitration of the 4-amino group had taken place rather than ring nitration was deduced from the reduction of VII with sodium hydrosulfite to give 4-amino-6-hydroxy-2-trifluoromethylpyrimidine rather than a diamine. Moreover, the primary amino absorption at 3400 and 3290 $cm.^{-1}$ in the infrared spectrum of the isomer, VIII, was missing in the spectrum of VII. Further evidence for the attack on the amino nitrogen was the observation that, at higher temperatures, oxidation as well as nitration took place to give crude 4,6-dihydroxy-5-nitro-2-trifluoromethylpyrimidine in poor yields. Fractional crystallization from acetone–benzene gave a specimen which was identical with an authentic sample.[2,7]

4-Amino-6-hydroxy-5-nitro-2-trifluoromethylpyrimidine (VIII) was prepared from VII and 96% sulfuric acid by a method analogous to that used for the rearrangement of N-nitroaniline.[10] The main product was removed from the diluted reaction mixture by filtration. The aqueous filtrate was extracted with ether and the ether was evaporated to yield more of VIII. After two recrystallizations from water, the crystals were combined with the main product which had also been recrystallized from water.

4-Amino-6-hydroxy-5-nitroso-2-trifluoromethylpyrimidine (IX) was prepared from I and ethyl hydroxyiminocyanoacetate by an adaptation of the method of Landauer and Rydon[12] for other pyrimidines. However, 2 *M* equivalents of sodium ethoxide had to be used for each mole of free base, indicating the presence of 2 acidic groups in IX. The salt formed was removed by filtration and dried by standing several days on a porous plate. Then, 4 ml. of water was added per g. of salt. The suspension was acidified until the solid turned yellow and the product (32%), m.p. 160–162° dec, was filtered and dried overnight on a porous

plate. Purification could not be effected and the crude product was used directly for the preparation of X. IX was also obtained by the nitrosation of 4-amino-6-hydroxy-2-trifluoromethylpyrimidine[2,7] but in unsatisfactory yields.

4,5-Diamino-6-hydroxy-2-trifluoromethylpyrimidine (X). Method A.—Sodium hydrosulfite (20 g.) was added to a suspension of 0.024 mole of IX in 125 ml. of water at room temperature and allowed to stir for 3 hr. After the mixture was cooled, the solid was removed by filtration and recrystallized from ethanol–benzene to give the anhydrous product.

Method B.—Sodium hydrosulfite (12 g.) was added in portions to 0.0089 mole of VIII in 80 ml. of hot water and boiled for several min. After cooling, the solid was collected by filtration and recrystallized. Samples of analytical purity from methods A and B gave the same melting and mixture melting points.

Evidence for the structure and activity of X was given by its reaction with formamide[24] to give 6-hydroxy-2-trifluoromethylpurine.[6] This was identical with a sample synthesized by the deamination[9] of 6-amino-2-trifluoromethylpurine[6] which had been prepared from 4,5,6-triamino-2-trifluoromethylpyrimidine[2,7] by the use of ethyl orthoformate and N,N-dimethylformamide,[25] ethyl orthoformate and acetic anhydride,[26] and formamide.[24]

4,6-Dihydroxy-5-nitroso-2-trifluoromethylpyrimidine (XI).—A solution of 0.0167 mole of 4,6-dihydroxy-2-trifluoromethylpyrimidine[2,7] and 0.66 g. of sodium hydroxide in 29 ml. of water was cooled to 0–5°. Then 1.26 g. of sodium nitrite was added, followed by 2.2 g. of concd. sulfuric acid in 3 ml. of water. After stirring at 0–5° for 105 min., the mixture was extracted with ether and the ether was evaporated. The solid was crystallized from acetone–benzene. Proof for the nitrosation was given by reduction with sodium hydrosulfite to 5-amino-4,6-dihydroxy-2-trifluoromethylpyrimidine,[2,7] identical with a specimen obtained by a similar reduction of 4,6-dihydroxy-5-nitro-2-trifluoromethylpyrimidine.[2,7]

4-Anilino-2-trifluoromethylpyrimidine (XII).—A solution of 0.0072 mole of crude 4-chloro-2-trifluoromethylpyrimidine[2,7] and 0.022 mole of aniline in 10 ml. of ethanol was refluxed for 1 hr. and left overnight. The product was obtained by filtering and recrystallizing from ethanol–water.

8-Hydroxy-2-trifluoromethyl purine (XIII).—A cold solution of 8 g. of bromine in 100 ml. of 2.5 *N* potassium hydroxide was added to 0.050 mole of 4-amino-2-trifluoromethyl-5-pyrimidinecarboxamide[3a] at 0–5° and then allowed to warm to room temperature. It was heated with a steam bath for 105 min., cooled, and acidified with 10% hydrochloric acid to pH 5–6. After standing, XIII was removed by filtration and dried in a vacuum desiccator. Further acidification of the mother liquor gave a crude solid which, after several recrystallizations, yielded white needles (4%) from ethanol–water. This was identified as 4-amino-2-trifluoromethyl-5-pyrimidinecarboxylic acid[3a] by comparison with an authentic sample.

Ethyl 4-Hydrazino-2-trifluoromethyl-5-pyrimidinecarboxylate (XIV).—A solution of 0.078 mole of hydrazine hydrate in 30 ml. of ethanol was added to 0.026 mole of ethyl 4-chloro-2-trifluoromethyl-5-pyrimidinecarboxylate in 20 ml. of ethanol at 0° and left overnight in the refrigerator. Then 200 ml. of water was added, the mixture was allowed to stand for several hr., and the product was removed by filtration. It was recrystallized from ethanol–water.

6-Trifluoromethylpyrazolono[3,4-d]pyrimidine (XV).—Sufficient 5% sodium hydroxide was added to dissolve 0.0080 mole of XIV. The solution then was acidified with 10% hydrochloric acid to precipitate XV which was removed by filtration after standing overnight. Recrystallization was from ethanol–water.

8-Chloro-2-trifluoromethylpurine (XVI) was prepared according to the method for 6-chloropurine[27] except that N,N-diethylwas substituted for N,N-dimethylaniline. Compound XVI was isolated from the crude reaction mixture by fractional crystallization from benzene.

4-Hydroxy-7-trifluoromethylpyrimido[4,5-d]pyrimidine (XVII).—A mixture of 0.0097 mole of 4-amino-2-trifluoromethyl-5-pyrimidinecarboxamide,[3a] 16 g. of ethyl orthoformate, and 11 g. of acetic anhydride was refluxed for 70 hr.[15] The solution was concentrated to about 6 ml. at reduced pressure but was not evaporated to dryness since then a different product resulted. Water was added and the precipitate was removed by filtration and crystallized from ethanol–water.

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