lized from methanol giving colorless needles or prisms, m.p. 258–260°.

Anal. Calcd. for $C_6H_8N_2O_2S$: C, 41.85; H, 4.68; N, 16.27; S, 18.62. Found: C, 41.84; H, 4.73; N, 16.10; S, 18.70.

5-Ethylthiomethyluracil (IX).—Ethyl iodide (0.70 ml.) was added to a solution of 5-mercaptomethyluracil (VI, 1.20 g., 7.6 mmoles) in 1% sodium hydroxide (50 ml.) and stirred at 5° for 48 hr. Treatment similar to that for compound VIII yielded 1.06 g. (74%) of a crystalline product, m.p. 232–234°. A sample recrystallized from methanol afforded colorless needles, m.p. 244–246°.

Anal. Calcd. for $C_7H_{10}N_2O_2S$: C, 45.15; H, 5.41; N 15.05; S, 17.22. Found: C, 45.17; H, 5.01; N, 15.08; S, 17.36,

Reactions of 5-chloromethyluracil (II) with thio derivatives are listed in Table III. Isolation procedure A consisted of filtering the precipitate, washing with ether, and drying in a desiccator *in vacuo* over P_2O_5. In procedure B, the reaction product was evaporated under reduced pressure at 50–60° and the residue was washed with ethyl acetate, ether, and water and dried as above.

Bis(thyminyl) Sulfone (XVI).—Hydrogen peroxide (30%, 6 ml.) was added slowly to a solution of bis(thyminyl) sulfide (XV, 2.0 g., 7 mmoles) in trifluoroacetic acid (20 ml.) at 25°. A precipitate appeared after addition of the peroxide; the resulting suspension was heated at 60° for 30 min. and kept at 25° overnight. The suspension was filtered and the residue was washed with cold water, ethanol, and ether to yield 1.05 g. (46%) of a colorless microcrystalline product, m.p. 340°.

XVI was sparingly soluble in boiling water and the usual organic solvents. Elemental analysis was performed on material obtained from a similar preparation using analytically pure bis(thyminyl) sulfide (XV).

Anal. Calcd. for $C_{10}H_{10}N_4O_6S$: C, 38.21; H, 3.21; N, 17.83; S, 10.20. Found: C, 38.13; H, 3.48; N, 17.61; S, 10.32.

Bis(thyminyl) Disulfide (XVII). A.—A stream of air was bubbled through a freshly prepared solution of 5-acetylthiomethyluracil (VII, 11.40 g., 0.057 mole) in concentrated aqueous NH_3 (250 ml.) at 25°. After 5 min. a copious precipitate appeared, and when the precipitation was complete (24 hr.), the product was collected, washed with water, and dried to yield 4.2 g. of white crystals, m.p. >320°. A second crop of the same material (3.4 g.) was obtained upon concentration of the filtrate, giving a total yield of 84%.

5-Mercaptomethyluracil (VI) in concentrated aqueous NH_3 gave a 78% yield of XVII in a similar manner to that described above.

Compound XVII was sparingly soluble in water and in the usual organic solvents at their boiling point. An analytical sample was prepared from recrystallized VII and concentrated aqueous NH_3 and oxidized with air for 16 hr. at 25°. Colorless needles were obtained, m.p. >320°.

Anal. Calcd. for $C_{10}H_{10}N_4O_4S_2$: C, 38.21; H, 3.21; N, 17.83; S, 20.40. Found: C, 38.20; H, 3.57; N, 17.61; S, 20.45.

Compound XVII gave a negative nitroprusside test, indicating the absence of a free thiol group, but after treatment with a solution of 1 *N* NaOH at 70° for 10 min., a strong positive thiol test with nitroprusside was obtained.

B.—5-(S-Dithiocarbamyl)methyluracil (XI, 2.17 g., 10 mmoles) was dissolved in concentrated aqueous NH_3 (20 ml.) and kept at 25° for 15 hr. The resulting crystalline precipitate was collected, washed with cold water, and dried to yield 1.50 g. (95%) of a crystalline product, m.p. >300° dec. This product was identified as XVII by means of ultraviolet spectra at different pH values, and R_f values in several solvent systems. It (0.314 g., 1 mmole) was treated with Raney nickel (0.8 g.) by a similar method to that described for VI. A yield of 0.090 g. (71%) of chromatographically pure thymine (XVIII) was obtained.

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Nucleosides. XXIX. 1-β-D-Arabinofuranosyl-5-fluorocytosine and Related Arabino Nucleosides[1]

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Reaction of the 5′-*O*-trityl derivative of uridine or 5-fluorouridine with thiocarbonyldiimidazole yielded crystalline 2,2′-anhydro-1-(β-D-arabinofuranosyl)uracils (III, R = H or F; R′ = trityl) *directly* in high yields. These derivatives (III) were converted to the 1-β-D-arabinofuranosyluracil (V, R = H) and 1-β-D-arabinofuranosyl-5-fluorouracil (FUA) (V, R = F) in high yield. FUA was acetylated, thiated, and then alkylated to the 4-methylmercapto derivative IX which was converted with liquid ammonia to 1-β-D-arabinofuranosyl-5-fluorocytosine (FCA, X). FUA (V), FCA (X), and 1-β-D-arabinofuranosylcytosine (CA) were active against Sarcoma 180 in mice. FCA was highly active against transplanted mouse leukemias P815 and P388, and FCA was more strongly active on a molar basis than CA against a 5-fluorouracil-resistant line of mouse leukemia P815. FCA and CA were effective against the 5-fluorouracil-resistant L1210 mouse leukemia. FCA, CA, and IUDR showed essentially the same activity in preventing the development of herpes keratitis in rabbits.

Pyrimidine nucleosides containing the 1-β-D-arabinofuranosyl moiety have exhibited interesting biological properties. 1-β-D-Arabinofuranosylcytosine (CA)[2] is effective against several experimental tumors,[3] was shown to inhibit several DNA viruses in cell cultures, and is effective against herpes simplex keratitis in the rabbit.[4] 5-Iodo-2′-deoxyuridine[5] (IUDR) also exerts antitumor[6] and antiviral activity,[4b,d] and recently it was shown that 1-β-D-arabinofuranosyl-5-iodouracil is active against herpes and vaccinia virus in cell cultures.[7]

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA 03190-09).

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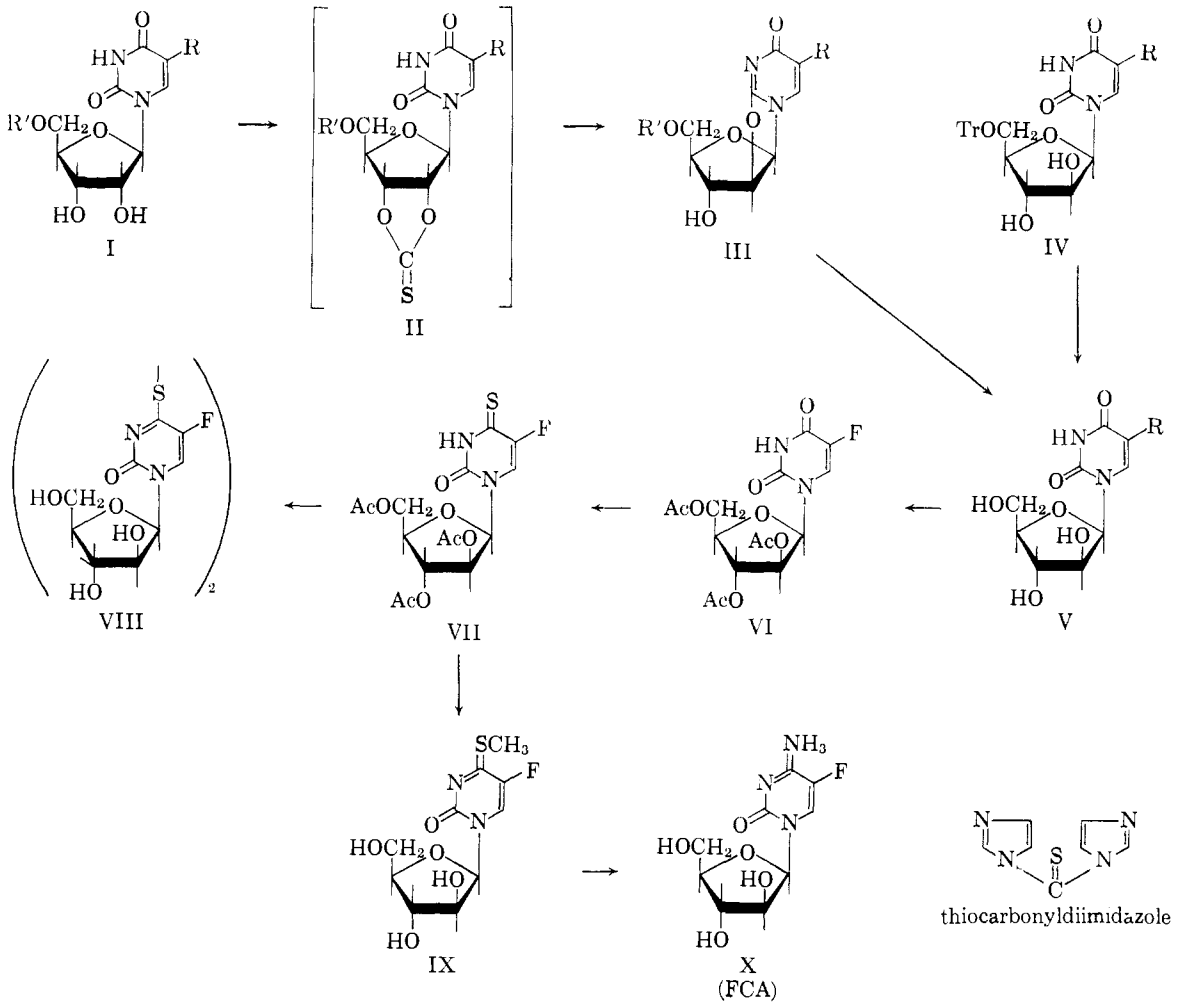
(3) J. S. Evans, E. A. Musser, G. D. Mengel, K. R. Forsblad, and J. H. Hunter, *Proc. Soc. Exptl. Biol. Med.*, **106**, 350 (1961); M. Y. Chu and G. A. Fischer, *Biochem. Pharmacol.*, **11**, 423 (1962); J. S. Evans and G. D. Mengel, *ibid.*, **13**, 989 (1964).

(4) (a) H. E. Renis and H. G. Johnson, *Bacteriol Proc.*, **62**, 140, V45 (1962); (b) G. E. Underwood, *Proc. Soc. Exptl. Biol. Med.*, **111**, 660 (1962); (c) H. E. Kaufman, *ibid.*, **109**, 251 (1962); (d) D. A. Buthala, *ibid.*, **115**, 69 (1864).

(5) W. H. Prusoff, *Biochim. Biophys. Acta*, **32**, 295 (1959).

(6) W. H. Prusoff, W. L. Holmes, and A. D. Welch, *Cancer Res.* **13**, 221 (1953).

SCHEME I



Finally 1-β-D-arabinofuranosyl-5-fluorouracil (FUA)[8] has demonstrated activity against leukemia B82 and Sarcoma 180 (*vide infra*).

These data suggest that 1-β-D-arabinofuranosyl-5-fluorocytosine (FCA), hitherto unknown, should be examined as a potentially interesting chemotherapeutic agent because of its structural similarity to CA and to 5-fluoro-2′-deoxycytidine[9] (FCDR). The synthesis and some biological properties of FCA are the subject of this paper. In addition, a new and direct synthesis of 2,2′-anhydropyrimidine nucleosides (the chemical precursors of arabino nucleosides) is described.

Though several approaches exist for the synthesis of FCA, a route proceeding *via* FUA was chosen, because the latter compound was also needed in large amounts for further biological investigation. Previous studies showed good activity by FUA against certain experimental tumors.[8] The synthesis of FUA from 1-β-D-ribofuranosyl-5-fluorouracil (FUR) has been described.[8] In a recent communication[10] we reported preliminary studies on a direct approach for the conversion of uridines (I, R = H; R′ = H or trityl) to their 2,2′-anhydro derivatives by reaction of I with thiocarbonyldiimidazole which led to a simple synthesis of 1-β-D-arabinofuranosyluracil (V, R = H). This procedure was elegantly applicable to the synthesis of FUA (see Scheme I).

Treatment of 5′-*O*-trityl-5-fluorouridine (I, R = F; R′ = trityl)[8] with 1 equiv. of thiocarbonyldiimidazole[11] in refluxing toluene yielded the crystalline 2,2′-anhydronucleoside (III, R = F; R′ = trityl) directly in 90% yield. As suggested previously,[10] this reaction proceeds *via* the 2′,3′-thionocarbonate (II) or a thiocarbamate derivative. Treatment of III with a slight excess of alkali followed by neutralization with acetic acid at room temperature afforded crystalline IV in 95% yield which upon detritylation yielded FUA (V, R = F) in nearly quantitative yields. Anhydronucleoside III may be converted to V directly without purification of IV. However, since IV may be obtained easily from III, this modification offered no practical advantage.

FUA (V, R = F) was converted to FCA (X) by modifications of the thiation process previously reported for pyrimidine nucleosides.[12] Acetylation of V proceeded quantitatively to the tri-*O*-acetate VI which was then thiated with phosphorus pentasulfide in pyridine to the 4-thione VII in high yield. Crystalline

(7) M. Privat and J. de Rudder, *Compt. rend.*, 259 (1964).

(8) N. C. Yung, J. H. Burchenal, R. Fecher, R. Duschinsky, and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 4060 (1961).

(9) I. Wempen, R. Duschinsky, L. Kaplan, and J. J. Fox, *ibid.*, **83**, 4755 (1961).

(10) J. J. Fox and I. Wempen, *Tetrahedron Letters*, **11**, 643 (1965).

(11) H. A. Staab and G. Walther, *Ann.*, **657**, 98 (1962); W. Reid and B. M. Beck, *ibid.*, **646**, 96 (1961). In our hands, the procedure described by Staab and Walther resulted in a major product containing no sulfur. Therefore, the procedure used is a modification of that described by Reid and Beck.

(12) J. J. Fox, D. van Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich, and G. B. Brown, *J. Am. Chem. Soc.*, **81**, 178 (1959).

VII could not be obtained in analytically pure form. However, it was quite satisfactory for subsequent conversions. Treatment of VII with iodine afforded a crystalline disulfide VIII which was analytically pure.

It was advantageous at this point to methylate the thione VII with methyl iodide and alkali to the methylmercapto derivative IX. Crystalline IX (85%) was obtained which, upon treatment with liquid ammonia[9,13,14] at room temperature, afforded 1-β-D-arabinofuranosyl-5-fluorocytosine (X) in *ca.* 80% yield.

Caution must be exercised in the treatment of FUA (or its 5′-*O*-trityl derivative, IV) and FCA with alkali. Treatment of FUA with 0.1 *N* sodium hydroxide for 1 day at room temperature caused a 30% decrease in the ultraviolet absorption spectrum. After 4 days in alkali, *all selective absorption was lost.* FCA also underwent chemical change with loss of extinction in alkaline solutions. These transformations of FUA and FCA in dilute alkali will be discussed in another paper in this series.

Screening Studies.[15]—FUA (V), CA, and FCA (X) were administered intraperitoneally to Sarcoma 180 tumor-bearing mice twice daily starting with the day following tumor implantation. With FUA, tumor hosts showed no toxic signs after 13 injections of 250 mg./kg./day. The ratio of the average diameter of treated tumors to the average diameter of the control tumors (T/C) was 0.38 indicating retardation of the growth of tumor by FUA. FCA and CA were considerably more toxic to tumor-bearing mice, the maximal tolerated dose of either of these two drugs being only 31 mg./kg./day. At this latter dose level, tumors treated with FCA were retarded to a greater extent (T/C = 0.42) than by CA (T/C = 0.59).[15a]

At doses of 6.25–25/mg./kg. i.p. daily for 10 doses, FCA was highly active in all experiments against transplanted mouse leukemias P815 and P388, producing doubling to quadrupling of survival times. It was somewhat more active on a molar basis than CA, 5-fluorouracil, or 5-fluoro-2′-deoxyuridine. FCA was also strongly active and more active on a molar basis than CA against a line of leukemia P815 made resistant to 5-fluorouracil.[15b] Doses of FCA at 50 and 100 mg./kg., which were nontoxic, were effective in increasing the median survival time by more than 100% of mice bearing the 5-fluorouracil resistant L1210 mouse leukemia.[15c]

Preliminary experiments were carried out to determine the prophylactic effects of a 1-mg./ml. solution of IUDR, CA, and FCA in herpes keratitis in rabbits. All three substances showed essentially similar activity in preventing the development of herpes keratitis. Further experiments will have to be carried out to determine the relative potencies of these substances.[15d]

These data would suggest that (where comparative data are available in these preliminary investigations) the behavior of FCA is more akin to that of CA than to that of the fluorinated pyrimidine nucleoside, FUA. A detailed study of FCA, CA, and FUA is presently underway in this institute to evaluate the relative efficacy of these drugs in several experimental systems.

Experimental Section[16]

***N,N*-Thiocarbonyldiimidazole.**[11]—A solution of predried imidazole (59 g., 0.87 mole) in 700 ml. of dry benzene was heated to *ca.* 60° and was thoroughly flushed with a slow stream of *dry* nitrogen, preferably by use of a gas sparger. The efficiently stirred solution was treated dropwise with thiophosgene (25 g., 0.22 mole) in dry benzene. The temperature was maintained as high as the exothermic reaction permitted. A continuous slow stream of nitrogen was maintained during the addition and during the reflux period which followed. (Omission of the nitrogen resulted in a 50% diminution of yield.) After addition of the thiophosgene, the reaction mixture was refluxed for 5 hr. Precipitation of solid usually started at the onset of the reflux period. The orange-red reaction mixture was finally allowed to stand overnight at room temperature under an atmosphere of nitrogen. The precipitate of imidazole hydrochloride was removed by rapid filtration on a large Büchner funnel preferably under a stream of nitrogen, and the solid was thoroughly washed with dry benzene until any occluded yellow solid was dissolved. The benzene filtrate was evaporated *in vacuo* to a thin syrup which was treated with *ca.* 50 ml. of *dry* tetrahydrofuran (THF) and re-evaporated. This procedure was repeated until the residual benzene was replaced by the THF. During this treatment, the syrup solidified to a bright yellow granular solid. After thorough chilling, the solid was filtered and washed with small portions of cold THF containing increasing amounts of dry ether and finally with dry ether alone. The residual solvent was removed in a continuously evacuated desiccator and the solid, m.p. 100–102°, was stored in a refrigerator under an atmosphere of nitrogen. A second crop was obtained by allowing the THF–ether filtrate (under nitrogen) to stand overnight at 0°. The yield averaged 80–90% and, under the above storage conditions, the product was usable for several weeks. The ultraviolet absorption spectrum of thiocarbonyldiimidazole in THF shows a strong maximum at 293 mμ.

2,2′-Anhydro-1-(5′-*O*-trityl-β-D-arabinofuranosyl)uracil (III, R = H; R′ = trityl).—A suspension of 58 g. (0.12 mole) of dry 5′-*O*-trityluridine[17] in 2500 ml. of anhydrous toluene was heated to *ca.* 80°, and, over a period of 10 min., a solution of 23 g. (0.13 mole) of thiocarbonyldiimidazole in anhydrous toluene was added. After the addition, solution occurred. The reaction was heated slowly to reflux temperature; a solid gradually precipitated. The reaction mixture was allowed to reflux for 1 hr. After thorough chilling of the reaction mixture, the solid was removed by filtration and washed with dry toluene. Recrystallization of the precipitate from methanol (decolorizing carbon) afforded a highly crystalline product, 33.4 g., m.p. 217–219°. From the methanol solution, a further 7.5 g. of material was obtained; m.p. 194–196°. Evaporation of the original toluene filtrate, followed by recrystallization of the residue from methanol, yielded a second crop, 11.3 g., m.p. 194–196°. The lower melting materials were combined and recrystallized again (yield, 15.3 g.), m.p. 206–209°.[10,18,19] The total yield of recrystallized III (R = H; R′ = trityl) was 85%.

2,2′-Anhydro-1-(β-D-arabinofuranosyl)uracil (III, R, R′ = H).

A. From Uridine.—Uridine (1.34 g., 0.0056 mole) was suspended in *ca.* 50 ml. of dry toluene, a solution of 1.0 g. (0.0056 mole) of thiocarbonyldiimidazole in toluene was added, and the

(13) It was noted previously[9] that treatment of 4-thio-FUDR with liquid ammonia required heating at 60° to effect replacement of the 4-thione by amine. Many by-products were obtained. Replacement of the methylmercapto group of IX by amine at room temperature was complete in several hours without the formation of troublesome by-products. The ease of replacement of a 4-methylmercapto by amines in the pyrimidine area had been noted by Mizuno, Ikehara, and Watanabe.[14]

(14) Y. Mizuno, M. Ikehara, and K. A. Watanabe, *Chem. Pharm. Bull.* (Tokyo), **10**, 653 (1962).

(15) The authors are indebted to the following investigators of this Institute for kindly providing their preliminary results: (a) Drs. G. S. Tarnowski and F. A. Schmid for their data on Sarcoma 180; (b) Dr. J. H. Burchenal for the data on mouse leukemias P815 and P388; (c) Dr. D. J. Hutchison for the study on L1210 (5-fluorouracil-resistant) mouse leukemia; and (d) Dr. E. Grundberg of Hoffmann-La Roche, Inc., Nutley, N. J., for his data on herpes keratitis in rabbits.

(16) All melting points were taken on a Thomas–Hoover capillary melting point apparatus and are corrected. Microanalyses were performed by Galbraith Laboratories Inc., Knoxville, Tenn., and by Spang Microanalytical Laboratory, Ann Arbor, Mich.

(17) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **104**, 385 (1934).

(18) The variance of this melting point with that previously reported[19] was discussed in paper XXVI of this series.[10]

(19) J. F. Codington, I. L. Doerr, and J. J. Fox, *J. Org. Chem.*, **29**, 558, 564 (1964).

reaction mixture was refluxed for 40 min. Gradually, solution occurred. After *ca.* 0.5 hr., a film of oil began to deposit on the wall of the flask and the color darkened appreciably. The reaction mixture was chilled, and the toluene was decanted. The brittle residue was dissolved in hot 95% EtOH, treated with decolorizing carbon, and after filtration allowed to cool slowly. The precipitate of fine needles was filtered and washed with cold methanol. The yield of pure product (III, R, R′ = H) was 460 mg. (36%), m.p. 240–244° (effervescent). A second crop, 0.5 g. (4%), m.p. 240–243° (effervescent), was obtained from the mother liquor. A recrystallization of the combined crops from 90% ethanol yielded stout needles, m.p. 244–247°, and showed no depression when admixed with an authentic sample.[18] The identity of the compound as 2,2′-anhydroarabinosyluracil was confirmed by comparison of the ultraviolet absorption properties with those of the known material.

B. From 2,2′-Anhydro-1-(5′-*O*-tritylarabinosyl)uracil.—Compound III (R = H; R′ = trityl) (0.95 g., 0.002 mole) was stirred with a methanolic solution of HCl at 0° for 1 hr. The methanol was removed *in vacuo*, and the resulting solid was dissolved in water and treated with Dowex-1 (acetate) to remove chloride ions. The resin was removed by filtration, and the filtrate was evaporated *in vacuo*. The residue was recrystallized from 90% ethanol. The yield of III (R, R′ = H) was 300 mg. (66%), m.p. 246–249°.

1-β-D-Arabinofuranosyluracil (V, R = H).—An ethereal suspension of 25.2 g. (0.05 mole) of IV (R = H) was treated with *ca.* 50 ml. of ether, previously saturated at 0° with HCl. The mixture was stirred for 2 hr. at 0°. The yellowish suspension was filtered, and the solid was triturated thoroughly with dry ether. The crude product was recrystallized from *ca.* 600 ml. of hot methanol containing 10 ml. of water. The yield of crystalline product was 9.2 g. (75%), m.p. 222–224°; Bergmann and Burke[20] reported 226–228°. A mixture melting point with an authentic specimen prepared by Miss I. L. Doerr of this laboratory by hydrolysis of 2,2′-anhydroarabinosyluracil[21] showed no depression. A further crop, 2.6 g. (21%), of equal purity was obtained from the mother liquor. The combined crops (11.7 g., 96%) were uridine-free and migrated identically with authentic 1-β-D-arabinosyluracil in paper electrophoresis (borate buffer, pH 9.2, 900 v.).

1-(5′-*O*-Trityl-β-D-arabinofuranosyl)uracil (IV, R = H).—This procedure is similar to that previously reported.[19] A solution of 30 g. (0.076 mole) of III (R = H) in 1500 ml. of ethanol–water (1:1) was treated with 112 ml. of 1 *N* NaOH and stirred at room temperature for 2 hr. During this time, the ultraviolet absorption maximum shifted to 260 mμ. The solution was neutralized with 2 *N* acetic acid, the ethanol was removed *in vacuo*, and the resulting precipitate was filtered. After thorough washing with water, the damp solid was dissolved in hot 50% ethanol–water, filtered, and allowed to cool slowly. The precipitated rosettes of crystals were chilled thoroughly, filtered, and dried in a vacuum desiccator overnight, then at 100° for 2 hr. *in vacuo*; yield of pure product, 25.2 g. (68%), m.p. 132–135° effervescent (lit.[19] m.p. 119–120° effervescent). The infrared and ultraviolet absorption spectra were identical with those of an authentic sample.[19]

2,2′-Anhydro-1-(5′-*O*-trityl-β-D-arabinofuranosyl)-5-fluorouracil (III, R = F; R′ = trityl).—Dry 1-(5′-*O*-trityl-β-D-ribofuranosyl)-5-fluorouracil[8] (I, R = F; R′ = trityl) (25 g., 0.05 mole) was suspended in *ca.* 1 l. of dry toluene and warmed to *ca.* 80°. To the well-stirred suspension, a solution of 9.4 g. (0.53 mole) of thiocarbonyldiimidazole in dry toluene was added in one portion. The reaction mixture was then heated to reflux. After *ca.* 10 min., solution occurred and usually, within an additional 10 min., precipitation began accompanied by a bleaching of the yellow-orange color. After a reflux time of 1 hr. the reaction mixture was chilled. The precipitate was removed by filtration and washed thoroughly with dry toluene. The crude product was recrystallized from boiling methanol and the color was removed by the use of decolorizing carbon. After drying, the yield of crystalline product was 19.8 g., m.p. 200–203°. A further amount of product, 2.4 g., m.p. 180–182°,[22] was obtained by evaporation of the original toluene filtrate and crystallization of the resulting syrup from hot methanol with liberal use of decolorizing carbon. The total yield of III (R = F; R′ = trityl) was 91%. An aliquot was recrystallized from ethanol for analysis.

Anal. Calcd. for $C_{28}H_{23}FN_2O_5 \cdot 0.5EtOH$: C, 68.37; H, 5.10; F, 3.73; N, 5.50. Found: C, 68.39; H, 4.98; F, 4.02; N, 5.13.

1-(5′-*O*-Trityl-β-D-arabinofuranosyl)-5-fluorouracil (IV, R = F).—Compound III (R = F; R′ = trityl) (12.0 g., 0.025 mole) in 1 l. of 50% ethanol was treated with 70 ml. of 1 *N* NaOH at room temperature for 1 hr. During this period, the ultraviolet absorption maximum shifted to 268 mμ. The solution was neutralized with 2 *N* acetic acid, and the alcohol was evaporated *in vacuo*. The resulting solid was washed thoroughly with water, and the damp solid was recrystallized from 50% ethanol. Pure IV (R = F) precipitated as micaceous flakes, 12.4 g., m.p. 172–174° (effervescent). An additional 0.5 g. was obtained from the alcohol filtrate; total yield of IV (R = F) 92%.

Anal. Calcd. for $C_{28}H_{25}FN_2O_6$: C, 66.66; H, 4.99; F, 3.77; N, 5.55. Found: C, 64.43; H, 5.10; F, 3.71; N, 5.34.

1-β-D-Arabinofuranosyl-5-fluorouracil (FUA) (V, R = F).—Compound IV (R = F) (12.0 g., 0.024 mole) was detritylated by a procedure analogous to that described above for the preparation of V (R = H). The crude product was recrystallized from a minimum of hot ethanol and allowed to cool slowly. The crystalline precipitate was filtered and washed with dry ether; 5.7 g. (92%), m.p. 187–188°. The product was identical with an authentic sample[5] as demonstrated by a comparison of ultraviolet absorption properties and of their migration in paper electrophoresis (borate buffer, pH 9.2, 900 v.).

1-(2′,3′,5′-Tri-*O*-acetyl-β-D-arabinosyl)-5-fluorouracil (VI).—Acetic anhydride (8.0 ml.) was added to 60 ml. of anhydrous pyridine containing 5.4 g. (0.02 mole) of I. The clear solution was stirred for 16 hr. at room temperature. Ethanol (~10 ml.) was added, and the solution was evaporated *in vacuo* to a thin syrup. Pyridine was removed by repeated evaporation and addition of 50% ethanol after which crystallization of II occurred. The mixture was cooled to complete crystallization and, after filtration, the solid was washed with water and dried; yield 7.78 g. (98%); m.p. 139–140°; recrystallization from 50% EtOH gave an analytically pure sample, m.p. 142–143°; $\lambda_{max}^{50\%\,EtOH}$ 205 and 275 mμ, minimum at 232 mμ.

Anal. Calcd. for $C_{18}H_{17}FN_2O_9 \cdot 0.5H_2O$: C, 45.35; H, 4.57; N, 7.05. Found: C, 45.26; H, 4.44; N, 6.78.

1-(2′,3′,5′-Tri-*O*-acetyl-β-D-arabinosyl)-5-fluoro-4-thiouracil (VII).—Phosphorus pentasulfide (4.4 g., 0.02 mole) was added to a solution of 40 ml. of pyridine (reagent grade) containing 3.9 g. (0.01 mole) of VI. The mixture was refluxed (stirring) for 4 hr. The reaction was monitored as follows. A small sample was removed from the reaction mixture and evaporated to dryness. Repeated addition and evaporation, using 50% ethanol, gave a gummy solid free from pyridine. The ultraviolet spectrum of the gummy residue in 50% ethanol gave two maxima, at 267 mμ for the starting material and at 334 for the thiated product. The ratio of extinctions at 267/334 mμ was ~4, indicating approximately 10% thiation. Phosphorus pentasulfide (4.4 g.) was again added to the stirred reaction mixture and refluxing was continued for 4 hr. The ratio at 267/334 mμ of ~0.7 indicated ~60% thiation. A third addition of 4.4 g. of phosphorus pentasulfide was made and the reaction was refluxed for 4 more hr. Ultraviolet examination of the reaction product gave a ratio, 267/334 mμ, of ~0.2. The pyridine mixture was cooled and decanted from an oily, crystalline deposit. The decantate was evaporated to dryness *in vacuo*. Water (250 ml.) was added to the residue and, on stirring, a yellow granular solid separated. The solid was dissolved in methylene chloride and filtered from some insolubles. The filtrate was evaporated to a syrup, and methanol was added. A small amount of insolubles was filtered. After cooling, crystallization took place. In several runs, yields of crude material ranged from 3.0–3.6 g. Recrystallization of several batches from methanol gave yellow needles of varying melting points ranging from 59–120°. The absorption spectra of all these recrystallized batches were identical (λ_{max} 334 and 244 mμ, ratio 334/224 mμ = 5.6 in 50% ethanol). Even after repeated recrystallization from different solvents (methanol, ethanol, ether, etc.) consistent analyses could not be obtained. It was found, however, that this material (as well as the crude material) was of sufficient purity for subsequent reactions.

1-β-D-Arabinofuranosyl-4-methylmercapto-5-fluoro-2-pyrimidone (IX).—The thione VII (13.6 g.) was dissolved in 250 ml. of methanol, 50 ml. of water, and 9.0 g. of methyl iodide. To the stirred solution, 34.5 ml. of 1 *N* NaOH was added dropwise over a

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(22) Comparison of the infrared spectra of the differently melting products showed complete identity except for a slight aberration in the 833-cm.$^{-1}$ region. When the respective KBr disks were heated at 101° for 15 min., complete spectral identity was obtained.

period of 40 min. The resulting solution (pH 8) was neutralized with acetic acid to ~pH 6 and the methanol was removed by evaporation *in vacuo* to give long light yellow needles. Three crops yielded 8.3 g. (87%) of IX. Recrystallization from hot water gave a pure, colorless material: m.p. 140–141°; $\lambda_{max}^{50\% EtOH}$ 202.5, 277.5, 315 mμ; minima at 237.5 and 292.5 mμ; shoulder at 220 mμ; ratio at 277.5/315 mμ = 0.79. The absence of acetyl groups in IX was verified by the absence of acetoxy resonances in the n.m.r. spectrum[23]; $[\alpha]^{23}D$ +219° (*c* 0.22, methanol).

Anal. Calcd. for $C_{10}H_{13}FN_2O_5S$: C, 41.08; H, 4.48; F, 6.50; N, 9.58; S, 10.97. Found: C, 40.99; H, 4.76; F, 6.82; N, 9.70; S, 10.82.

1-β-D-Arabinofuranosyl-5-fluorocytosine (FCA, X).—Compound IX (5.0 g., 0.017 mole) was left at room temperature in 25 ml. of anhydrous ammonia overnight. Ammonia was removed *in vacuo*. About 50 ml. of water was added and the residual ammonia was neutralized to ~pH 5 with acetic acid. The solution was evaporated to a crystalline residue, and 10 ml. of water was added to give X (3.4 g. in two crops), m.p. 232–233° dec. (effervescent with previous browning at 210°). X was purified on a column of Dowex 50 (H^+) 100–200 mesh, by first washing with water until free of ultraviolet-absorbing material, then eluting with 1 *N* NH_4OH. The ammonia eluates containing ultraviolet-absorbing material were combined and evaporated to a crystalline residue, which was recrystallized in 90% ethanol. Two crops of colorless needles yielded 3.2 g., m.p. 237–238° (to a brown liquid with previous browning at 225°), $[\alpha]^{23}D$ +163 ± 2 (*c* 0.18, in water). FCA was examined in thin layer chromatography using Brinkmann GF 254 silica gel and was found to be free from FUA, 5-fluorocytosine, and all other pyrimidine derivatives listed in Table I in two chromatographic systems.

Ultraviolet absorption properties of FCA are: in 1 *N* HCl, maxima at 221 and 290.5 mμ (ε 10,300 and 11,900), minimum at 246 mμ (ε 1160); at pH 5–7, maxima at 235 and 280 mμ (ε 7860 and 8240), minima at 225 and 257.5 mμ (ε 7550 and 5240); pK_a (spectrophotometrically determined)[24] = 2.33 ± 0.05.

Anal. Calcd. for $C_9H_{12}FN_3O_5$: C, 41.38; H, 4.63; F, 7.27; N, 16.08. Found: C, 41.53; H, 4.62; F, 7.32; N, 16.05.

(23) The authors are indebted to Dr. Floie Vane of Hoffmann-La Roche, Inc., Nutley, N. J., for the n.m.r. spectrum.

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TABLE I

THIN LAYER CHROMATOGRAPHY DATA

Compd.	R_f in solvent system[a] A	B
FCA (X)	0.06	0.11
Cytosine	0	0
5-Fluorocytosine	0.22	0.09
CA	0	0
5-Fluorocytidine	0.03	0
5-Fluorouracil	0.42	0.74
FUA (V)	0.31	0.69
5-Fluorouridine	0.21	0.04

[a] Solvent system A, ethyl acetate–acetic acid (22:3); B, 5% boric acid in ethanol–ethyl acetate (1:3). The authors are indebted to Dr. R. Duschinsky of Hoffmann-La Roche, Inc., for these solvent systems.

1-β-D-Arabinofuranosyl-4-thio-5-fluorouracil Disulfide (VIII).—Crude VII (300 mg.) was allowed to stand overnight at room temperature in 20 ml. of methanol saturated with HCl. The solution was evaporated to dryness and azeotroped repeatedly with ethanol. The syrup did not crystallize. It was treated with 20 ml. of phosphate buffer (pH 6.8). Iodine solution (1 *N*, 1.3 ml.) was added dropwise to pH 4. Immediately a white amorphous material precipitated. The solid was collected and washed with water, ethanol, and ether (yield 120 mg., m.p. 199–203° dec.). Recrystallization from 50% ethanol gave minute crystals: 50 mg.; m.p. 213–214° (to amber liquid with effervescence, with previous browning at 210°); $\lambda_{max}^{50\% EtOH}$ 215, 260, 327.5 mμ; minima at 205, 234, 288 mμ; ratio at 260/327.5 mμ = 0.66.

Anal. Calcd. for $C_{18}H_{20}F_2N_4O_{10}S_2$: C, 39.13; H, 3.65; N, 10.14; S, 11.24. Found: C, 38.93; H, 3.98; N, 9.98; S, 11.32.

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3-Deaza-6-methylthiopurine Ribonucleoside[1]

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The synthesis and biologic evaluation of 4-methylthio-1-β-D-ribofuranosyl-1*H*-imidazo[4,5-*c*]pyridine, the 3-deaza analog of 6-methylthiopurine ribonucleoside, a highly cytotoxic purine nucleoside, is described.

6-Mercaptopurine [purine-6(1*H*)-thione, I] is converted *in vivo* by inosine monophosphate–guanosine monophosphate (IMP–GMP) pyrophosphorylase to its ribonucleotide, thioinosinic acid (III),[2] a potent allosteric inhibitor of phosphoribosyl pyrophosphate (PRPP)–glutamine amidotransferase.[3] Furthermore, there is good evidence that inhibition of this enzyme is responsible for the cytotoxicity of 6-mercaptopurine.[4]

In an effort to learn more about the binding sites of all the enzymes involved in this process of growth inhibition by 6-mercaptopurine, we first synthesized its four deaza analogs.[5] None of these compounds are cytotoxic except at high levels. This lack of biologic activity could be due to the fact that the possible deaza analogs of thioinosinic acid do not bind to the allosteric site of phosphoribosyl pyrophosphate–glutamine amidotransferase, but it is equally likely that the deazapurines simply are not metabolized to ribonucleotides by cells.

Recently we have found that, although 6-mercaptopurine ribonucleoside is not a substrate for cellular purine kinase (presumably adenosine kinase), 6-methylthiopurine ribonucleoside (II) is, and is readily converted *in vivo* to 6-methylthiopurine ribonucleotide (IV) (see Chart I). As a consequence II is highly

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