(VII, 500 mg, 1.77 mmoles) was dissolved in 4 ml of 14% aqueous NH_3. Benzyl chloride (230 mg, 1.8 mmoles) in 2.0 ml of dioxane was added to the basic solution and the mixture was stirred at room temperature for 3 hr. The mixture was diluted with 6 ml of water and then extracted five times with 7.5-ml portions of ethyl acetate. The ethyl acetate solution, after drying ($MgSO_4$), was evaporated *in vacuo* to yield an oil which solidified when triturated with ligroin (bp 90–120°). The crude solid was recrystallized from an acetone–ligroin mixture to yield 500 mg (76%) of analytically pure product, mp 143–144°, $[\alpha]^{22}D$ −66.3° (c 1, ethanol).

Anal. Calcd for $C_{18}H_{19}N_3O_4S$: C, 57.9; H, 5.13; N, 11.3. Found: C, 58.1; H, 5.03; N, 11.1.

4-Methylthio-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (IXa).—To a solution of 0.55 g of $NaOCH_3$ in 30 ml of methanol was added 2.8 g of 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-4-thiol (VII). After VII had dissolved, 0.6 ml of methyl iodide in 5 ml of methanol was added and the solution was stirred at room temperature for 2 hr. The pH was then adjusted to 6 and the solution (some solid present) evaporated to dryness *in vacuo* at room temperature. The resulting yellow oil was triturated with acetone. The white solid was collected by filtration, dried, and recrystallized from methanol to yield 2 g (65%) of analytically pure compound, mp 193–194°.

Anal. Calcd for $C_{12}H_{15}N_3O_4S$: C, 48.4; H, 5.05; N, 14.14. Found: C, 48.53; H, 5.20; N, 14.27.

Nucleosides. V. 2-Thiopyrimidine β-D-Arabinofuranosides

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The reaction of 2,2′-anhydro-1-(5-*o*-trityl-β-D-arabinofuranosyl)uracil (IIa) with H_2S in alkaline medium followed by detritylation produced 1-(β-D-arabinofuranosyl)-2-thiouracil (IX). Thiation of the triacetate of IV yielded the corresponding 2,4-dithiouracil nucleoside VI. Reaction of VI with NH_3 yielded the 2-thiocytosine arabinoside VII, cytosine arabinoside VIII, or the 2,2′-anhydrocytosine nucleoside IX depending on the conditions used. Reaction of IV or VII with bromine water resulted in the formation of 2,2′-anhydronucleosides IIb and IX, respectively. Iodination of IV gave a similar result. 1-(β-D-Arabinofuranosyl)-2-thiocytosine (VII) showed antiviral activity against vaccinia in tissue culture. The other thiopyrimidine nucleosides were inactive as antiviral agents.

Interest in thiopyrimidine nucleosides was heightened recently by the isolation of 4-thiouridylic acid[1] and a 2-thiopyrimidine nucleotide[2,3] from *E. coli* t-RNA as "odd" nucleotides and by the demonstration of an enzymatic thiolation of t-RNA.[4] It has been suggested that[2] the facile formation and cleavage of a disulfide bond in t-RNA may provide a chemical mechanism to the "adapter modification hypothesis,"[5] although no difference in the maximum tyrosine-accepting ability was detectable between the native and disulfide forms of *E. coli* tyrosine t-RNA.[6] More recently, evidence was presented for reversible conformational changes and ribosome binding efficiency upon iodine oxidation of lysyl t-RNA from *B. subtilis*.[7] A model involving sulfhydryl–disulfide interconversion of thiopyrimidines was again postulated. In our study of nucleoside antimetabolites, it was noted that the free base, 2-thiouracil, has been reported to suppress the production of infective turnip yellow mosaic viral nucleoprotein,[8] possibly *via* a preferential inhibition of viral-RNA-dependent RNA synthesis.[9] It was also incorporated into RNA of tobacco leaves and tobacco mosaic virus. The physicochemical difference between 2-thiouracil and uracil was indicated by recent nmr studies which concluded that 2-thiouracil exists essentially in the thiol form.[10] The ultraviolet absorption spectrum of 2-thiouridine suggests that it may act as an analog of cytidine as well as of uridine in RNA synthesis.[9] Following our previous investigations of pyrimidine nucleosides as potential antiviral agents the synthesis of 2-thiopyrimidine β-D-arabinosides was considered of some interest.

In contrast to the 4-thiopyrimidine nucleosides which are readily available from the corresponding 4-oxopyrimidine nucleosides by thiation with P_2S_5,[11,12] the 2-thiopyrimidine nucleosides have been prepared by more circuitous routes.[13] Methods involving the use of glycosyl amines[14,15] and glycosylthioureas[16] as starting points for building the 2-thiouracil ring system have been reported.

The formation of 2-thiouridine by the reaction of H_2S with 2,5′-anhydrouridine under mildly alkaline conditions has been described by Todd[17] and by others.[18,19] The bacterial synthesis of thiouridylic acid has been demonstrated.[20]

Taking advantage of our recent experience with 2,2′-

(1) M. N. Lipsett, *J. Biol. Chem.*, **240**, 3975 (1965).

(2) J. A. Carbon, L. Hung and D. S. Jones, *Proc. Natl. Acad. Sci. U. S.*, **53**, 979 (1965).

(3) T. Schleich and J. Goldstein, *Science*, **150**, 1168 (1965).

(4) R. S. Hayward and S. B. Weiss, *Proc. Natl. Acad. Sci., U. S.*, **55**, 1161 (1966).

(5) N. Sueoka and T. Kano-Sueoka, *ibid.*, **52**, 1535 (1964).

(6) M. N. Lipsett and A. Peterkofsky, *ibid.*, **55**, 1169 (1966).

(7) B. Goehler and R. H. Doi, *ibid.*, **56**, 1047 (1966).

(8) R. I. B. Francki and R. E. F. Matthews, *Virology*, **17**, 367 (1962).

(9) P. K. Ralph, R. E. F. Matthews, and A. I. Matus, *Biochim. Biophys. Acta*, **108**, 53 (1965).

(10) J. Kokko, L. Mandell, and J. Goldstein, *J. Am. Chem. Soc.*, **84**, 1042 (1962).

(11) J. J. Fox, D. V. Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich, and G. B. Brown, *ibid.*, **81**, 178 (1959).

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

(13) Very recently, T. Ueda, Y. Iida, K. Ikeda, and Y. Mizuno, *Chem. Pharm. Bull.* (Tokyo), **14**, 666 (1966), have reported the preparation of 2,4-dithiouridine by the thiation of 2′,3′,5′-tri-O-benzoyl-4-thiouridine under forcing conditions. Conversion of 2,4-dithiouridine to 2-thiocytidine was accomplished by ammonolysis in a manner similar to the one reported here for the arabinonucleoside.

(14) G. Shaw and R. N. Warrener, *J. Chem. Soc.*, 153 (1958).

(15) G. Shaw, R. N. Warrener, M. H. Maguire, and R. K. Ralph, *ibid.*, 2294 (1958).

(16) M. Sano, *Bull. Chem. Soc. Japan*, **10**, 308 (1962).

(17) D. M. Brown, D. B. Parihar, A. Todd, and S. Varadarajan, *J. Chem. Soc.*, 3028 (1958).

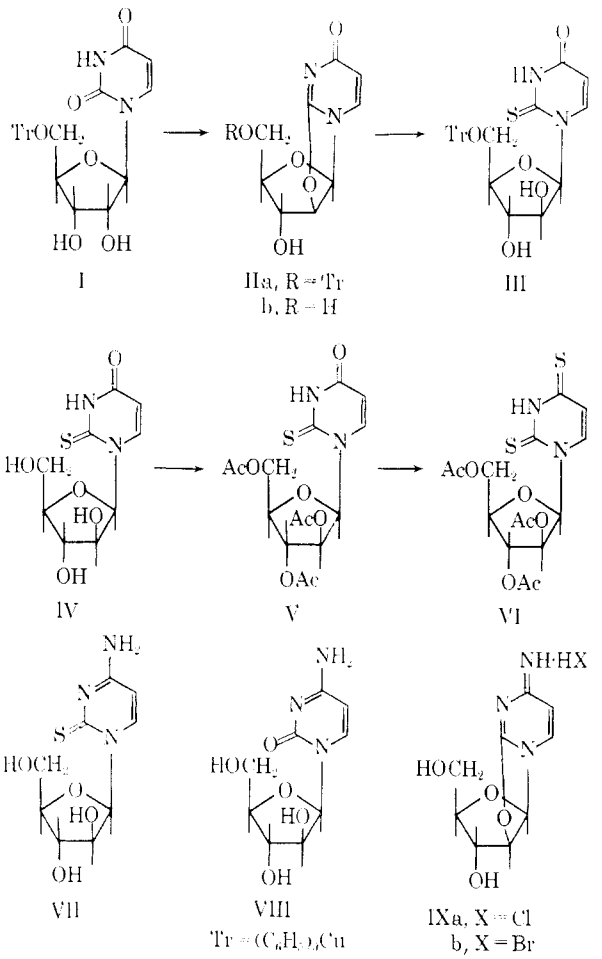
(18) R. W. Chambers and V. Kurkov, *J. Am. Chem. Soc.*, **85**, 2160 (1963).

(19) N. K. Kochetkov, E. I. Budonsky, and V. N. Shibaev, *Tetrahedron*, **19**, 1207 (1963).

(20) H. Amos, E. Vollmayer, and M. Korn, *Arch. Biochem. Biophys.*, **77**, 236 (1958).

anhydronucleosides, a feasible synthetic pathway for the preparation of 2-thiopyrimidine β-D-arabinosides appeared to be by way of the anhydro ring cleavage of 2,2′-anhydronucleosides with H_2S in a manner analogous to the reaction of 2,5′-anhydronucleosides.[17-20] Initially, when 2,2′-anhydro-(5-trityl-1-β-D-arabinofuranosyl)uracil (IIa) was treated with H_2S at room temperature[17] only the starting material was recovered. Nonetheless, when the temperature was raised to 100–110°, the desired reaction took place, and 5′-trityl-1-β-D-arabinofuranosyl-2-thiouracil (III) was obtained in good yield (Chart I). By-products corresponding

CHART I



to the 5′-thio or 5′6-epithio structures[18,19] obtained in the reaction of 2,5′-anhydrouridine with H_2S were apparently not formed in appreciable amounts. Detritylation of III furnished 1-β-D-arabinofuranosyl-2-thiouracil (IV). The ultraviolet spectra of IV in acid and base were very similar to those reported for 2-thiouridine.[15] Hydrolysis of IV with aqueous chloroacetic acid[15] yielded a mixture which contained, as a major component, a substance running at the same rate as 1-β-D-arabinofuranosyluracil (spongouridine) in thin layer chromatography. Acetylation and thiation of IV yielded 2′,3′,5′-tri-O-acetyl-1-β-D-arabinofuranosyl-2,4-dithiouracil (VI).

The course of the reaction of VI with ammonia was quite dependent upon the conditions used. By heating VI with aqueous NH_4OH at 100° for 3 hr, amination was accompanied by complete desulfurization; 1-β-D-arabinofuranosylcytosine (VIII) was formed as the major product and isolated as the hydrochloride in 74% yield. When VI was heated in anhydrous methanolic ammonia at 100° for 3 hr, the desired 1-β-D-arabinofuranosyl-2-thiocytosine (VII) was obtained in 30% yield. However, when VI was heated in anhydrous methanolic NH_3 at the same temperature for 49 hr, a complex reaction mixture was obtained instead and from which only 2,2′-anhydro-1-(β-D-arabinofuranosylcytosine could be isolated as the hydrochloride (IXa)[21] in very low yield.

An aqueous solution of IV discharged the color of bromine water very rapidly. Somewhat unexpectedly the product of the bromination was found to be the hydrobromide of 2,2′-anhydro-1-(β-D-arabinofuranosyl)uracil (IIb). Similarly, the bromination of VII yielded 2,2′-anhydro-1-(β-D-arabinofuranosylcytosine hydrobromide (IXb).[22]

In the course of our limited investigation of the reactions of 2-thionucleosides an attempt was made to prepare the disulfide of VII by reaction with iodine. As indicated above, formation of disulfides of this type has been suggested to have some biological significance. The reversible inactivation of s-RNA by iodine is believed to involve such changes.[2] Compound IV, when treated with 0.8 equiv of iodine in near neutral solution (conditions which result in the conversion of 4-thiouridine to 4-thiouridine disulfide[11]) yielded only unchanged IV and the 2,2′-anhydronucleoside IIb in the ratio of about 2:1. It is presumed that the mechanism of the formation of IIb in this case is similar to that involved in the bromination reaction. Compound VII consumes iodine very rapidly under the same conditions but unfortunately no recognizable compounds could be isolated from the reaction mixture. Chambers and co-workers[15] were also unsuccessful in attempts to prepare the disulfide of acetone 2-thiouridine.

The attempted desulfurization of IV with Raney nickel was also abortive; only an intractable gum was obtained.

Preliminary evaluation of these nucleosides indicated that 1-β-D-arabinofuranosyl-2-thiocytosine (VII) possessed selective antiviral activities in the tissue culture assay[24] against vaccinia at 6.25 μg/ml with a therapeutic index of greater than 64 (see Table I). It was slightly active against herpes and adeno 2 viruses. It was not active against the human adenocarcinoma (HAd) No. 1 grown in the embryonated egg; a dose of 2 mg/egg was not toxic to the chick embryo.[25]

(21) E. R. Walwick, W. K. Roberts, and C. A. Dekker, *Proc. Chem. Soc.*, 81 (1959).

(22) *The formation of 2,2′-anhydronucleosides from nucleosides of the arabino configuration appears to be rare.* I. L. Doerr, J. F. Codington, and J. J. Fox, *J. Org. Chem.*, **30**, 167 (1965), report the formation of IIb as a secondary product from the hydrolysis of 5′-O-mesyl-1-β-D-arabinofuranosyluracil. In the present case a possible explanation of the bromination product could be in the formation of 2-bromosulfonyl or 2-bromo intermediates which could then undergo displacement by the 2′-hydroxyl group. In this connection, it is of interest that Robdin and Clapp[23] found that the chlorination of certain 2-thiopyrimidines yielded the corresponding 2-chlorosulfonylpyrimidines, and the latter decomposed readily to the 2-chloropyrimidines with loss of SO_2.

(23) R. P. Robdin and J. W. Clapp, *J. Am. Chem. Soc.*, **72**, 4890 (1950).

(24) We are indebted to Dr. M. M. Nemes of these laboratories for these results. Methods used are given by M. M. Nemes and M. R. Hilleman, *Proc. Soc. Exptl. Biol. Med.*, **119**, 515 (1965).

(25) This experiment was carried out by Dr. Charles Bittenman of these laboratories, following the procedure published previously: C. O. Gitterman, E. L. Dulaney, E. A. Kaczka, D. Heeffin, and H. R. Wiendrab, *ibid.*, **109**, 852 (1962).

TABLE I

ANTIVIRAL ACTIVITY OF 1-β-D-ARABINOFURANOSYL-2-THIOCYTOSINE

Virus	Antiviral concn, μg/ml	Therap index[a]
Vaccinia	6.25	>64
Herpes	200	>2
Adeno 2	200	>2

[a] Cytotoxic concentration for GMK cells was greater than 400 μg/ml.

At 1 m*M* concentration it showed no effect on the incorporation of hypoxanthine or orotic acid into RNA in intact Ehrlich ascites cells.[26] No antiviral activity was found with 1-β-D-arabinofuranosyl-2-thiouracil (IV).

Experimental Section[27,28]

2,2′-Anhydro-1-(5-O-trityl-β-D-arabinofuranosyl)uracil (IIa).[29]—To a solution of 9.8 g (0.144 mole) of imidazole in 83 ml of CH_2Cl_2 was added, dropwise, a solution of 4.12 g (0.036 mole) of thiophosgene in 34 ml of benzene. The mixture became warm spontaneously and was stirred without cooling for 2 hr. The precipitated imidazole hydrochloride was filtered, and the solid was washed with 35 ml of CH_2Cl_2 and 160 ml of toluene. The filtrate was filtered again to remove a slight turbidity. 5′-O-Trityluridine (I) (15.9 g, 0.033 mole) was added to the filtrate, and the mixture was heated to distil the CH_2Cl_2 and benzene. When the temperature of the distillate reached 107° (in approximately 0.75 hr) precipitation of the product appeared complete. The mixture was cooled and filtered, and the product was washed with cold ethanol. The yield was 14.3 g (94%), mp 200–205°. This material is sufficiently pure for most synthetic purposes, but may be recrystallized from ethanol to give pure material, mp 215–219°, with very good recovery.

1-(5-O-Trityl-β-D-arabinofuranosyl)-2-thiouracil (III).—A stream of H_2S was passed into a solution of 8.0 g (17 mmoles) of 2,2′-anhydro-1-(5-O-trityl-β-D-arabinofuranosyl)uracil in 75 ml of dry dimethylformamide (DMF) and 5.6 ml of triethylamine while the mixture was heated to 95° during 1.5 hr. Heating was continuous while the temperature was raised to 115° during 4.5 hr. After keeping at room temperature overnight, the reaction mixture was poured into 300 ml of water. The product was extracted twice with 100-ml portions of ethyl acetate. The addition of saturated NaCl solution was helpful in breaking the emulsion that formed. The ethyl acetate solution was washed with water, dried ($MgSO_4$), and concentrated to 9.0 g of a glassy foam *in vacuo*. Chromatography on 450 g of silica gel yielded 7.8 g (90%) of amorphous pale yellow solid in one peak, eluted with 4% methanol in CH_2Cl_2. The product showed a single spot on thin layer chromatography (silica gel, methanol–CH_2Cl_2, 1:9).

1-(β-D-Arabinofuranosyl)-2-thiouracil (IV).—A mixture of 7.8 g (15.5 mmoles) of 1-(5-O-trityl-β-D-arabinofuranosyl)-2-thiouracil and 80 ml of 80% acetic acid was heated on the steam bath for 20 min. The mixture was concentrated to dryness *in vacuo* and the residue was partitioned between water and ether. Concentration of the aqueous phase yielded 4.0 g of a crystalline solid. Trituration with ethanol gave 3.2 g (80%) of product, mp 199–204°. Recrystallization from ethanol furnished an analytical sample, mp 203–205°, $[\alpha]^{25}_{589}$ +110° (H_2O, *c* 1.0), $\lambda_{max}^{H_2O}$ 276 mμ (ε 14,700), $\lambda_{min}^{H_2O}$ 245 mμ (ε 4500), $\lambda_{max}^{0.1MNaOH}$ 270 mμ (ε 14,000) and 241 mμ (ε 21,700), $\lambda_{min}^{0.1MNaOH}$ 262 mμ (ε 13,600).

Anal. Calcd for $C_9H_{12}N_2O_5S$: C, 41.54; H, 4.65; N, 10.77; S, 12.2. Found: C, 41.65; H, 4.64; N, 10.37; S, 12.3.

1-(2,3,5-O-Triacetyl-β-D-arabinofuranosyl)-2-thiouracil (V).—A solution of 2.5 g (9.6 mmoles) of 1-(β-D-arabinofuranosyl)-2-thiouracil in 4 ml of pyridine and 20 ml of acetic anhydride was heated on the steam bath for 1 hr. The mixture was concentrated *in vacuo* to an oil, and the concentration was repeated twice after the successive addition of 20-ml portions of ethanol and toluene. Recrystallization from ethanol yielded 3.31 g (89%) of crystals, mp 140.5–141.5°.

Anal. Calcd for $C_{15}H_{18}N_2O_8S$: C, 46.63; H, 4.70; N, 7.25; S, 8.3. Found: C, 46.48; H, 4.63; N, 7.27; S, 8.4.

1-(2,3,5-O-Triacetyl-β-D-arabinofuranosyl)-2,4-dithiouracil (VI).—A mixture of 3.2 g of 1-(2,3,5-O-triacetyl-β-D-arabinosyl)-2-thiouracil, 55 ml of dry pyridine, and 7.4 g of P_2S_5 was heated at reflux for 3.5 hr. The cooled mixture was poured into 300 ml of water, and after 20 min of stirring, the solid product was filtered and washed well with water. The moist product was taken up in 25 ml of pyridine and warmed on the steam bath until the evolution of H_2S was complete. The product was again precipitated by the gradual addition of 200 ml of cold water. After filtering, washing, and drying at 110° *in vacuo* the crude product weighed 3.26 g (98%), mp 145–147°. After recrystallization from toluene–hexane, 3.01 g (91%) of bright yellow crystals was obtained; mp 146–147°; $\lambda_{max}^{H_2O}$ 281 mμ (ε 20,400), inflections at 360, 340, 300, and 198 mμ.

Anal. Calcd for $C_{15}H_{18}N_2O_7S$: C, 44.78; H, 4.51; S, 15.9. Found: C, 44.68; H, 4.32; S, 15.9.

1-(β-D-Arabinofuranosyl)-2-thiocytosine (VII).—A solution of 2.0 g of 1-(2,3,5-O-triacetyl-β-D-arabinofuranosyl)-2,4-dithiouracil in 100 ml of methanol was saturated with anhydrous NH_3 at 0°. The mixture, in a glass liner, was heated in a pressure bomb at 100° for 3 hr. The reaction mixture was concentrated to a gum *in vacuo*, and most of the by-product acetamide was removed by sublimation at 60° (0.1 mm). The residue was chromatographed on 100 g of silica gel. Elution of the column with CH_2Cl_2–methanol mixtures with methanol concentrations of 2–25% gave fractions containing acetamide and a series of brown gums. The desired product was eluted with 30% methanol–CH_2Cl_2; a total of 0.386 g (30%), mp 175–180° dec, was obtained. Recrystallization from methanol–2-propanol furnished an analytical sample, mp 180–182° dec, λ_{max}^{pH1} 278 mμ (ε 17,800) and 230 mμ (ε 16,800), λ_{min}^{pH1} 252 mμ (ε 7900), λ_{max}^{pH13} 252.5 mμ (ε 22,900), pK_a = 3.20 (from uv data).

Anal. Calcd for $C_9H_{13}N_3O_4S$: C, 41.70; H, 5.06; N, 16.21; S, 12.3. Found: C, 41.76; H, 4.98; N, 16.49; S, 12.7.

Prolonged Reaction of VI with Methanolic NH_3. 2,2′-Anhydro-1-(β-D-arabinofuranosyl)cytosine Hydrochloride (IXa).—A solution of 250 mg of VI in 10 ml of methanol saturated with NH_3 at 0° was heated in a bomb at 100° for 45 hr. The dark brown solution was filtered to remove a small amount of black solid. The filtrate was concentrated *in vacuo* and the acetamide was sublimed at 60° (0.5 mm). The residue was taken up in ethanol (8 ml) and treated with charcoal, and the filtrate was diluted with 10 ml of ether. Thin layer chromatography showed a multiplicity of components. Upon treatment with excess ethereal HCl and nearly complete evaporation, a crystalline solid was deposited. After recrystallization from methanol–2-propanol, 4.4 mg of material was collected which showed the properties of 2,2′-anhydro-1-(β-D-arabinofuranosyl)cytosine hydrochloride,[21] mp 250–260° dec, $\lambda_{max}^{H_2O}$ 231 mμ (ε 8900) and 263 mμ (ε 9900).

Anal. Calcd for $C_9H_{12}ClN_3O_4$: C, 41.47; H, 4.64; N, 16.12. Found: C, 41.48; H, 4.49; N, 16.62.

Reaction of VI with Aqueous NH_3. 1-β-D-Arabinofuranosylcytosine (VIII).—A solution of 200 mg of VI in 15 ml of concentrated NH_4OH was heated in a bomb for 3 hr at 100°. After removal of the solvent *in vacuo* and the acetamide by sublimation at 60° (0.1 mm), the crude amorphous product showed an uv spectrum typical of a cytosine nucleoside. After the addition of a slight excess of ethanolic HCl, and recrystallization from ethanol, 110 mg (75%) of 1-β-D-arabinofuranosylcytosine hydrochloride was obtained; mp 188–190°, not depressed on admixture with an authentic sample. The infrared spectrum in Nujol was identical with that of an authentic spectrum.

Bromination of IV. 2,2′-Anhydro-1-(β-D-arabinofuranosyl)-uracil (IIb).—To a solution of 520 mg (2 mmoles) of IV in 12 ml of water was added dropwise 3 ml of 1 *M* Br_2 solution in CCl_4.

(26) This experiment was carried out by Dr. H. T. Shigeura of these laboratories following the procedure published previously: H. T. Shigeura and C. N. Gordon, *J. Biol. Chem.*, **237**, 1932 (1962).

(27) All melting points were determined on a Kofler hot stage equipped with a calibrated thermometer.

(28) We are indebted to Mr. R. N. Boos and his associates for microanalytical data, and to Mr. E. A. MacMullan and his associates for the ultraviolet spectral data.

(29) This preparation of IIa is reported here as a more convenient procedure than those previously reported;[30,31] isolation of bisimidazole thione is avoided.

(30) J. J. Fox, N. Miller, and I. Wempen, *J. Med. Chem.*, **9**, 101 (1966).

(31) W. V. Ruyle, T. Y. Shen, and A. A. Patchett, *J. Org. Chem.*, **30**, 4855 (1965).

At this point the color of the bromine persisted for 2–3 min after each addition. The unreacted bromine was blown off with a stream of nitrogen, and the reaction mixture was concentrated to a syrup *in vacuo*, bath temperature less than 50°. The residue was evaporated three times with 10-ml portions of ethanol, whereupon it crystallized. The product was triturated with cold ethanol and with ether to obtain 340 mg of a crystalline hydrobromide salt, mp 135–138°. Recrystallization from methanol–ether gave an analytical sample, mp 136–138°. *Anal.* Calcd for $C_9H_{10}BrN_3O_5$: Br, 26.0. Found: Br, 25.71. Concentration of the original mother liquors yielded 170 mg of the starting material, 2-thiouracilarabinoside. Treatment of a concentrated ethanolic solution of the hydrobromide with a slight excess of ethanolic NH_3 yielded 2,2′-anhydro-1-(β-D-arabinofuranosyl)-uracil (IIb), mp 238–241°, identical in all respects with an authentic sample.[4]

Bromination of 1-(β-D-Arabinofuranosyl)-2-thiocytosine. 2,2′-Anhydro-1-(β-D-arabinofuranosyl)cytosine Hydrobromide (IXb).—Bromination of 80 mg of VII in the manner described in the previous example yielded, after recrystallization from ethanol, 17 mg of IXb, mp 240° dec, $\lambda_{max}^{H_2O}$ 264 mμ (ε 9900) and 231 mμ (ε 9100), $\lambda_{min}^{H_2O}$ 244 mμ (ε 6550), λ_{max}^{pH13} 275 mμ (ε 9200), λ_{min}^{pH13} 251 mμ (ε 4800).

Anal. Calcd for $C_9H_{10}BrN_3O_5$: C, 35.31; H, 3.95; N, 13.71; Br, 26.11. Found: C, 34.55; H, 3.76; N, 13.69; Br, 25.84.

After acidification of the alkaline uv solution the following constants were obtained: λ_{max}^{pH2} 281 mμ (ε 12,400), λ_{min}^{pH2} 242 mμ (ε 15001).

Iodination of IV. A solution of 260 mg (1 mmole) of IV in a mixture of 28 ml of water and 7 ml of pH 6.84 buffer was treated dropwise with 0.8 ml of 1 *N* I_2 solution which was 2.4 *N* in KI. A solution of *N* K_2CO_3 was added simultaneously to maintain the pH near neutrality. The slightly turbid solution was deionized by treatment with Dowex 3 (OH^-) and Dowex 50 WX (H^+) resins. Concentration *in vacuo* yielded a mixture from which IIb (66 mg) was separated by virtue of its very limited solubility in ethanol. From the mother liquor, 123 mg of the starting material (IV) was obtained.

Substituted 2,3-Dihydro-4(1H)-quinazolinones. A New Class of Inhibitors of Cell Multiplication

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The synthesis of 23 2-aryl-2,3-dihydro-4(1H)-quinazolinones is reported. A number of these are highly active in inhibiting the multiplication of Earle's L cells growing in suspension; nine have $ED_{50} \leq 6$ μg/ml and of these two have $ED_{50} = 0.1$ μg/ml in this screening procedure.

The literature contains a limited number of references to 2,3-dihydro-4(1H)-quinazolinones, and several of these reports are concerned with the evaluation of these compounds for possible pharmacodynamic, insecticidal, and antifungal activity.[1] Our objective in synthesizing a series of 2-aryl-substituted derivatives of that heterocycle was to study them as inhibitors of multiplication of the Earle's L cell line of mouse fibroblasts growing in suspension.[2] It will be seen in Table I that a significant number of these derivatives showed very high *in vitro* activity: compounds **1** and **11** with $ED_{50} = 0.1$ μg/ml and **2–5**, **10**, **13**, and **21** with $ED_{50} \leq 6$ μg/ml[3] were the most potent. For comparison, 2,3-dihydro-2-phenyl-4H-1,3-benzoxazin-4-one,[4] the 1-oxa analog of **1**, had $ED_{50} > 50$ μg/ml; actinomycin IV, one of the highly cytotoxic antibiotics, had $ED_{50} = 0.006$ μg/ml.[5]

The compounds were screened as inhibitors of cell multiplication by the procedure of Perlman, *et al.*[6] To aliquots of sterile cells were added serial dilutions of aqueous dimethyl sulfoxide solutions of the quinazolone and the mixtures were incubated for 3 days at 37°. The amount of compound needed to give a 50% inhibition was determined graphically by means of dose-response curve.

Several methods were employed for the synthesis of the 2-aryl-2,3-dihydro-4(1H)-quinazolinones. Method A, anil formation in ethanol between the 2-aminobenzamide and the aromatic aldehyde followed by the base-catalyzed cyclization[1a] was the most generally applicable. This method failed with **13** and **22**; the successful procedure (method C) employed catalytic amounts of *p*-toluenesulfonic acid in boiling chlorobenzene and made use of a special device which allowed the condensed solvent to be dried by percolation through a bed of calcium hydride before returning to the reaction flask. In method B, saturated ethanolic hydrogen chloride was the reaction medium; while successful with **1** and **12**, the procedure failed with several other compounds. The two amino derivatives, **14** and **17**, were obtained by catalytic hydrogenation of the corresponding nitro compounds.

Structure–Activity Relationships.—The only structural modification of **1** which did not adversely affect activity was the replacement of a hydrogen at position 6 by a chlorine atom; somewhat decreased activity was found when the substituent in that position was NO_2 or Br and even less activity when the substituent was H_2N or CH_3CONH. A high order of activity was retained when the 2-phenyl group of **1** was replaced by

(1) (a) T. A. Kilroe Smith and H. Stephen, *Tetrahedron*, **1**, 38 (1957); (b) H. Böhme and H. Bäing, *Arch. Pharm.*, **293**, 1011 (1960); (c) H. Gurien and B. B. Brown, *J. Pharm. Sci.*, **52**, 1102 (1963); H. Gurien and T. P. Gordon, U. S. Patent 3,162,636 (Dec 22, 1964); (d) Instituto de Angeli S.p.A., French Patent M1893 (Aug 5, 1963); *Chem. Abstr.*, **60**, 3956*h* (1964); (e) C. H. Boehringer Sohn, French Patent M2588 (July 6, 1964); *Chem. Abstr.*, **61**, 16075*h* (1964); Nederland Application 302,479; *Chem. Abstr.*, **64**, 9743 (1966); (f) Farbenfabriken Bayer A.-G., Belgian Patent, 632,578 (Nov 20, 1963); *Chem. Abstr.*, **61**, 8321*d* (1964); (g) G. Pala and A. Mantegani, *Gazz. Chim. Ital.*, **94**, 595 (1964); (h) V. S. Dighe and S. L. Mukherjee, *Current Sci.* (India), **33**, 645 (1964); *Chem. Abstr.*, **62**, 2775*e* (1966); Rexall Drug Co., U. S. Patent 3,257,397 (June 21, 1966); Shulton, Inc., U. S. Patent 3,265,697 (Aug 9, 1966).

(2) The inhibition studies were carried out by Dr. D. Perlman of these laboratories.

(3) J. Leitner, B. J. Abbott, and S. A. Schepartz, *Cancer Res.*, **25**, 1779 (1965), state that in the CCNSC cell culture screening procedure for the selection of compounds for further study, $ED_{50} \leq 6$ μg/ml is the requirement to pass the first stage of evaluation.

(4) A. W. Titherley, *J. Chem. Soc.*, **91**, 1419 (1907).

(5) D. Perlman, *Hindustan Antibiot. Bull.*, **8**, 175 (1966).

(6) D. Perlman, N. A. Guiffre, and P. W. Jackson, *Proc. Soc. Exptl. Biol. Med.*, **102**, 290 (1959).