[CONTRIBUTION FROM THE DIVISIONS OF NUCLEOPROTEIN CHEMISTRY AND EXPERIMENTAL CHEMOTHERAPY, SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH; SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK 21, NEW YORK AND HOFFMANN LA ROCHE, INC., NUTLEY, NEW JERSEY]

Nucleosides. XI. Synthesis of 1-β-D-Arabinofuranosyl-5-Fluorouracil and Related Nucleosides[1]

BY NAISHUN C. YUNG, JOSEPH H. BURCHENAL, RONALD FECHER, ROBERT DUSCHINSKY AND JACK J. FOX

RECEIVED APRIL 24, 1961

Methods are described for the synthesis in good yields of 1-β-D-arabinofuranosyl- and 1-β-D-lyxofuranosyl-5-fluorouracils from 5-fluorouridine *via* anhydro nucleoside intermediates. Synthesis of 5-fluorouridine in improved yield from 5-fluorouracil *via* the mercuri procedure is described. Preliminary studies on the effect of these nucleosides against transplanted mouse leukemia B82 are reported.

The usefulness of 5-fluoro-2′-deoxyuridine[2] as an anti-tumor agent in experimental tumors[3] and in clinical trials[4] is believed to be due to the inhibition of the metabolic conversion of 2′-deoxyuridylic acid to thymidylic acid by the nucleotide of 5-fluoro-2′-deoxyuridine.[5] The efficacy of 5-fluoro-2′-deoxyuridine is weakened, however, by catabolic processes (*e.g.*, cleavage by nucleosidases to 5-fluorouracil and subsequently to products derived therefrom).[6] The discovery[7] that 1-β-D-arabinofuranosyluracil is phosphorylated enzymically to the 5′-nucleotide which, further, can partake in the enzymic methylation step (albeit poorly) is of significance. Since 1-β-D-aldopentofuranosylpyrimidines other than ribosyl are generally poor substrates for nucleosidase activity (glycosyl cleavage),[7,8] the 1-β-D-arabinofuranosyl-5-fluorouracil and related nucleosides were synthesized to determine whether they could exert anti-tumor activity but with decreased toxicity relative to 5-fluoro-2′-deoxyuridine. A preliminary report dealing with the syntheses of these compounds has appeared.[9]

The synthesis of 1-β-D-arabinofuranosyl-5-fluorouracil (VI) was accomplished by modifications of the procedures[10,11] used for the epimerization of uridine and 1-β-D-ribofuranosylthymine[12] *via* 2,2′-anhydronucleoside intermediates to their corresponding arabino nucleosides. The preparation of 5-fluorouridine[2a] by the mercuri process[12] was improved by use of 5-fluorouracilmercury[2b] in the condensation reaction with tri-*O*-benzoyl-D-ribofuranosyl chloride.[13] Removal of the benzoyl blocking groups with dilute alkali afforded 5-fluorouridine (I) in 65% yield (based upon 5-fluorouracil). Tritylation of I afforded an almost quantitative yield of the 5′-*O*-trityl derivative (II). Tosylation of II with *p*-toluenesulfonyl chloride in pyridine afforded a mixture from which the 2′-*O*-tosyl derivative (III) crystallized in 52% yield.[14] Detritylation of III with ethanolic hydrogen chloride at 60° for ten minutes yielded the 2′-*O*-tosyl derivative (IV) which was converted to the 2,2′-anhydronucleoside (V, R = F) by treatment with one equivalent of alkali. Treatment of V with dilute alkali at room temperature afforded VI.

A more practical approach to VI was effected by treatment of III with two equivalents of alkali in 50% ethanol. By this procedure, the anhydronucleoside (VII) is formed and cleaved *in situ* by the excess alkali to VIII which precipitates. VIII was detritylated with dilute acid in 50% ethanol to form VI. Though intermediates were not purified, the over-all yield of VI (based upon III) was 88%.

Proof that VI is 1-β-D-arabinofuranosyl-5-fluorouracil rests on the following data: The ultraviolet absorption spectrum of VI was similar to that given by I. When treated with metaperiodate, VI consumed one mole of reagent per mole of nucleoside *slowly*[12] without the liberation of formic acid in accord with a furanosyl structure containing a *trans* α-glycol system. Finally, reduction of VI with palladium–charcoal afforded 1-β-D-arabinofuranosyluracil.

The lyxo nucleoside (XII) also was prepared from 5′-*O*-trityl-5-fluorouridine (II). Mesylation of II afforded a di-*O*-mesyl derivative (IX) as an

(1) This investigation was supported in part (to the Sloan-Kettering Institute) by funds from the National Cancer Institute of the National Institutes of Health, Public Health Service (Grant No. CY 3190), the Cancer Chemotherapy National Service Center, Research Contract SA-43-ph-2445 and the American Cancer Society.

(2) (a) R. Duschinsky, E. Pleven, E. Malbica and C. Heidelberger, Abstr. 132nd Meeting, Am. Chem. Soc., 1957, p. 19-C; (b) M. Hoffer, R. Duschinsky, J. J. Fox and N. Yung, *J. Am. Chem. Soc.*, **81**, 4112 (1959).

(3) (a) C. Heidelberger, L. Griesbach, O. Cruz, R. J. Schnitzer and E. Grunberg, *Proc. Soc. Exp. Biol. Med.*, **97**, 470 (1958); (b) J. H. Burchenal, E. A. D. Holmberg, J. J. Fox, S. C. Hemphill and J. A. Reppert, *Cancer Research*, **19**, 494 (1959).

(4) A. R. Curreri and F. Ansfield, *Cancer Chemotherapy Reports* (Cancer Chemotherapy National Service Center), **2**, 8 (1959); F. Ansfield and A. R. Curreri, *ibid.*, **6**, 21 (1960).

(5) S. S. Cohen, J. G. Flaks, H. D. Barner, M. R. Loeb and J. Lichenstein, *Proc. Natl. Acad. Sci.*, **44**, 1004 (1958); L. Bosch, E. Harbers and C. Heidelberger, *Cancer Research*, **18**, 335 (1958).

(6) N. K. Chaudhuri, K. L. Mukherjee and C. Heidelberger, *Biochemical Pharmacology*, **1**, 328 (1959).

(7) L. I. Pizer and S. S. Cohen, *J. Biol. Chem.*, **235**, 2387 (1960); Abstr. 136th Meeting, Am. Chem. Soc., 1959, p. 9C.

(8) J. J. Fox, J. F. Codington, N. C. Yung, L. Kaplan and J. O. Lampen, *J. Am. Chem. Soc.*, **80**, 5155 (1958).

(9) (a) J. J. Fox, N. Yung, I. Wempen, R. Duschinsky and L. Kaplan, Abstr. Intl. Union Pure and Applied Chemistry (Symposium on Natural Products) Australia, 1960, p. 66; (b) the synthesis of 1-β-D-arabinofuranosyl-5-fluorouracil by an alternate route has been reported as a communication recently by E. J. Reist, J. H. Osiecki, L. Goodman and B. R. Baker, *J. Am. Chem. Soc.*, **83**, 2208 (1961).

(10) D. M. Brown, A. R. Todd and S. Varadarajan, *J. Chem. Soc.*, 2388 (1956).

(11) J. J. Fox, N. C. Yung and A. Bendich, *J. Am. Chem. Soc.*, **79**, 2775 (1957).

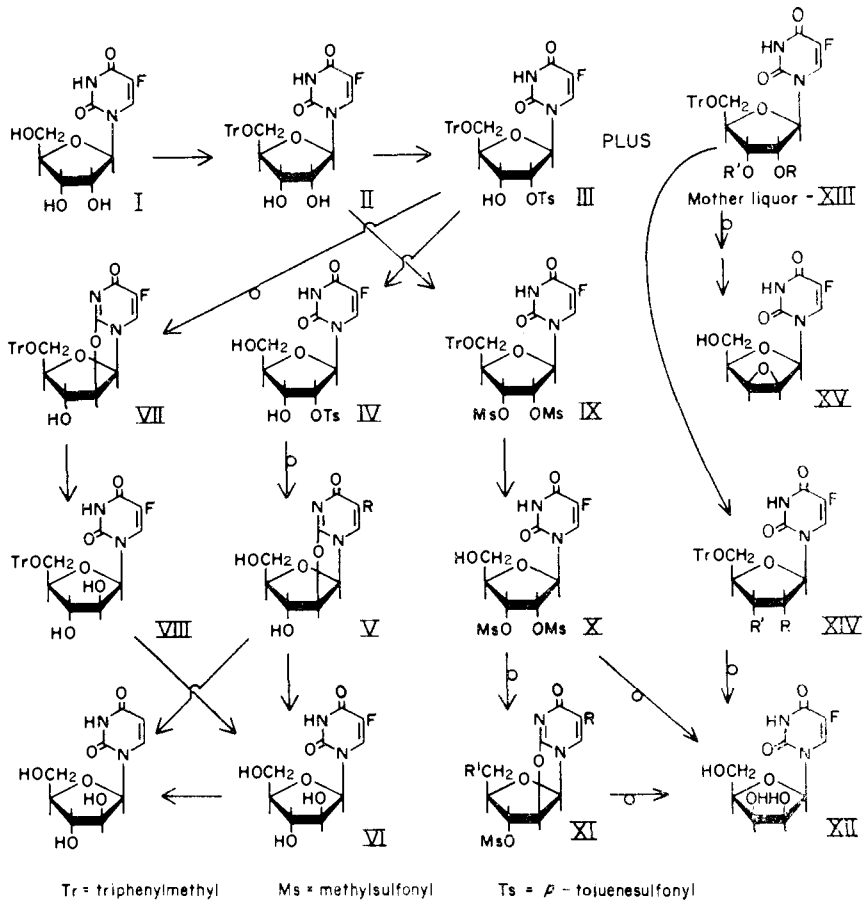
(12) J. J. Fox, N. Yung, J. Davoll and G. B. Brown, *ibid.*, **78**, 2117 (1956).

(13) 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-D-ribose (R. K. Ness, H. W. Diehl and H. G. Fletcher, Jr., *ibid.*, **76**, 763 (1954); H. M. Kissman, C. Pidacks and B. R. Baker, *ibid.*, **77**, 18 (1955), was used for the preparation of this halogenose.

(14) The mother liquor from this reaction is rich in nucleoside derivatives. The composition of this mother liquor and its use in further syntheses are described later in the text.

amorphous solid. (Attempts to prepare a mono-*O*-mesyl derivative of II using one molecular equivalent of methanesulfonyl chloride were unsuccessful. The product obtained was invariably the di-*O*-mesylated derivative (IX) along with starting material.) Detritylation of IX afforded the 2′,3′-di-*O*-mesyl derivative (X) of 5-fluorouridine which, upon boiling in water for 4 hr., was converted to 1-β-D-lyxofuranosyl-5-fluorouracil (XII). Treatment of X with one equivalent of alkali formed the 2,2′-anhydronucleoside (XI, R = F, R′ = OH) which, upon refluxing with dilute acid, also gave XII.

When treated with metaperiodate, 1-β-D-lyxofuranosyl-5-fluorouracil consumed one mole of oxidant per mole *rapidly*[12] without the liberation of formic acid in accordance with a pentofuranosyl structure containing an *α-cis* glycol system. A comparison of the molecular rotations of these fluorinated nucleosides *versus* their non-fluorinated analogs (see Table I) is in accord with configurational assignments made for I, VI and XII.



Flow Chart

The conversion of XI (R = F, R′ = OH) to the lyxo nucleoside (XII) was not unexpected in light of previous studies in this Laboratory[15] which demonstrated conclusively that 5′-substituted derivatives of XI (R = H, R′ = *O* mesyl, *O*-benzoyl or hydrogen) are converted to 1-β-D-lyxofuranosyluracils by merely boiling in water. The mechanism elaborated[15] involves first the hydrolytic cleavage of the 2,2′-anhydro linkage to the arabino nucleoside followed by formation of a 2,3′-anhydro-lyxosyl nucleoside with the liberation of one mole of methylsulfonic acid. Under the acidic conditions engendered, the 2,3′-anhydro bond is hydrolyzed with the formation of lyxo nucleosides.

Whereas the non-fluorinated derivatives (XI, R = H, R′ = *O*-benzoyl, *O*-mesyl or hydrogen) are converted by refluxing in water for 4–5 hr. to lyxosyluracils,[15] the fluorinated derivative (XI, R = F, R′ = OH) remained unaltered under these reaction conditions. However, if the refluxing aqueous solution of XI (R = F) is "primed" with dilute acid (one molecular equivalent of methylsulfonic acid), the formation of the 5-fluorolyxosyl nucleoside is complete in 4 hr. That the 3′-mesyloxy function plays no significant role in the hydrolytic cleavage of the anhydro linkage is shown by the fact that V (R = F) is also inert in refluxing water for 4 hr., and, again, acid "priming" of the refluxing solution is essential for its conversion to VI. On the other hand, the non-fluorinated analog of V (R = H) undergoes appreciable conversion to 1-β-D-arabinofuranosyluracil without acid "priming" under identical reflux conditions. In the conversion of X to XII, however, acid priming is not necessary since methylsulfonic acid is liberated by the formation of the 2,2′-anhydronucleoside intermediate (XI, R = F, R′ = OH). The liberated acid catalyzes the cleavage of the anhydro linkage.

The acid requirement needed for the cleavage of the anhydro linkages of the 5-fluorinated derivatives (V and XI) as *versus* their un-fluorinated analogs is probably a reflection of the electron withdrawing properties of the 5-fluoro atom exerted through the conjugated system in the aglycon. It is probable that the electronegative 5-fluoro atom renders the formation of the conjugate acid of the anhydro nucleoside more difficult by water. This obstacle would be overcome as the hydrogen ion concentration in the refluxing medium is increased.

As mentioned above, a 52% yield of III was obtained upon tosylation of II. The mother liquor from this reaction (designated as XIII-mother liquor on the flow chart) was also utilized for the

(15) R. Fecher, J. F. Codington and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 1889 (1961).

synthesis of 1-β-D-lyxofuranosyl-5-fluorouracil (XII). XIII-Mother liquor could contain four (or less) possible nucleosides: starting material (II), additional 2′-*O*-tosyl nucleoside (III), the isomeric 3′-*O*-tosyl-5′-*O*-trityl derivative and, finally, a 5′-*O*-trityl-2′,3′-di-*O*-tosylated 5-fluorouridine (XIII, R,R′ = *p*-tosyl).

Treatment of XIII-mother liquor with an excess of methylsulfonyl chloride in pyridine afforded a sirup (XIV, R,R′ = mesyloxy or tosylosy) which did not crystallize. This sirup should contain (regardless of the relative composition of nucleosides in XIII-mother liquor) only 5′-*O*-trityl-5-fluorouridines bearing sulfonyloxy groups on *both* the 2′- and 3′-positions. The sirup was detritylated with acid and the detritylated nucleosides refluxed in water for 20 hr. A 73% yield of crystalline 1-β-D-lxyofuranosyl-5-fluorouracil (based upon XIII-mother liquor) was obtained.

A good indication of the composition of nucleosides contained in XIII-mother liquor was obtained by treatment of this mother liquor with excess alkali at room temperature. Under these conditions, the following nucleosides would be expected to form: From the remaining 2′-*O*-tosyl derivative (III), anhydro formation followed by cleavage of the anhydro bond with the excess alkali would lead (*via* VII and VIII) to 1-β-D-arabinofuranosyl-5-fluorouracil (VI). If any 3′-*O*-tosyl isomer of III were present in XIII-mother liquor, only 3′-*O* - tosyl - 5′ - *O* - trityl - 5 - fluorouridine should result since it had been demonstrated previously[16] that *under these conditions* 3′-*O*-sulfonyloxyuridines (such as 3′-*O*-mesyluridine and its 2′,5′-di-*O*-trityl derivative) are unaffected by aqueous alkali. Should any 2′3′-di-*O*-tosylate of II be present in XIII-mother liquor, alkaline treatment should form a 2,2′-anhydro nucleoside which, upon cleavage of the anhydro linkage, would provide a *trans* system at 2′,3′ amenable to formation of a 2′,3′-epoxylyxosyl derivative. Epoxy-nucleosides of this type have been synthesized previously in this Laboratory[17] by alkaline treatment of XI (R = H, R′ = OBz, OMs, or H). Finally, if starting material II were present in the mother liquor, alkaline treatment followed by detritylation with acid should yield I.

XIII-Mother liquor was treated with three equivalents of dilute alkali at room temperature for one day and the reaction mixture detritylated with dilute hydrochloric acid. A precipitate (XV) was obtained which exhibited a nucleoside spectrum but which did not consume metaperiodate. The melting point, optical rotation and absorption spectrum differed from that exhibited by the anhydronucleoside V(R = F) while the elemental analysis was consonant with that for a 5-fluoro nucleoside minus one molecule of water. A comparison of the molecular rotation of XV (see Table I) with that for the 2′,3′-epoxide of 1-β-D-lyxofuranosyluracil[17] permits the designation of XV as the 2′,3′-epoxide of 1-β-D-lyxofuranosyl-5-fluorouracil.

The formation of this epoxide by alkaline treatment of XIII-mother liquor signified that this mother liquor contains the di-*O*-tosylated derivative (XIII, R,R′ = *p*-tosyl). Since only one equivalent of *p*-toluenesulfonyl chloride was used in the reaction of II to III, it may be concluded that an appreciable amount of unreacted II is also contained in XIII-mother liquor.

After the isolation of XV, the remaining solution (hereinafter designated as "XV-mother liquor") was examined by paper electrophoresis (borate buffers, *p*H 6 and 9.2).[18] Only two spots of approximately equal intensities were revealed, neither of which corresponded to XV (in *p*H 9.2 borate). The spot migrating the farther (anodically) corresponded to 5-fluorouridine (I) while the second spot migrated similarly to VI. (The lyxo isomer (XII) is absent and the xylo isomer may be excluded on the basis of previous studies by Gordon, Intrieri and Brown[18] who demonstrated that a good electrophoretic separation of 1-β-D-aldopentofuranosylthymine isomers (borate *p*H 6) is achieved by this method.) The ultraviolet absorption spectra of eluates of the two spots resembled those given by I and VI. Neither of these spectra resemble that expected for 3′ - *O* - tosyl - 5 - fluorouridine.[19]

Thus, in the over-all tosylation of II with one mole of *p*-toluenesulfonyl chloride, the major product (III) is formed in approximately 60% yield (of which 54% was isolated in crystalline form). A small amount of 2′,3′-di-*O*-*p*-tosylate is formed leaving some unreacted II. The 3′-*O*-tosyl isomer of III is not formed in detectable amounts.

These data differ from the results obtained by Brown, *et al.*[20] They tosylated 5′-*O*-acetyluridine under similar conditions and isolated 2′-*O*-tosyl-5′-*O*-acetyluridine as a major product as well as an approximately 20% yield of the 3′-*O*-tosyl isomer. (The presence of a 2′,3′-di-*O*-tosylate was not reported by them.)

TABLE I

1-β-D-Aldopentofuranosyluracils	$[\alpha]_D$	$[M]_D$	Difference in $[M]_D$
Uridine	+ 10[a]	+ 2,440	−2020
Ribosyl-5-fluorouracil (I)	+ 17	+ 4,460	
Arabinosyluracil	+126[21]	+30,770	−2790
Arabinosyl-5-fluorouracil (VI)	+128	+33,560	
Lyxosyluracil	+ 95[16]	+23,200	−2230
Lyxosyl-5-fluorouracil (XII)	+ 97	+25,430	
2′,3′-Epoxy-lyxosyluracil[17]	+ 34[b]	+ 7,690	
2′,3′-Epoxy-lyxosyl-5-fluorouracil (XV)	+ 42	+10,260	−2570

[a] D. T. Elmore, *J. Chem. Soc.*, 2084 (1950). [b] The authors are indebted to Dr. J. F. Codington for this value.

(16) N. C. Yung and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 3060 (1961).

(17) J. F. Codington, R. Fecher and J. J. Fox, Abstr. 139th Meeting, Am. Chem. Soc., St. Louis, April 1961, p. 13D.

(18) M. P. Gordon, O. M. Intrieri and G. B. Brown, *J. Am. Chem. Soc.*, **80**, 5161 (1958).

(19) 3′-*O*-Tosyl-5-fluorouridine is unfortunately not available for comparison. However, it is reasonable to assume that the spectrum of this compound would resemble that for its isomer, 2′-*O*-tosyl-5-fluorouridine (IV). The spectrum of the 2′-*O*-tosyl isomer is quite different from that for I or VI (see experimental).

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

(21) W. Bergmann and D. C. Burke, *J. Org. Chem.*, **20**, 1501 (1955).

Chemotherapy Studies.—Studies on these compounds were initiated against transplanted mouse leukemia B82.[3b] This line of leukemia has in the past demonstrated sensitivity to various 5-fluorinated pyrimidine derivatives,[22,23] and the results of these studies are shown in Table II. As can be seen, 1-β-D-arabinofuranosyl-5-fluorouracil (VI) had approximately as good a chemotherapeutic index as 5-fluoro-2′-deoxyuridine but required four times as much. VI showed a considerably better chemotherapeutic index than 5-fluorouridine (I). The lyxosyl analog (XII) was totally inactive at all dose levels tried.[24] It is to be noted that there was no weight loss with XII at any of the doses that were used so that it appears likely that much higher doses could be given.

TABLE II

DOSE RESPONSE OF LEUKEMIA B82 TO 5-FLUOROURACIL NUCLEOSIDES

Nucleoside	Dose, mg./kg./d × 10	Δ wt. *Rx/c*	Tumor wt. *Rx/c*	Inhibition, %
5-Fluoro-2′-deoxy-uridine	100	−4.4/+1.0	24/1229	98
	50	−0.5/+1.3	77/852	91
	25	−0.4/+1.8	159/902	82
	12.5	+0.2/+2.0	419/981	57
5-Fluorouridine (I)	3.3	−3.6/+1.1	35/453	92
	1.6	+0.1/+1.1	199/453	56
	0.8	+1.7/+1.1	341/453	24
1-β-D-Arabino-furanosyl-5-fluorouracil (VI)	471	−5.8/+2.0	0/1032	100
	393	+0.5/+4.0	46/723	94
	225	+1.7/+3.9	10/658	98
	150	+1.4/+3.9	38/658	94
	100	+2.0/+3.9	170/658	74
1-β-D-Lyxo-furanosyl-5-fluorouracil (XII)	210	+0.9/+1.0	975/931	−5
	105	+0.6/+1.0	935/931	0
	52	+1.4/+1.0	895/931	4

It would appear from these preliminary data that the arabinosyl analog (VI) is behaving more like 5-fluoro-2′-deoxyuridine than 5-fluorouridine, both in its over-all toxicity and in the relationship of toxicity to chemotherapeutic effect. 1-β-D-Lyxofuranosyl-5-fluorouracil, on the other hand, appears to be totally inactive at the levels tested and to be inactive at a dose level twice that at which VI is effective. These data would appear to demonstrate the importance of alterations in the sugar moiety on the toxicity and therapeutic activity of these 5-fluorinated nucleoside analogs.

Acknowledgments.—The authors wish to thank the Cancer Chemotherapy National Service Center for some of the 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose used in this investigation. The authors are deeply indebted to Dr. George Bosworth Brown of this Institute for helpful suggestions and continued interest.

(22) J. H. Burchenal and H. F. Oettgen, *Cancer Chemotherapy Reports* (Cancer Chemotherapy National Service Center), **2**, 16 (1959).

(23) J. H. Burchenal, H. F. Oettgen, J. A. Reppert and V. Coley, *ibid.*, **6**, 1 (1960).

(24) Higher doses of XII will be tried when more is available.

Experimental[25]

5-Fluorouracilmercury.[2b,26]—Mercuric acetate (0.1 mole) was dissolved in 600 ml. of methanol under stirring and refluxing. A hot solution of 13.0 g. (0.1 mole) of 5-fluorouracil in 250 ml. of water was added causing an immediate precipitation of 5-fluorouracilmercury. The mixture was allowed to cool while stirring. The fine precipitate was filtered and dried in a desiccator. The yield (33 g.) was quantitative. The compound does not melt below 360°.

Anal. Calcd. for $C_4HFN_2O_2Hg$: N, 8.52. Found: N, 8.02.

1-(2′,3′,5′-Tri-*O*-benzoyl-β-D-ribosyl)-5-fluorouracil.—A mixture of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose[13] (0.02 mole) in 250 ml. of anhydrous ether was saturated with hydrogen chloride at 0°. After six days at 5°, the solvent was removed *in vacuo* and the light sirup treated with 10 ml. of benzene. The benzene was removed *in vacuo* (bath temperature not exceeding 40°) and the sirup treated again with benzene. After removal of the solvent, the sirup was treated with toluene and the solution added to a previously azeotroped, refluxing mixture of 5-fluorouracilmercury (0.01 mole) in 350 ml. of dry toluene. The well-stirred mixture became homogeneous with slight yellowing. After 0.5 hr. of refluxing, the hot toluene was filtered and the filtrate concentrated *in vacuo* to a glass. The glass was dissolved in 100 ml. of ethyl acetate and washed twice with 50-ml. portions of 30% potassium iodide and finally with water. The organic layer was dried over sodium sulfate, filtered and concentrated under vacuum to a glass. The glass was treated with 25 ml. of warm chloroform whereupon crystallization occurred. After cooling, the solid was removed and washed with a small amount of cold chloroform and ether. From two crops, a yield of 74% was obtained, m.p. 207–209D°, $[\alpha]^{28}$ −33° (*c* 2.0, $CHCl_3$). Recrystallization from ethyl acetate–ether gave an analytical sample, m. p. 207–209°

Anal. Calcd. for $C_{30}H_{23}FN_2O_9$: C, 62.72; H, 4.04; N, 4.88. Found: C, 62.54; H, 4.01; N, 4.86.

5-Fluorouridine (I).—The above tri-*O*-benzoate (4.5 g.) in 100 ml. of 50% ethanol was treated with stirring with 20 ml. of 1 *N* sodium hydroxide. After 2 hr. the *p*H of the light-yellow solution was between 10–12. (It is important that the consumption of alkali with time ceases.) The solution was neutralized with glacial acetic acid and most of the ethanol was removed. The aqueous solution was treated batchwise with Dowex 50 (H^+). (During the successive treatments with Dowex, benzoic acid precipitated.) The precipitated benzoic acid was removed along with the Dowex resin by filtration. (The resin treatment is complete when benzoic acid no longer precipitates and the filtrate exhibits a negative flame test for sodium ion.) The aqueous filtrate was washed three times with chloroform and then taken to dryness *in vacuo*. The semi-solid obtained was azeotroped with toluene to remove water and dissolved in a minimum amount of hot absolute ethanol. Upon cooling, 5-fluorouridine crystallized, m.p. 180–182° (88%). Recrystallization from absolute ethanol did not alter the melting point. This material exhibited only one spot (paper electrophoresis, borate, *p*H 6.2) and was sufficiently pure for use in the further syntheses described below. For chemotherapeutic studies and elemental analyses, a sample of 5-fluorouridine was chromatographed on Dowex 1 (formate). The material obtained melted at 184–185°, $[\alpha]^{26}D$ +17° (*c* 2.0, water). Light absorption data: in 1 *N* hydrochloric acid, maximum at 269 mμ, ϵ_{max} 8950; minimum at 234 mμ, ϵ_{min} 1680. Spectrophotometrically determined pK_a = 7.57.[27]

Anal. Calcd. for $C_9H_{11}FN_2O_6$: C, 41.22; H, 4.23; F, 7.25; N, 10.68. Found: C, 41.04; H, 4.67; F, 7.12; N, 10.73.

1-(5′-*O*-Trityl-β-D-ribofuranosyl)-5-fluorouracil (II).—5-Fluorouridine (8.3 g.) was treated with 9.8 g. of triphenylmethyl chloride in 80 ml. of dry pyridine. The amber

(25) Melting points are corrected unless stated otherwise. Analytical data by Spang Microanalytical Laboratories, Ann Arbor, Mich., and by Dr. Al Steyermark of Hoffmann LaRoche, Inc.

(26) The authors are indebted to Dr. Max Hoffer of Hoffmann LaRoche, Inc., for his unpublished data for this preparation.

(27) The authors are indebted to Miss Iris Wempen for spectrophotometric determination of *p*Ka values.

solution was kept at 5° for 16 hr. after which it was heated on a steam-bath for 2 hr. The cooled solution was poured in a thin stream into one liter of stirred ice-water whereupon a reddish gum separated. After decantation, the gum was treated again with ice-water. After decantation, the gummy residue was dissolved in ether and dried over sodium sulfate, filtered and the solution was evaporated to a glass *in vacuo*. The glass was dissolved in a minimum amount of ether (~100 ml.). Benzene was added to faint opalescence and with cooling and scratching long, colorless needles separated (94%), m.p. 115–119°. (This product contains solvent of crystallization.) Recrystallization from ethanol gave colorless rosettes, m.p. 202–203°. Ultraviolet absorption data: in ethanol, maximum at 268.5 mμ, ϵ_{max} 9350; minimum at 245 mμ, ϵ_{min} 4780; $[\alpha]^{24}D$ +42° (*c* 0.4, ethanol).

Anal. Calcd. for $C_{28}H_{25}FN_2O_6$: C, 66.67; H, 5.00; N, 5.55. Found: C, 66.77; H, 5.12; N, 5.76.

1-(2′-*O*-*p*-Tosyl-5′-*O*-trityl-β-D-ribosyl)-5-fluorouracil (III).—A solution of II (4.5 g.) in 20 ml. of dry pyridine was treated with 1.87 g. of *p*-toluenesulfonyl chloride. The mixture was agitated until a clear solution was obtained and allowed to remain at room temperature for 16 hr. Ethanol (20 ml.) was added and the solvents removed. The reddish sirup was dissolved in ethanol (60 ml.) with warming (40°) whereby precipitation of a granular solid occurred. Complete crystallization was effected by cooling. The solid (52%) was collected on a filter and washed thoroughly with cold ethanol. Recrystallization from ethanol gave colorless rosettes, m.p. 178–179°; $[\alpha]^{24}D$ + 24° (*c* 0.24, EtOH). Ultraviolet absorption data: in ethanol, maximum at 263 mμ, ϵ_{max} 6800; minimum at 248 mμ, ϵ_{min} 4650.

Anal. Calcd. for $C_{35}H_{31}FN_2O_8S$: C, 63.81; H, 4.74; N, 4.25; S, 4.88. Found: C, 63.98; H, 5.20; N, 4.49; S, 4.85.

The ethanolic mother liquors (designated as XIII-mother liquor on the flow chart) were combined and used in subsequent reactions.

1-(2′-*O*-*p*-Tosyl-β-D-ribofuranosyl)-5-fluorouracil (IV).—III (1.5 g.) was added to 50 ml. of ethanol (previously saturated with hydrogen chloride) and warmed for 10 min. at 50–60°. The solution was concentrated *in vacuo* to a sirup (bath temperature below 40°). The sirup was triturated with ether repeatedly in order to remove triphenylcarbinol. A granular solid remained (0.84 g.) which was crystallized from water–ethanol (3:1) to give pure IV, m.p. 162–163°, $[\alpha]D^{24}$ −34° (*c* 0.55, EtOH). Ultraviolet absorption data: in ethanol, maximum at 266 mμ, ϵ_{max} 7120; minimum at 245 mμ, ϵ_{min} 4000.

Anal. Calcd. for $C_{16}H_{17}FN_2O_8S$: N, 6.73; S, 7.71. Found: N, 6.67; S, 7.43.

2,2′-Anhydro-1-(β-D-arabinofuranosyl)-5-fluorouracil (V, R = F).—Sodium hydroxide (0.5 *N*, 0.9 ml.) was added to 0.2 g. of IV in 10 ml. of ethanol. The solution was allowed to stand for 10 min., then adjusted to *p*H 5–6 with dilute acetic acid and the solvent evaporated *in vacuo*. The residue was dissolved in 5 ml. of water, and the solution was passed through a small column of Dowex 50 (H^+ form) (12 × 1.5 cm). The column was washed with water until no ultraviolet absorbing material was found in the eluate. The acidic eluates were combined and evaporated *in vacuo* at room temperature. Repeated ether extractions of the sirup gave a residue free of acid (ether extractions discarded). Product crystallized readily from absolute ethanol as colorless platelets, 100 mg., m.p. 196–197°, $[\alpha]^{24}D$ −61° (*c* 0.28, EtOH). Ultraviolet absorption data: at *p*H 5–6, maxima at 222.5 and 254 mμ, ϵ_{max} 7210 and 9050, respectively; minimum at 234 mμ, ϵ_{min} 5620.

Anal. Calcd. for $C_9H_9FN_2O_5$: C, 44.28; H, 3.73; N, 11.47. Found: C, 44.48; H, 3.95; N, 11.42.

1-β-D-Arabinofuranosyl-5-fluorouracil (VI) from III.—Sodium hydroxide (1 *N*, 12 ml.) was added to a stirring suspension of 4 g. of III in 100 ml. of 50% ethanol. The mixture became clear after the addition of alkali and was stirred at room temperature for 3 hr. The solution was adjusted to *p*H 5–6 with acetic acid and most of the ethanol was removed *in vacuo*. The precipitated VIII was collected on a filter and washed thoroughly with water (to remove sodium tosylate). The crude nucleoside was placed in 100 ml. of 50% ethanol, containing 5 drops of concentrated hydrochloric acid and refluxed for 1 hr. Approximately 75 ml. of solvent was removed *in vacuo* and the precipitated triphenylcarbinol was filtered and discarded. The aqueous filtrate was extracted three times with 15-ml. portions of chloroform and finally concentrated to a light sirup (bath temperature was kept below 50°). Ethanol was added and evaporated *in vacuo* repeatedly. The neutral sirup was dissolved in about 10 ml. of absolute ethanol in which crystallization of colorless prisms occurred. A crude yield (88%) was obtained in three crops. Recrystallization from ethanol gave an analytical sample, m.p. 187–188°, $[\alpha]^{24}D$ +128° (*c* 0.21, H_2O). (Reist, *et al.*, report +108°[9b]). Ultraviolet absorption data: at *p*H 5–6, maximum at 269.5 mμ, ϵ_{max} 9170; minimum at 234 mμ, ϵ_{min} 1780. Spectrophotometrically determined pK_a = 7.63.[27] Treatment of VI with sodium metaperiodate and titration of aliquots with arsenite showed a slow uptake of one mole of oxidant per mole which was essentially complete after approximately 24 hr. Formic acid was not liberated.

Anal. Calcd. for $C_9H_{11}FN_2O_6$: C, 41.23; H, 4.23; F, 7.25; N, 10.68. Found: C, 41.21; H, 4.48; F, 7.09; N, 10.68.

1-β-D-Arabinofuranosyl-5-fluorouracil (VI) from V (R = F).—A sample of V (R = F, 4 mg.) in 2 ml. of water was refluxed for 4 hr. The reaction was followed spectrophotometrically and showed no alteration of the spectrum during this time period. Hydrochloric acid (2 *N*, 2 drops) was added and refluxing continued for 4 hr. Paper electrophoresis (3 hr., 700 volts, *p*H 9.2, borate buffer[18]) of the refluxed solution showed only one spot at an identical distance from the origin as an authentic sample of VI.

1-β-D-Arabinofuranosyluracil.[21]—Palladium–charcoal (5%, 100 mg.) was added to 100 mg. of VI in 10 ml. ethanol containing one drop of triethylamine. The mixture was shaken under hydrogen at room temperature and atmospheric pressure for 20 hr. The mixture was passed through a filter and the filtrate concentrated to a light sirup *in vacuo*. Dilute ammonium hydroxide was added to the sirup and the solution refluxed for 20 min. Solvents again were removed, then absolute ethanol was added and the solution cooled. Colorless prisms were collected, m.p. 217–219°. A mixed melting point with a sample of 1-β-D-arabinofuranosyluracil prepared by another route[28] showed no depression.

1-β-D-Arabinofuranosyluracil from V (R = H).—A sample of V[10,29] (R = H, 2 mg.) was refluxed for 4 hr. in 2 ml. of water and the reaction followed spectrophotometrically. Spectral shifts were noted when reflux temperature was reached. The solution was chromatographed electrophoretically (3 hr., 700 volts, *p*H 9.2 borate buffer) after 4 hr. of reflux. Two spots were found, one at the same distance as a known sample of 1-β-D-arabinofuranosyluracil.[28] The other spot (like V) did not leave the origin.

1-(2′,3′-Di-*O*-mesyl-5′-*O*-trityl-β-D-ribosyl)-5-fluorouracil (IX).—A solution of II (3 g.) in 30 ml. of dry pyridine at 0–5° was treated while stirring with 0.9 ml. of methylsulfonyl chloride. The mixture was allowed to stand at 5° for 16 hr., then poured into 500 ml. of ice-water and stirred for 1 hr. An ochre-colored solid separated and was collected on a filter and washed thoroughly with water. After drying *in vacuo* the amorphous solid weighed 3.15 g. Crystalline IX was not obtained, and the amorphous solid was used in the following reaction without further purification.

1-(2′,3′-Di-*O*-mesyl-β-D-ribofuranosyl)-5-fluorouracil (X).—Concentrated hydrochloric acid (10 drops) was added to crude IX (3.15 g.) in 150 ml. of ethanol and was refluxed for 10 min. The ethanolic solution was evaporated to dryness and the residue triturated repeatedly with hot ether until it became crystalline. A yellow solid, 2.0 g., m.p. 160–163°, was obtained. Recrystallization, using preheated 50% ethanol, gave a pure sample, m.p. 172–173°, $[\alpha]^{24}D$ +26° (*c* 0.2, EtOH). Ultraviolet absorption data: in ethanol, maximum at 263 mμ, ϵ_{max} 9190; minimum at 234 mμ, ϵ_{min} 3400.

Anal. Calcd. for $C_{11}H_{16}N_2O_{10}S_2$: C, 31.57; H, 3.61. Found: C, 31.39; H, 3.77.

(28) J. F. Codington, R. Fecher and J. J. Fox, *J. Am. Chem. Soc.*, **82**, 2794 (1960).

(29) The authors are indebted to Dr. D. M. Brown of the University Chemical Laboratory, Cambridge, England, for a sample of this compound.

2,2′-Anhydro-1-(3′-*O*-mesyl-β-D-arabinofuranosyl)-5-fluorouracil (XI, R = F, R′ = OH).—Sodium hydroxide (0.1 *N*, 2 ml.) was added to a solution of 80 mg. of X in 6 ml. of water. The resulting solution was neutral. Crystallization occurred on standing and the colorless prisms (60 mg.) were collected, m.p. 192–197° dec. Recrystallization from water gave a pure sample, m.p. 196–198° dec., $[\alpha]^{24}D$ −75° (*c* 0.26, H_2O). Ultraviolet absorption data: at *p*H 5–6, maxima at 222.5 and 254 mμ, ϵ_{max} 6730 and 8850, respectively; minimum at 233 mμ, ϵ_{min} 5540.

Anal. Calcd. for $C_{10}H_{11}FN_2O_7S$: C, 37.26; H, 3.44; N, 8.69; S, 9.96. Found: C, 37.20; H, 3.79; N, 8.91; S, 9.93.

1-β-D-Lyxofuranosyl-5-fluorouracil (XII). Method A from X.—Water (50 ml.) was added to 1 g. of X and refluxed for 4–5 hr. Titration of an aliquot showed that two equivalents of methylsulfonic acid were liberated. The solvent was concentrated to a light sirup and triturated repeatedly with ether to extract the acid. Crystallization occurred on addition of ethanol, 0.45 g., m.p. 195–196°. Recrystallization from 95% ethanol afforded a pure sample, m.p. 203.5–204°, $[\alpha]^{26}D$ + 97° (*c* 0.3, H_2O). Ultraviolet absorption data: at *p*H 5–6, maximum at 269 mμ, ϵ_{max} 9190; minimum at 234 mμ, ϵ_{min} 1760. When treated with metaperiodate, XII consumed one mole of oxidant per mole of nucleoside *rapidly* (within 3 minutes) without the liberation of formic acid. Titration of aliquots after this 3 minute period showed no further uptake of metaperiodate.

Anal. Calcd. for $C_9H_{11}FN_2O_6$: C, 41.22; H, 4.23; F, 7.25; N, 10.69. Found: C, 40.98; H, 4.62; F, 7.11; N, 10.70.

Method B from XI (R = F, R′ = OH).—Methylsulfonic acid (1 *N*, 0.06 ml.) was added to 20 mg. of XI (R = F, R′ = OH) in 5 ml. of water and refluxed for 4 hr. Only one ultraviolet absorbing spot was found, migrating identically as 1-β-D-lyxofuranosyl-5-fluorouracil (XII), in paper electrophoresis (4 hr., 700 volts, *p*H 6, borate buffer[18]).

1-β-D-Lyxofuranosyl-5-fluorouracil (XII) from XIII-Mother Liquor.—The ethanolic mother liquor (containing theoretically 0.009 mole of products) from the reaction of II to III (*vide supra*) was concentrated *in vacuo* to a sirup and dried azeotropically with small portions of toluene. The sirup was dissolved in 50 ml. of anhydrous pyridine, cooled to 5° and treated dropwise with an excess (3 ml.) of methylsulfonyl chloride. After storage at 5–10° overnight, the contents were poured in a thin stream into 1 l. of stirred ice-water. The amorphous solid was collected and washed with water. The solids were added to 300 ml. of 30% ethanol and treated with 1 ml. of concentrated hydrochloric acid. This mixture was refluxed for 20 hr., after which the clear, yellow solution was concentrated to a small volume (10 ml.) *in vacuo* (bath temperature not exceeding 40°). An amorphous solid formed, was collected and triturated with warm diethyl ether to remove sulfonic acids and triphenylcarbinol. The solid was dissolved in ~250 ml. of warm ethanol, treated with charcoal, filtered and the filtrate cooled. Colorless needles were obtained, 73% (based upon XIII-mother liquor). Recrystallization from 95% ethanol gave a pure sample of XII, m.p. 203.5–204°. The product was identical with XII obtained previously.

Determination of Components in XIII-Mother Liquor. A. Presence of 5′-*O*-Trityl-2′,3′-di-*O*-*p*-tosyl-5-fluorouridine Proved by Synthesis of 1-(2′,3′-Epoxy-β-D-lyxofuranosyl)-5-fluorouracil (XV).—XIII-Mother liquor (0.028 mole) in ethanol was concentrated to a light sirup and treated repeatedly with water to remove salts. The aqueous triturates were discarded and the amorphous solids treated with 400 ml. of 50% ethanol. Sodium hydroxide (50 ml. of 1 *N*) was added to the stirred mixture at room temperature. After stirring overnight, the clear solution was neutralized with acetic acid (to *p*H 6) and evaporated *in vacuo* to a gum. The gum was dissolved in 500 ml. of chloroform and the chloroform solution washed several times with water. The aqueous washings were discarded and the organic layer was taken to a sirup (14 g.). The sirup was treated with 200 ml. of 50% ethanol containing 1 ml. of concentrated hydrochloric acid and the mixture refluxed for 1 hr. The clear solution was reduced in volume *in vacuo* to approximately 10 ml., 50 ml. of water added and the solution extracted several times with chloroform. (The chloroform extract contains 6.5 g. of triphenylcarbinol, theor = 7.3 g.) The aqueous layer was concentrated to a light sirup and treated with *ca.* 15 ml. of ethanol. Colorless short needles precipitated, 1.0 g., (14% yield based on XIII-mother liquor or 6.3% based on the over-all reaction from II), m.p. 193–197°. One recrystallization from ethanol gave an analytical sample of XV, m.p. 198–200°, $[\alpha]^{23}D$ +42° (*c* 0.75, water). Ultraviolet absorption data: at *p*H 5–6, maximum at 266 mμ, ϵ_{max} 8740; minimum at 232.5 mμ, ϵ_{min} 1910. The epoxide (XV) did not consume metaperiodate over a period of one day.

Anal. Calcd. for $C_9H_9FN_2O_6$: C, 44.26; H, 3.71; N, 11.47. Found: C, 44.50; H, 3.46; N, 11.50, 11.69.

The mother liquors after epoxide removal (hereinafter designated as "XV-mother liquor") were used in the next experiment.

The isolation of this epoxide permits the conclusion (see text) that XIII (R,R′ = *p*-tosyl) is a component of XIII-mother liquor in amounts equivalent to the percentage of XV.

B. Presence of II and III in XV-Mother Liquor Proved by Determination of I and VI.—The ethanolic mother liquors remaining after isolation of epoxide (XV) above was examined electrophoretically in two systems (borate buffers, 700 volts, *p*H 6, 4 hr.; *p*H 9.2, 3 hr.[18]). Only two ultraviolet absorbing spots were revealed. The farther spot (anodically) corresponded to 5-fluorouridine (I) while the other corresponded to VI. (Epoxide XV appears to be absent in this mother liquor. Its electrophoretic migration differs from I and VI in the *p*H 9.2 borate system.) Each of these spots was eluted and its spectrum determined. The spectra were similar to those for I and VI and did not resemble the spectrum of IV.[19] For the spot corresponding to I, maximum at 268 mμ, minimum at 234 mμ, ratio max./min. = 5.7. For pure I (see above), ratio max./min. = 5.3. For the spot corresponding to VI, maximum at 268 mμ, minimum at 234 mμ, ratio max./min. = 4.4. For pure VI (see above), ratio max./min. = 5.15. For pure IV (see above) ratio max./min. = 1.78.

Spectrophotometric Studies.—The pK_a values[27] were determined spectrally by methods previously employed[30,31] and are accurate to within 0.05 *p*H unit.

Metaperiodate Studies.—Periodate oxidations were performed according to procedures employed in previous papers in this series.[8,12]

(30) D. Shugar and J. J. Fox, *Biochim. et Biophys. Acta*, **9**, 199 (1952).

(31) J. J. Fox and D. Shugar, *Bull. soc. chim. Belges*, **61**, 44 (1952).