Fluorinated Pyrimidines. XXIX. Syntheses of 2′,3′-Dehydro-5-fluoro-2′-deoxyuridine and 2′,3′-Dideoxy-5-fluorouridine[1]

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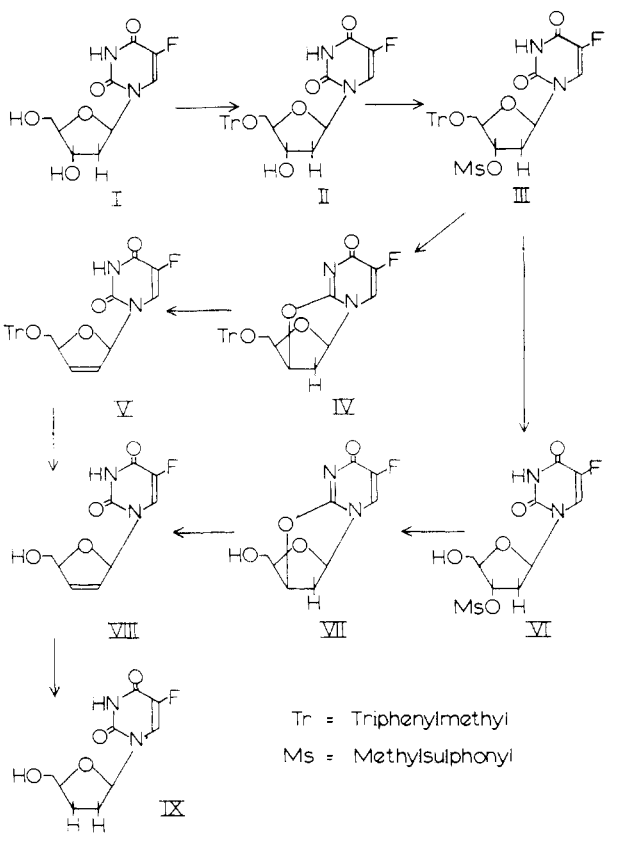
2,3′-Anhydro-1-(2-deoxy-β-D-lyxofuranosyl)-5-fluorouracil (VII) on treatment with potassium *t*-butoxide in anhydrous dimethyl sulfoxide gave 1-(2,3-dideoxy-2,3-didehydro-β-D-*glycero*-pentofuranosyl)-5-fluorouracil (VIII, DHFUDR). DHFUDR was phosphorylated with β-cyanoethyl phosphate, followed by an alkaline treatment to obtain the corresponding 5′-phosphate (DHFUDRP). The nucleoside (VIII) was hydrogenated with palladium on charcoal catalyst to provide 1-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-5-fluorouracil (IX, 2′,3′-dideoxy-5-fluorouridine) which has little biological activity. In a minimal medium, DHFUDR is bactericidal against *Escherichia coli* B; it is not a substrate for nucleoside phosphorylase, uridine kinase, or thymidine kinase. In cell culture experiments thymidine kinaseless Novikoff hepatoma cells are 5000-fold resistant to FUDR (I) but only 50-fold resistant to DHFUDR. Similarly, mouse leukemia L5178BF cells (lacking thymidine kinase) are 1000-fold resistant to FUDR and not at all resistant to DHFUDR. *In vivo* DHFUDR is a powerful inhibitor of Sarcoma 180 and mouse leukemia L1210. Animals with L5178BF (FUDR-resistant) transplanted leukemias showed an equally good response to VIII as those with L5178Y (FUDR-sensitive) transplants. A preparation of DHFUDR-2-^{14}C is described, and a new color test for 2′,3′-unsaturated nucleosides is reported.

A number of fluorinated pyrimidines and their nucleosides have been synthesized in this laboratory.[3,4] 5-Fluorouracil[5] (FU) and 5-fluoro-2′-deoxyuridine[6,7] (I, FUDR) have been shown to produce significant objective responses in patients suffering from advanced solid tumors, particularly with gastrointestinal and breast carcinomas.[8,9] These compounds are metabolized to 5-fluoro-2′-deoxyuridine 5′-phosphate (FUDRP), which inhibits thymidylate synthetase,[10-12] the enzyme responsible for the conversion of 2′-deoxyuridine 5′-phosphate to thymidine 5′-phosphate. Thus these analogs inhibit DNA synthesis and the growth of rapidly dividing normal tissues and tumors. Both FU and FUDR are more rapidly catabolized by normal cells than by tumors, and thus some selectivity against tumors is achieved.[13] Two factors prevent more successful chemotherapeutic efficacy of FUDR: (a) its cleavage to FU by nucleoside phosphorylase,[14] and (b) the emergence of cellular resistance.[3] The former reduces the potency of the drug, whereas the latter somehow prevents the formation of FUDRP, the active drug. The mechanism of resistance to FUDR thus far has been shown to result from the loss of thymidine kinase (which also phosphorylates FUDR) in the resistant cells.[15]

CHART I



Various attempts have been made (*cf.* ref 3 and 4) to modify the base, sugar, or both of these moieties of FUDR (I) to produce an analog that would not be susceptible to cleavage by nucleoside phosphorylase[14] (thus increasing the potency of the drug) or that would be phosphorylated in a different way and hence overcome the problem of resistance. This report describes the preparation (Chart I) and biological properties of two such derivatives, 1-(2,3-dideoxy-2,3-didehydro-β-D-*glycero*-pentofuranosyl)-5-fluorouracil (VIII, 2′,3′-

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(2) American Cancer Society Professor of Oncology.

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dehydro-5-fluoro-2′-deoxyuridine, DHFUDR) and 1-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-5-fluorouracil (IX, 2′,3′-dideoxy-5-fluorouridine).

Although 2′,3′-dideoxynucleoside derivatives have been known for a long time,[16] their importance was only recently pointed out by Robins and co-workers[17,18] as possible chain terminators of DNA biosynthesis. The elucidation of the structure of nucleoside antibiotics, such as blasticidin S (a 2′,3′-unsaturated pyranoside),[19] and angustmycin A (a furanoside with an exocyclic 4′,5′ double bond),[20] along with the observation of Novak and Sorm[21] that 9-(2,3-dideoxy-2,3-didehydro-β-*erythro*-hex-2-enosyl)adenine has considerable carcinostatic activity against a transplanted leukemia 1210 in mice, prompted us to synthesize DHFUDR. We used to method of Horwitz, *et al.*,[22,23] to convert the 2,3′-anhydronucleosides (IV and VII) to the corresponding 2′3′-unsaturated derivatives (V and VIII). The unsaturated compound (VIII) was catalytically hydrogenated to obtain 2′,3′-dideoxy-5-fluorouridine (IX).

1-(2-Deoxy-5-*O*-trityl-β-D-ribofuranosyl)-5-fluorouracil[24] (II) was treated with an equivalent amount of methanesulfonyl chloride in pyridine to obtain its 3′-*O*-mesyl derivative (III). The crude III was treated with potassium *t*-butoxide (2 equiv) in dimethyl sulfoxide at room temperature for 30 min to obtain 1-(5-*O*-trityl-2,3-dideoxy-2,3-didehydro-β-D-*glycero*-pentofuranosyl)-5-fluorouracil (V) in over 80% yield. The latter could also be prepared by converting the mesylate (III) to 2,3′-anhydro-1-(2-deoxy-5-*O*-trityl-β-D-lyxofuranosyl)-5-fluorouracil (IV) by alkaline treatment[25] (58%), followed by base-catalyzed decyclization with potassium *t*-butoxide (1 equiv) in dimethyl sulfoxide (89.3%). Detritylation of V gave VIII in good yields. The pmr spectra of VIII showed two adjacent vinyl protons located at δ 6.50 and 5.96, respectively. The anomeric proton was a multiplet (spectra taken in D_2O) centered at δ 6.96 with a coupling constant of 2 cps. The structure of VIII was further confirmed by its elemental analysis and conversion to the corresponding 2′,3′-dideoxynucleoside (IX). DHFUDR was found to be quite sensitive to acid hydrolysis. At 95–100° in 0.1 *N* HCl VIII was completely converted to FU within 5 min. However, at room temperature it was stable under similar acid conditions up to 30 min; after 1 hr, traces (5–7%) of FU could be detected. DHFUDR is stable to alkaline hydrolysis; at 95–100° aqueous 0.1 *N* NaOH produced no hydrolysis up to 30 min, whereas under similar conditions at room temperature VIII was stable for more than 3 hr.

It was found that acid-catalyzed detritylation of V invariably resulted in DHFUDR contaminated with traces of FU; this was more apparent in large-scale preparations. The known biological activity of FU forced us to devise a route that would eliminate the acid treatment after generation of the 2′,3′ double bond. 2,3′-Anhydro-1-(2-deoxy-β-D-lyxofuranosyl)-5-fluorouracil (VII) was prepared from FUDR by the method of Fox and Miller[24] and treated at room temperature with potassium *t*-butoxide in dimethyl sulfoxide for 2.15 hr to obtain DHFUDR (64%) in a high state of purity. This constitutes the first instance of the conversion of a 2,3′-anhydronucleoside unprotected at the 5′ position to a 2′,3′-unsaturated nucleoside derivative. It may be noted that the presence of a trityl group at the 5′ position puts a considerable strain on 2,3′-ether linkage of an anhydronucleoside which, as a consequence, makes the base-catalyzed rearrangement to 2′,3′-unsaturated nucleosides much more favorable. The same effect has been demonstrated by other workers[23] when the bulky 5′-*O*-trityl group was replaced by a mesyl group, or by using a more stable 3′,5′-oxetane derivative. Thus, rearrangement of anhydronucleosides unprotected at the 5′ position invariably took 2 or more hr for completion, whereas under similar conditions a 5′-mesylanhydronucleoside[23a] required 60 min, and our tritylated derivative (IV) was completely converted to V in 10–15 min.[26] The 2′,3′-unsaturated nucleoside, DHFUDR, gave a purple color on chromatograms when sprayed and heated (3–5 min) with Hanes–Isherwood reagent.[27] This color test appears to be universal for all the 2′,3′-unsaturated nucleosides and may prove to be a useful tool for the chemical study of such compounds.[28]

The biological activity of DHFUDR made it necessary to prepare it labeled with ^{14}C (starting with FUDR-2-^{14}C) as well as its 5′-phosphate derivative. The nucleotide was synthesized by phosphorylating DHFUDR with β-cyanoethyl phosphate and dicyclohexylcarbodiimide in pyridine according to the method of Tener;[29] subsequent alkaline treatment gave 1-(2,3-dideoxy-2,3-didehydro-β-D-*glycero*-pentofuranosyl)-5-fluorouracil 5′-phosphate (X, DHFUDRP), which was isolated as its barium salt. The structure of X was proved by its hydrolysis with prostatic phosphomonoesterase to DHFUDR, which was characterized by paper chromatography, electrophoresis, and the color test with molybdate spray mentioned previously.

2′,3′-Dideoxy-5-fluorouridine (IX) was prepared by hydrogenation of DHFUDR (VIII) in the presence of

(16) A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, 816 (1955).

(17) M. J. Robins and R. K. Robins, *J. Am. Chem. Soc.*, **86**, 3585 (1964).

(18) M. J. Robins, J. R. McCarthy, and R. K. Robins, *Biochemistry*, **5**, 224 (1966).

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(20) H. Hoekrema, G. Slomp, and E. E. von Tamelen, *ibid.*, 1787 (1964).

(21) J. J. K. Novak and F. Sorm, *Experientia*, **18**, 213 (1962).

(22) Treatment of 1-(2-deoxy-3-*O*-mesyl-5-*O*-trityl-β-D-ribofuranosyl)-5-fluorouracil (III) with excess sodium methoxide in anhydrous DMF (refluxed at 100–110° for 24 hr), followed by an acid treatment led us to an independent synthesis of DHFUDR in low yields. The structure of this compound was later confirmed by its preparation by the method of Horwitz, *et al.*[23a]

(23) (a) J. P. Horwitz, J. Chua, M. A. DaRooge, M. Noel, and I. L. Klundt, *J. Org. Chem.*, **31**, 205 (1966); (b) J. P. Horwitz, J. Chua, M. Noel, and J. T. Donatti, *ibid.*, **32**, 817 (1966), and references cited therein.

(24) J. J. Fox and N. C. Miller, *ibid.*, **28**, 936 (1963).

(25) J. P. Horwitz, J. Chua, J. A. Urbanski, and M. Noel [*J. Org. Chem.*, **28**, 942 (1963)] used the same method to prepare the corresponding thymine derivative.

(26) Similar treatment of 1-(2-deoxy-3-*O*-mesyl-5-*O*-trityl-β-D-ribofuranosyl)-5-trifluoromethyluracil with potassium *t*-butoxide in anhydrous dimethyl sulfoxide gave 1-(5-*O*-trityl-2,3-dideoxy-2,3-didehydro-β-D-*glycero*-pentofuranosyl)-5-trifluoromethyluracil. The reaction was complete in 10–15 min and the product was characterized by its pmr and ultraviolet absorption spectra (T. A. Khwaja and C. Heidelberger, unpublished results).

(27) C. S. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949).

(28) The chromatograms were sprayed with molybdate reagent, dried in a current of warm air to remove most of the moisture, and then heated in a drying oven at 100° for 2–3 min. The 2′,3′-unsaturated derivatives of uridine, thymidine, cytidine, and adenosine (kindly provided by Dr. J. P. Horwitz) all gave a positive color test. The presence of a protecting group at 5′ position of a nucleoside, *e.g.*, trityl (in DHFUDR), benzoyl (in uridine derivative), or thiethyl [9-(2,3-dideoxy-5-*S*-ethyl-5-thio-β-D-*glycero*-pentofuranosyl)adenine (kindly provided by Dr. R. K. Robins)], did not interfere with the color reaction.

(29) G. M. Tener, *J. Am. Chem. Soc.*, **83**, 159 (1961).

a palladium–charcoal catalyst in dioxane. The reaction was complete in a short time, but invariably resulted in some concomitant hydrogenolysis of the glycosidic linkage and of the fluoro group from the 5′ position of the nucleoside, because the reaction product always contained small amounts of FU and an unknown nucleoside (its ultraviolet absorption maximum was 262 mμ at pH 12). This facile hydrogenolysis of the glycosidic linkages of 2′,3′-unsaturated nucleosides had not been mentioned in the literature[23a,30] until quite recently.[23b] The pmr spectrum of IX showed a multiplet at δ 1.75–2.5 corresponding to four 2′,3′-protons. The anomeric proton was a multiplet (Robins, *et al.*,[18] obtained a triplet in the corresponding adenosine analog) centered at δ 5.96. This along with the elemental analysis confirms the structure of compound IX.

Biological Activity.—In bacterial studies DHFUDR (VIII) was found to be lethal to *E. coli* B in a minimal media (M9) at a concentration of less than 50 μg/ml (FUDR at the same concentration showed more toxicity).[31] The killing effect of DHFUDR was partially reversed by thymidine (500 μg/ml) and completely reversed by uridine (250 μg/ml). By contrast, the toxic effect of FUDR was completely reversed by thymidine and addition of uridine enhanced its toxicity to the bacterium.[31] This in not surprising as uridine has been shown to prevent the cleavage of FUDR to FU by nucleoside phosphorylase.[14] 2′,3′-Dideoxy-5-fluorouridine (IX) killed *E. coli* B at a concentration of 50 μg/ml, but it was considerably less active than either DHFUDR or FUDR; its toxic effect was partially reversed by thymidine (500 μg/ml) and completely by uridine (250 μg/ml).

In cell-culture studies[32] 10^{-5} *M* DHFUDR inhibited the growth of Hela cells (10^{-6} *M* FUDR produced a similar effect). The inhibition, as with FUDR, was reversed by thymidine, but not by uridine. The growth of Novikoff hepatoma cells was 50% inhibited by 10^{-6} *M* DHFUDR, and 10^{-7} *M* DHFUDR completely inhibited L5178Y mouse leukemia cells.[33]

DHFUDR, incubated *in vitro* with Ehrlich ascites cells, inhibited the incorporation of ^{14}C-formate into DNA-thymine.[34] The concentration of DHFUDR necessary for 50% inhibition was $10^{-4.5}$ *M* as compared to 10^{-8} *M* for FUDR. DHFUDR is not phosphorylated[33] by either thymidine kinase or uridine kinase, as demonstrated by the lack of competition with the normal substrates, even at a concentration 10^3 times that of thymidine or uridine. Unlike FUDR, DHFUDR is not a substrate for nucleoside phosphorylase.[34] These observations suggested the study of this drug against FUDR-resistant tumor cells. Thus a cell line of Novikoff hepatoma lacking thymidine kinase[15,35] was $10^{3.5}$ times resistant to FUDR, but only $10^{1.5}$ times resistant to DHFUDR.[33] Similarly, mouse leukemia L5178SBF cells[36] (also lacking thymidine kinase) were 1000-fold resistant to FUDR, but not at all resistant to DHFUDR.[33] It should be pointed out that the inhibition of L5178Y (FUDR-sensitive) cells by 10^{-6} *M* DHFUDR is reversed by thymidine (10^{-5} *M*) but not by uridine (10^{-5} *M*) or 2′-deoxyuridine (10^{-5} *M*). Under the same conditions, the inhibition of L5178SBF (FUDR-resistant) cells by 10^{-6} *M* DHFUDR is not reversed by any of the above-mentioned pyrimidine nucleosides (10^{-5} *M*).[33]

2′,3′-Dideoxy-5-fluorouridine also inhibited the growth of HeLa cells at 10^{-4} *M*; the inhibitory effect was reversed by thymidine (10^{-4} *M*), but not by uridine (10^{-5} *M*). This compound (IX) did not inhibit the incorporation of ^{14}C-formate into DNA-thymine and is not a substrate for nucleoside phosphorylase.[34] The growth of vaccinia virus in HeLa cells was not affected by 10^{-5} *M* DHFUDR (this concentration inhibits HeLa cell growth).[37]

The effect of DHFUDR against transplanted mouse tumors was studied by methods previously described.[37] As shown in Table I, DHFUDR at the proper dosage is as effective as FUDR at inhibiting the growth of Sarcoma 180. A series of experiments was also carried out in various ascites leukemias, as shown in Table II. In leukemia L1210 DHFUDR caused a longer prolongation of survival than did FUDR under optimal dosage. DHFUDR was also more effective than FUDR in the L5178 tumors that were sensitive and resistant to FUDR. It is of interest that, although *in vitro* the L5178 cells are 1000-fold resistant to FUDR, *in vivo* FUDR was equally effective against the sensitive and resistant tumors. Two lines of L1210 489 leukemias, sensitive and resistant to FU,[38] were also studied. In the sensitive line, DHFUDR was somewhat more effective than FU. The resistant line showed no increase in survival with FU or FUDR, but a slight increase in survival was produced by DHFUDR, which suggests that there was some cross-resistance with FU. It is evident from these experiments that DHFUDR has appreciable activity at inhibiting transplanted tumors in mice, including those that are resistant to FUDR because they lack thymidine kinase.[39]

TABLE I
ACTIVITY OF FUDR AND DHFUDR AGAINST SARCOMA 180 IN FEMALE SWISS MICE[a]

	Δ wt, g	Tumor vol, mm^3	T/C
Control	−3.1	1550	
FUDR, 40 mg/kg × 7	+0.1	64	0.13
DHFUDR, 150 mg/kg × 7	−3.2	1117	0.53
DHFUDR, 400 mg/kg × 3	−3.1	120	0.06

[a] The mice (six/group) received the drugs (by intraperitoneal injections) 1 day after bilateral transplantation. The mice were measured on the 12th day after transplantation.

Experimental Section

All melting points are corrected. Thin layer chromatography was done on plastic plates coated with silica gel (Eastman Chromagram sheet 6060), with fluorescent indicator) or cellulose (MN-Polygram cell/300/UV, Macherey Nagel and Co., Düren,

(30) J. R. McCarthy, M. J. Robins, L. B. Townsend, and R. K. Robins, *J. Am. Chem. Soc.*, **88**, 1549 (1966).

(31) T. Corbett and C. Heidelberger, unpublished data.

(32) M. Umeda and C. Heidelberger, unpublished data.

(33) C. Heidelberger, T. A. Khwaja, M. Umeda, and R. Kent, Abstract, 7th International Congress on Biochemistry, Tokyo, Japan, Aug 1967, p 1033.

(34) R. J. Kent and C. Heidelberger, unpublished data.

(35) Kindly supplied by Dr. V. R. Potter.

(36) Kindly supplied by Dr. Glenn Fisher, Department of Pharmacology, Yale University Medical School.

(37) C. Heidelberger, L. Griesbach, R. J. Montag, D. Mooren, O. Cruz, R. J. Schnitzer, and E. Grunberg, *Cancer Res.*, **18**, 305 (1958).

(38) Kindly provided by Dr. Doris Hutchison of the Sloan-Kettering Institute, Rye, N. Y.

(39) The L5178 resistant tumor still lacks thymidine kinase *in vivo*, suggesting that the activity of FUDR may result from its cleavage to FU in this line.

TABLE II
SURVIVAL OF BDF MICE WITH VARIOUS LEUKEMIAS IN ASCITES FORM[a]

Leukemia	Mean survival, days	Extremes, days	T/C
L1210			
Control	10.1	8–12	
FUDR, 40 mg/kg × 7	11.8	8–15	1.17
FUDR, 200 mg/kg × 3	10.5	9–12	1.04
DHFUDR, 400 mg/kg × 3	14.1	12–17	1.40
L5178Y, FUDR-sensitive			
Control	13.8	11–18	
FUDR, 40 mg/kg × 7	20.9	16–24	1.51
DHFUDR, 400 mg/kg × 3	23.5	16–31	1.70
L5178BF, FUDR-resistant			
Control	13.9	13–15	
FUDR, 40 mg/kg × 7	20.8	18–27	1.50
DHFUDR, 400 mg/kg × 3	21.4	13–30	1.54
DHFUDR, 150 mg/kg × 7	22.9	19–29	1.65
DHFUDR, 250 mg/kg × 5	34.4	17–150 (1 survived)	2.47
L1210 489, FU-sensitive			
Control	9.0	7–10	
FUDR, 40 mg/kg × 7	10.0	9–12	1.11
FU, 25 mg/kg × 7	12.9	10–15	1.43
DHFUDR, 400 mg/kg × 3	12.8	11–16	1.42
DHFUDR, 175 mg/kg × 7	15.0	11–21	1.60
L1210 XIII, FU-resistant			
Control	10.7	9–14	
FUDR, 40 mg/kg × 7	10.6	8–13	0.99
FU, 25 mg/kg × 7	9.1	8–10	0.84
DHFUDR, 400 mg/kg × 3	12.2	8–24	1.14
DHFUDR, 175 mg/kg × 7	14.4	10–35	1.35

[a] There were ten mice/group, and the drugs were given by intraperitoneal injection 1 day after transplantation.

Germany). The following solvent systems were used: A, EtOH–1-PrOH–H_2O (4:1:2, v/v); B, MeOH–C_6H_6 (1:3, v/v); C, Me_2CO–cyclohexane (1:1, v/v); D, 2-PrOH–NH_4OH–H_2O (7:1:2, v/v). The uv absorption spectra were run on a Cary spectrophotometer Model 15. The analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

2,3′-Anhydro-1-(2-deoxy-5-*O*-trityl-β-D-lyxofuranosyl)-5-fluorouracil (IV).—1-(2-Deoxy-5-*O*-trityl-β-D-ribosyl)-5-fluorouracil[24] (II) (19.6 g, 40.17 mmoles) was twice evaporated with dry pyridine (50-ml portions), and the residue was dissolved in 150 ml of dry pyridine, cooled to 0°, and mixed with cold methanesulfonyl chloride (4.98 ml, freshly distilled). The sealed reaction mixture was kept in a refrigerator overnight. Absolute EtOH (18 ml) was added, and after 1 hr (0°) the dark yellow solution was poured over ice water (2 l.). The solution was stirred and the pale, granular precipitate of 1-(2-deoxy-3-mesyl-5-*O*-trityl-β-D-ribofuranosyl)-5-fluorouracil[24] (III) was collected by filtration, washed with excess cold water, and dried *in vacuo* (P_2O_5). The crude material weighed 23.6 g (103.9%) and was used as such for further reactions.

Crude III (5.62 g, 10 mmoles) was dissolved in EtOH (100 ml), 1 *N* aqueous NaOH (10.5 ml) was added, and solution was heated under reflux for 3 hr. The reaction mixture was evaporated to dryness on a rotary evaporator. The residual solid was triturated with ice–water (150 ml) and filtered to give 4.38 g of the product. This was recrystallized from methanol (charcoal) to get colorless tiny needles: mp 237–238°; yield 2.712 g (57.9%); uv absorption, $\lambda_{max}^{CH_3OH}$ 254 mμ (ε 6727), $\lambda_{min}^{CH_3OH}$ 247 mμ (ε 6668), and shoulders at 230 and 265 mμ. The ir spectrum showed no band at 1170 cm^{-1} (absence of mesyl group).

Anal. Calcd for $C_{28}H_{23}FN_2O_4$: C, 71.49; H, 4.89; N, 5.96. Found: C, 71.21; H, 5.07; N, 5.72.

1-(2-Deoxy-3-*O*-mesyl-β-D-ribofuranosyl)-5-fluorouracil (VI) was prepared according to the method of Fox and Miller.[24] Crude III (23.6 g) gave 10.354 g (87.0% based on II) of VI as colorless long needles: mp 158°; uv absorption, $\lambda_{max}^{CH_3OH}$ 267.5 mμ (ε 9486), $\lambda_{min}^{CH_3OH}$ 234 mμ (ε 2402).

2,3′-Anhydro-1-(2-deoxy-β-D-lyxofuranosyl)-5-fluorouracil (VII).—Compound VI (11.00 g, 31.97 mmoles) following the method of Fox and Miller[24] gave 7.2 g (98.7%) of VII as colorless crystalline material: mp 166–170°; uv absorption, $\lambda_{max}^{H_2O}$ 255.5 mμ (ε 8008) and 236 mμ (ε 6603), $\lambda_{min}^{H_2O}$ 238 mμ (ε 6256) and 220 mμ (ε 5630).

1-(5-*O*-Trityl-2,3-dideoxy-2,3-didehydro-β-D-*glycero*-pentofuranosyl)-5-fluorouracil (V). Method 1.—III (0.651 g, 1.15 mmoles) was dissolved in anhydrous DMSO (5 ml) and added to a stirred solution of KO-*t*-Bu (0.432 g, 2.4 mmoles) in 5 ml of DMSO over a period of 5 min. The reaction was protected from moisture and maintained at room temperature for 30 min. The contents were then poured into ice–water (250 ml) and stirred vigorously. The aqueous solution was neutralized with 6 *N* AcOH (phenolphthalein) and filtered. The yellowish powder was vacuum desiccated over P_2O_5 and KOH overnight and recrystallized from absolute EtOH (charcoal) to yield 0.405 g (82.7%) of colorless needles of V: mp 192–193°; uv absorption, $\lambda_{max}^{CH_3OH}$ 267 mμ, $\lambda_{min}^{CH_3OH}$ 244 mμ.

Anal. Calcd for $C_{28}H_{23}FN_2O_4$: C, 71.49; H, 4.89; N, 5.96. Found: C, 71.62; H, 5.00; N, 6.04.

Method 2.—IV (2.35 g, 5 mmoles) was dissolved in anhydrous DMSO (20 ml) and added to a stirred solution of KO-*t*-Bu (0.588 g, 5.25 mmoles) in DMSO (20 ml) over a period of 10 min in the absence of moisture. After 30 min (room temperature) the light brown reaction mixture was poured into ice–water (1 l.) and stirred. The aqueous solution was neutralized with dilute AcOH (pH 5). The pale gelatinous precipitate was filtered, washed with excess cold water, and dried *in vacuo* over P_2O_5 and KOH to yield 2.1 g (89.3%) of pale crystalline material. This was recrystallized from absolute EtOH (charcoal) to furnish colorless crystalline material, mp 192–193°, no mixture melting point depression with the sample prepared by method 1. Both samples had identical uv absorption spectra and their ir spectra were superimposable.

1-(2,3-Dideoxy-2,3-didehydro-β-D-*glycero*-pentofuranosyl)-5-fluorouracil (VIII). Method 1.—V (0.2 g, 0.425 mmoles) was swirled with 2.5 ml of cold formic acid (98–100%). After 5 min (room temperature) the acid was quickly evaporated on an oil pump. The last traces of formic acid were removed by evaporation of the residue with dioxane (two 2-ml portions). The residue was twice evaporated from absolute EtOH (two 2-ml portions) and finally anhydrous ether (3 ml). The yellowish powder was extracted with warm water (5 ml) and filtered, and the filtrate was evaporated to dryness under reduced pressure (temperature less than 40°). The residue was dissolved in boiling Me_2CO (40–50 ml) and decolorized (charcoal). The colorless solution was evaporated to 5 ml and C_6H_6 (10 ml) was added. The turbid solution was warmed and allowed to crystallize in the cold. VIII was obtained as colorless microcrystals (0.068 g, 70.1%). This on recrystallization from EtOH–C_6H_6 (by evaporating the EtOH solution with excess C_6H_6 until slightly turbid) gave long colorless needles: mp 138–139° (resolidifies after a colorless melt); uv absorption, pH 1 λ_{max} 267.5 mμ (ε 7600), pH 12 λ_{max} 267.5 mμ (ε 5924).

Anal. Calcd for $C_9H_9FN_2O_4$: C, 47.37; H, 3.95; N, 12.28. Found: C, 47.54; H, 3.96; N, 12.20.

Method 2.—To a stirred solution of VII (3.176 g, 13.93 mmoles) in anhydrous DMSO (55 ml), KO-*t*-Bu (1.75 g, 15.62 mmoles) was added. The reaction was maintained in the absence of moisture for 2.15 hr. Then the solution was diluted with cold anhydrous MeOH (600 ml) and passed through an Amberlite IRC-50 (H^+) column (three 20-cm portions); cold MeOH was used to elute all of the uv-absorbing material. The MeOH eluate was concentrated on a rotary evaporator, and the DMSO was distilled (55°, 0.5 mm) to obtain a pale gum. The gum was dissolved in hot absolute EtOH (10 ml) and passed through a thin bed of charcoal and Supercel (25 ml of boiling EtOH was used as wash liquid). The filtrate and the washings were mixed with 15 ml of C_6H_6 and evaporated to 30 ml, the process was repeated twice after addition of C_6H_6 (two 25-ml portions), and finally the solution was concentrated to 30 ml. This solution was seeded and allowed to cool to room temperature when VIII started to crystallize, mp 136–138°. The solution was cooled overnight, filtered, and washed with the filtrate and then C_6H_6 (15 ml); yield 2.035 g (64.07%). The product had the same R_f values on tlc as that obtained by method 1; their ir spectra were superimposable. The filtrate and the washings were evaporated and the residual gum was evaporated to remove traces of DMSO. Finally, the gum was crystallized as above to obtain two more crops (0.60 and 0.110 g). The last two crops had traces of 5-fluorouracil impurity (tlc in systems A, C, and D).

1-(2,3-Dideoxy-2,3-didehydro-β-D-*glycero*-pentofuranosyl)-5-fluorouracil 5′-Phosphate (X).—VIII (0.114 g, 0.5 mmole) was phosphorylated with β-cyanoethyl phosphate (0.15 g, 1.00 mmole) and N,N′-dicyclohexylcarbodiimide (1.03 g, 5 mmoles) according to the method of Tener.[39] After removing the precipitated N,N′-cyclohexylurea, the filtrate and washings (6 ml of dry pyridine used as wash liquid) were evaporated to a gum under reduced pressure (temperature below 35°). The gum gave a single homogeneous, phosphate-positive, uv-absorbing spot on tlc (systems A and D). The material was suspended in aqueous NaOH (0.1 *N*, 5 ml) and kept under reflux (bath temperature 100°) for 7 min. Then the solution was cooled and diluted (100 ml) with distilled water. Amberlite IR-120 (H^+) (5 ml) was added and after swirling, the cold solution was quickly filtered and neutralized to pH 7.5 with dilute NH_4OH. The resulting solution was diluted with an equal volume of EtOH and carefully evaporated under reduced pressure to a gum. The gum was taken up in 1 ml of H_2O and the pH was adjusted to 9 with dilute NH_4OH. The solution was cooled to 5° and absorbed on a Dowex 1 (formate, 200–400 mesh) column (3 × 7 cm). The column was first washed with 600 ml of distilled H_2O (chromatography was done in a cold room at 5–7°). Then it was eluted with 700 ml of ammonium formate (0.05 *M*, pH 6.5); 10-ml fractions were collected. Fractions 8–15 had some uv-absorbing nonphosphorylated material. Then the column was eluted with 0.5 *M* ammonium formate (pH 6.5), and fractions 78–110 were combined (made up to pH 7.5 with NH_4OH) and carefully evaporated under reduced pressure. The residual gum was taken in water (50 ml) and desalted with the help of a charcoal column (2 × 3 cm). The filtrate containing the product was carefully deionized with Amberlite IR-120 (H^+) and immediately neutralized with aqueous $Ba(OH)_2$ (0.2 *M*). CO_2 was passed through, and the solution was filtered (10 ml), absolute EtOH (20 ml) was added, and the precipitated material was left in the refrigerator for 3 days. Then the precipitate was centrifuged, washed (EtOH, Et_2O), and air-dried to obtain barium 1-(2,3-dideoxy-2,3-didehydro-β-D-*glycero*-pentofuranosyl)-5-fluorouracil 5′-phosphate (X-Ba) as a white powder (0.4 g) which was purified by reprecipitation from EtOH. The product gave a single uv-absorbing ($\lambda_{max}^{H_2O}$ 267.5 mμ, $\lambda_{min}^{H_2O}$ 235 mμ) spot on tlc (system D) and its electrophoretic mobility was comparable to 5-fluoro-2′-deoxyuridine 5′-phosphate at pH 4.3 (acetate buffer) and 7.5 (phosphate buffer).

Anal. Calcd for $C_9H_8BaFN_2O_7P \cdot H_2O$: P, 6.47. Found: P, 6.31.

Enzymatic Dephosphorylation of X.—Compound X (1.5 mg, diammonium salt) was dissolved in 0.2 ml of acetate buffer (pH 5), prostatic phosphomonoesterase (0.5 mg) was added, and the mixture was incubated at 37.5° for 30 min; a blank without enzyme was also run. Tlc (systems A and D) revealed material corresponding to VIII as the sole uv-absorbing product (also inorganic phosphate), whereas the starting material X remained unaffected in the control (without enzyme).

1-(2,3-Dideoxy-β-D-*glycero*-pentofuranosyl)-5-fluorouracil (IX).—VIII (115 mg, 0.5 mmole) was dissolved in dioxane (25 ml) and hydrogenated (1.55 kg/cm²) in the presence of 5% Pd–C (200 mg) for 30 min (room temperature). The catalyst was removed by filtration, washed with 25 ml of hot EtOH, and the combined filtrate and washings were evaporated to dryness. Some ethanol-insoluble material (16 mg, shown to be 5-fluorouracil by melting point and uv spectra) was removed. The filtrate was evaporated and the residue was absorbed (2 ml of H_2O solution) on a Dowex 1 formate[40,41] (200–400 mesh) column (2.5 × 22 cm). The column was eluted with H_2O and 10-ml fractions were collected. Fractions 100–135 gave traces of impurity ($\lambda_{max}^{pH\ 12}$ 262 mμ), fractions 147–300 (mostly eluted with 30% aqueous MeOH) were combined and evaporated to dryness under reduced pressure. The residue was dissolved in 10 ml of Me_2CO, decolorized (charcoal), and then evaporated with C_6H_6 (10 ml) to 8 ml, then 5 ml of petroleum ether (bp 30–60°) was added to yield colorless needles IX: 35 mg (31.0%); mp 115–117°; uv absorption, $\lambda_{max}^{pH\ 1}$ 270.5 mμ (ε 9050), $\lambda_{min}^{pH\ 1}$ 235 mμ (ε 1292), $\lambda_{max}^{pH\ 12}$ 270.5 mμ (ε 6620), $\lambda_{min}^{pH\ 12}$ 248 mμ (ε 3810).

Anal. Calcd for $C_9H_{11}FN_2O_5$: C, 46.96; H, 4.48; N, 12.17. Found: C, 47.26; H, 4.41; N, 12.26.

(40) In another experiment DHFUDR (600 mg) was hydrogenated in the presence of Pd–C (400 mg) in absolute EtOH at 1.83 kg/cm². The hydrogenation was complete in 5 min and IX (420 mg) was isolated by chromatography on a Dowex 1 (OH^-) column (the product was eluted with 0.1 *M* aqueous $(NH_4)HCO_3$).

(41) C. A. Dekker, *J. Am. Chem. Soc.*, **87**, 4027 (1965).

Preparation of 1-(2,3-Dideoxy-2,3-didehydro-β-D-*glycero*-pentofuranosyl)-5-fluorouracil-2-^{14}C.—5-Fluoro-2′-deoxyuridine-2-^{14}C (1 mcurie) (obtained from CalBiochem) was diluted with nonradioactive 5-fluoro-2′-deoxyuridine (10 mg and dissolved in dry pyridine (5 ml). The solution was heated under reflux (bath temperature 100°) with trityl chloride (134 mg) for 2 hr. The cooled solution was poured over ice–water (100 ml) and the water was extracted with $CHCl_3$ (three 20-ml portions) and dried ($MgSO_4$). The $CHCl_3$ solution was evaporated to a gum, which crystallized on trituration with cold anhydrous ether, and was recrystallized from aqueous EtOH to give 160 mg of 1-(2-deoxy-5-*O*-trityl-β-D-ribofuranosyl)-5-fluorouracil-2-^{14}C. The tritylated compound was dissolved in dry pyridine (1.3 ml) and the solution was cooled to 0° and treated with cold, freshly distilled (0.04 ml) methanesulfonyl chloride. The reaction solution was maintained at 5° overnight (anhydrous conditions), then EtOH (0.05 ml) was added. After 1 hr at 5° the solution was poured over ice–water (150 ml), and the precipitated product was filtered, washed with water, and dried (vacuum, P_2O_5) (160 mg). The crude, 1-(2-deoxy-3-*O*-mesyl-5-*O*-trityl-β-D-ribofuranosyl)-5-fluorouracil-2-^{14}C was dissolved in 8 ml of $CHCl_3$–Et_2O (1:1) and the cooled solution (−5°) was saturated with dry HCl for 40 min. After 2 hr at 5° the precipitated material was filtered, washed with cold anhydrous ether (10 ml), and recrystallized from absolute EtOH (charcoal) to yield 45.5 mg of 1-(2-deoxy-3-*O*-mesyl-β-D-ribofuranosyl)-5-fluorouracil-2-^{14}C; the mother liquor gave 13 mg more of colorless needles. The mesylate (58.5 mg) was suspended in H_2O (1 ml), half a drop of methyl red was added, and the stirred solution was heated under reflux (bath temperature 100°). The pH of the solution was maintained between 4–5 by addition of 1 *M* Et_3N (in 50% aqueous EtOH) until the solution stayed yellow (10 min, 0.17 ml). The reaction was maintained for 50 min, then the contents were evaporated to dryness on a rotary evaporator and the residual gum was recrystallized from absolute EtOH to obtain 2,3′-anhydro-1-(2-deoxy-β-D-lyxofuranosyl)-5-fluorouracil-2-^{14}C (35 mg). The anhydronucleoside was dried and dissolved in anhydrous DMSO (0.6 ml). To this solution KO-*t*-Bu (19.3 mg) was added and solution was stirred at room temperature. After 2 hr the reaction solution was poured over 20 ml of anhydrous MeOH, stirred 10 min with 1 ml of Amberlite IRC-50 (H^+), and filtered. The combined filtrate and washings (10 ml of MeOH) were evaporated under reduced pressure; the last traces of solvents were removed at 55° (0.5 mm). The residual gum was dissolved in MeOH (charcoal), concentrated to a small volume, and purified by preparative thin layer chromatography (system B used as developing solvent). The major uv bands which corresponded to 1-(2,3-dideoxy-2,3-didehydro-β-D-*glycero*-pentofuranosyl)-5-fluorouracil-2-^{14}C were cut and eluted to furnish 22 mg of the product, which gave a single radioactive spot in four different chromatographic systems (A–D). The over-all yield based upon 5-fluoro-2′-deoxyuridine-2-^{14}C was 21.5%. The specific activity was 1.0 μcuries/mmole 18.35 μcuries/mg).

The chromatographic behavior of the various compounds is given in Table III.

TABLE III

CHROMATOGRAPHIC BEHAVIOR OF COMPOUNDS ON THIN LAYER CHROMATOGRAPHY

No.	Compd	R_{FU}[a] in solvent system— A (cellulose)	B (silica gel)	C (silica gel)	D (cellulose)
1	FU	1.00	1.00	1.00	1.00
2	I	1.14	0.89	0.68	1.08
3	II	1.32	1.56	2.05	1.75
4	III	1.31	1.73	1.91	1.80
5	IV	1.32	1.56	0.86	1.84
6	V	1.33	1.78	2.28	1.82
7	VI	1.19	1.38	1.36	1.27
8	VII	0.99	0.38	0.99	1.25
9	VIII	1.19	1.20	1.41	1.16
10	X				0.08
11	5′-β-Cyanoethyl-DHFUDRP	0.95			0.79
12	IX	1.21	1.24	1.36	1.22

[a] R_{FU} relative to that of FU. For composition of solvent systems, see text.