Nucleosides. LXIV. Fluoro Sugar Analogs of Arabinosyl- and Xylosylcytosines[1]

JOHN A. WRIGHT, DAN P. WILSON, AND JACK J. FOX

Division of Biological Chemistry, Sloan-Kettering Institute for Cancer Research, Sloan-Kettering Division of Cornell University Medical College, New York, New York 10021

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The syntheses of 1-(3-deoxy-3-fluoro- and -2-deoxy-2-fluoro-β-D-xylofuranosyl)cytosines (X and XI, respectively) and 1-(2-deoxy-2-fluoro-α- and -β-D-arabinofuranosyl)cytosines (XIIa and XIIb) from their corresponding suitably protected halogenoses are described. The susceptibility of these cytosine nucleosides (X, XI, XII) to cytidine deaminase was studied along with the susceptibility of several corresponding fluoro sugar adenine nucleosides to adenosine deaminase. Preliminary studies showed that in L1210 mouse leukemia suspension culture, XIIb has a growth inhibitory effect comparable with that of *ara*-C.

As part of a program of synthesis of nucleosides with biochemical and chemotherapeutic potential,[2] we have undertaken the preparation of a number of nucleosides containing F in the carbohydrate moiety. Previous studies dealt with the synthesis of 2′-deoxy-2′-fluoro analogs of uridine, 5-fluorouridine, ribothymidine,[3] and cytidine[4] by reaction of 2,2′-anhydro nucleosides with HF in dioxane. Conventional condensation of preformed deoxyfluoro sugar derivatives with 2,6-dichloropurine led to 9-(3-deoxy-3-fluoro-β-D-xylofuranosyl)-adenine[5] and 9-(2-deoxy-2-fluoro-α- and -β-D-arabinofuranosyl)adenines.[6] The present report describes the synthesis, *via* the "silyl" procedure,[7] of 1-(3-deoxy-3-fluoro- and -2-deoxy-2-fluoro-β-D-xylofuranosyl)cytosines(X and XI), and 1-(2-deoxy-2-fluoro-α- and -β-D-arabinofuranosyl)cytosines (XIIa and XIIb).

Compound XIIb is of particular interest as a 2′-fluoro analog of the chemotherapeutically active *ara*-C.[8] Pharmacological studies in man and mouse have shown that *ara*-C is rapidly deaminated to 1-β-D-arabinosyluracil, an inactive metabolite.[2,8] The present report examines the susceptibility of XIIb and related fluoro sugar cytosine nucleosides to cytidine deaminase. Additionally, the susceptibility of the 2′- and 3′-fluoro analogs of chemotherapeutically active[8] adenine nucleosides, *ara*-A and *xylo*-A, to adenosine deaminase is reported. Finally, some preliminary screening studies of these fluoro sugar cytosine nucleosides against L1210 mouse leukemia suspension culture are given.

The glycosides, methyl 3-deoxy-3-fluoro-β-D-xylofuranoside (I),[5] methyl 2-deoxy-2-fluoro-β-D-xylofuranoside (II),[9] and methyl 2-deoxy-2-fluoro-α-D-arabinofuranoside (III),[6] were treated with PhCOCl in pyridine to give 75–85% yields of the di-*O*-benzoyl derivatives IV, V, and VI, respectively. These were readily converted into the bromo-sugars VII, VIII, and IX by reaction with saturated HBr in glacial AcOH. Halogenose VII could also be obtained from IV by a less vigorous procedure, namely treatment with HBr in CH_2Cl_2 at 0°, whereas similar treatment of V and VI failed to afford bromo sugars. Compounds VII, VIII, and IX were unstable and were prepared immediately before use.

Initial attempts to prepare cytosine nucleosides from these bromo sugars using the generalized mercuric cyanide–nitromethane method[10] failed, due, probably, to the instability of the bromo sugars and the relative insolubility of *N*-acylcytosines in nitromethane. Addition of DMF to the solvent increased the solubility of the pyrimidine base, but yields of nucleosides were still low and extremely variable. Therefore this approach was abandoned in favor of a procedure using the bis(trimethylsilyl) derivative of cytosine.

A standardized procedure was adopted for the synthesis of the cytosine nucleosides. Thus, the bromo sugar was dissolved in a small volume of MeCN and added with stirring to a twofold excess of bis(trimethylsilyl)cytosine,[11] partly dissolved in MeCN at room temperature. After a few minutes the solution became clear, and the reaction was allowed to proceed for several days. The nucleosidic products, without characterization, were debenzoylated to the free nucleosides by treatment with methanolic NaOMe. In all cases, glycosylation occurred on N-1 of the base, as adduced from the close similarity of the uv spectra of the products to that of cytidine in acidic, neutral, and basic solution.

Using the above procedure, bromo sugar VII afforded a 76% yield of 1-(3-deoxy-3-fluoro-β-D-xylofuranosyl)-cytosine (X), isolated as the crystalline HCl salt. Since the 2-*O*-benzoyl substituent could participate during the condensation, the β anomeric configuration was expected, resulting in a 1′,2′-*trans* configuration, as previously obtained in the condensation of bromo sugar VII with *N*-benzoyladenine.[5] Firm evidence for the β configuration was obtained from the nmr spectrum of X (in DMSO-d_6), in which the signal for the 1′-proton appeared at δ 5.69 as a slightly broadened singlet ($J_{1',2'} < 1.0$ Hz). This small coupling is characteristic of the *trans* 1′,2′ configuration in furanose derivatives,[12] and hence of the β configuration in this

(1) This work was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant 08748).

(2) See M. R. Dollinger, J. H. Burchenal, W. Kreis, and J. J. Fox, *Biochem. Pharmacol.*, **16**, 689 (1967), and references therein.

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

(4) I. L. Doerr and J. J. Fox, *ibid.*, **32**, 1462 (1967).

(5) J. A. Wright and N. F. Taylor, *Carbohyd. Res.*, **6**, 347 (1968).

(6) J. A. Wright, N. F. Taylor, and J. J. Fox, *J. Org. Chem.*, **34**, 2632 (1969).

(7) T. Nishimura and I. Iwai, *Chem. Pharm. Bull.* (Tokyo), **12**, 357 (1964); M. W. Winkley and R. K. Robins, *J. Org. Chem.*, **33**, 2822 (1968).

(8) For a review of the biochemistry of arabinosyl nucleosides, see S. S. Cohen, *Progr. Nucleic Acid Res. Mol. Biol.*, **5**, 1 (1966).

(9) J. A. Wright and J. J. Fox, *Carbohyd. Res.*, in press.

(10) N. Yamaoka, K. Aso, and K. Matsuda, *J. Org. Chem.*, **30**, 149 (1965); K. A. Watanabe and J. J. Fox, *J. Heterocycl. Chem.*, **6**, 109 (1969).

(11) T. Nishimura and I. Iwai, *Chem. Pharm. Bull.* (Tokyo), **12**, 352 (1964).

(12) R. U. Lemieux and D. R. Lineback, *Ann. Rev. Biochem.*, **32**, 155 (1963).

instance. The CD (circular dichroism) spectrum of X·HCl in H_2O provided further confirmation of the β configuration in that it displayed a positive Cotton effect centered at 278 mμ; this conformed to the rule proposed by Ulbricht, *et al.*,[13] that 1-(β-D-pentofuranosyl)uracils and -cytosines show positive Cotton effects in the region of the so-called B_{2g} spectral band (260–280 mμ for most nucleosides) if the nucleoside possesses a preferred conformation owing to restricted rotation about the glycosyl bond. This rule appears to be valid for ribo-, deoxyribo-, and arabinonucleosides, regardless of whether the sugar OH groups are substituted. The *magnitude*, but not the *sign*, of the Cotton effect may be varied by changes in the sugar. Thus the rule, in our opinion, is applicable also to deoxyfluoro sugar nucleosides, and we will present evidence (see below) in support of this application.

Reaction of the 2-deoxy-2-fluoroxylosyl bromide VIII with bis(trimethylsilyl)cytosine gave a complex mixture of products, from which blocked nucleoside (in 65% yield) was isolated as an amorphous solid by preparative tlc. After debenzoylation, tlc showed the reaction mixture contained two components, the α and β nucleosides, in a ratio of 1:6. The predominant isomer was obtained by fractional crystallization from MeOH, and, on the basis of a positive Cotton effect at 270 mμ, was assigned the β configuration (XI). The other isomer could not be obtained in pure form.

The most plausible explanation for predominance of the β-nucleoside product in this condensation, in view of the absence of a participating group at C-2 of the sugar, is that bromo sugar VIII exists largely as the α anomer, and undergoes direct S_N2 attack by N-1 of the base to give a preponderance of the β nucleoside.

As in the case of the 2-deoxy-2-fluoro isomer VIII, 3,5-di-*O*-benzoyl-2-deoxy-2-fluoroarabinosyl bromide IX gave, as expected, a mixture of products from which the free anomeric nucleosides XIIa and XIIb were obtained after chromatography and deblocking in a combined yield of 45%. The α:β ratio obtained in this instance was approximately 1:1, as was previously experienced in the condensation by the fusion procedure of a derivative of this sugar with 2,6-dichloropurine.[6] Compounds XIIa and XIIb were characterized as the HCl salts.

Assignment of the α- and β-anomeric configurations to XIIa and XIIb, respectively, was made using nmr spectra. A close structural relationship exists between cytosine and adenine nucleosides in the C-6(C-8)-N-1-(N-9)C-1′-C-2′ part of the molecule. In the nmr spectrum of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-adenine,[6] long-range (^5J) coupling to the 2′-F causes a splitting of 2.0 Hz in the signal arising from H-8 of the base. This splitting is absent in the α anomer. A similar splitting of 1.5 Hz occurs in the signal of the C-6 proton of XIIb, thus permitting assignment of the β configuration.

In the circular dichroism spectra, XIIa and XIIb showed negative and positive Cotton effects, respectively (around 270 mμ), providing evidence for the applicability of Ulbricht's rule to deoxyfluoro nucleosides.

In the case of purine nucleosides, Cotton effects are generally much smaller, and their direction is opposite to those of pyrimidine nucleosides.[14] Thus, α and β nucleosides show small positive and negative Cotton effects, respectively, in the region of 260 mμ. That this also held for deoxyfluoro nucleosides was indicated by the fact that 9-(3-deoxy-3-fluoro-β-D-xylofuranosyl)-adenine[6] and 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-adenine[6] showed weak negative Cotton effects, while for 9-(2-deoxy-2-fluoro-α-D-arabinofuranosyl)adenine,[6] a positive Cotton effect was seen.

Enzymatic Studies.—The fluoro sugar β-D-nucleosides described herein along with their nonfluorinated analogs as well as cytidine and 2′-deoxycytidine were examined for their ability to act as substrates for partially purified cytidine deaminase from pig kidney. The results (Table I) show that *ara*-C and its 2′-fluoro derivative

TABLE I

SUSCEPTIBILITY OF 1-β-D-PENTOFURANOSYLCYTOSINES TO CYTIDINE DEAMINASE

Compound[a]	Configuration at 3′,2′	Relative rate[b,c]
Cytidine	+ HO, + OH	1
2′-Deoxycytidine[6]	+ H, + OH	1.41
ara-C	+ HO, + F	0.15
2′-F-*ara*-C (XIIb)	+ HO, + HO	0.13
xylo-C	+ H, + OH	1
2′-F-*xylo*-C (XI)	+ H, + F	0
3′-F-*xylo*-C (X)	+ F, + OH	1

[a] Assayed according to the method of R. Tomchick, *et al.*, *J. Biol. Chem.*, **243**, 2534 (1968). [b] Incubated 20 min at 37°. [c] Incubated 60 min at 37°. The products were identified by paper chromatography on Whatman No. 1 paper, using 5-PrOH–H_2O–HCl (30:37:33, v/v) as the developing solvent.

(XIIb) were deaminated more slowly than cytidine. Moreover, the substitution of F for OH in the 2′ position of *ara*-C did not change the relative rate of deamination. If, as in 2′-deoxycytidine, H replaced the 2′OH of *ara*-C or the F of 2′-F-*ara*-C, the rate of deamination increased threefold. Previous studies from this and other laboratories have demonstrated that 2′-deoxycytidine is deaminated by bacterial deaminase at about the same rate[15] or at a greater rate[16,17] than cytidine, whereas the rate of deamination of 2′-deoxycytidine by mammalian deaminase is 50% that of cytidine.[18,19] On the other hand, 1-β-D-arabinosylcytosine is converted into 1-β-D-arabinosyluracil at one-

(13) T. L. V. Ulbricht, T. R. Emerson, and R. J. Swan, *Tetrahedron Lett.*, 1561 (1966); T. R. Emerson, R. J. Swan, and T. L. V. Ulbricht, *Biochemistry*, **6**, 843 (1967).

(14) For a review of ORD and CD of nucleic acids, see I. Tinoco and T. Samejima, *Progr. Nucleic Acid Res. Mol. Biol.*, **9**, 223 (1969).

(15) I. Wempen, R. Duschinsky, L. Kaplan, and J. J. Fox, *J. Amer. Chem. Soc.*, **83**, 4755 (1961).

(16) T. P. Wang, H. Z. Sable, and J. O. Lampen, *J. Biol. Chem.*, **184**, 17 (1950).

(17) S. S. Cohen and H. D. Barner, *ibid.*, **226**, 631 (1957).

(18) W. A. Creasey, *ibid.*, **238**, 1772 (1963).

(19) R. Tomchick, L. D. Sqslaw, and V. S. Waravdekar, *ibid.*, **243**, 2534 (1968).

fourth or less the rate at which cytidine is converted into uridine.[17,18,19] Camiener[20] has reported that human liver deaminase will deaminate 2′-deoxycytidine and arabinosylcytosine approximately as rapidly as cytidine.

Epimerization of the 3′ position and/or substitution of the OH by F gave nucleosides which were not deaminated by the enzyme. It is apparent, therefore, that the enzyme can tolerate a configurational change at the 2′ position of the nucleoside, while at the 3′ position the "down" (ribo or arabino) configuration must be maintained in order for deamination to occur. These results are in agreement with those of Fox, *et al.*,[21] and Camiener,[20] who found that cytosine nucleosides containing a 3′-OH in the "up" (xylo) configuration are not substrates for cytidine deaminase.

It is of interest to note that while the 2′- or 3′-fluoro-substituted pyrimidine nucleosides were either poor substrates or nonsubstrates for cytidine deaminase, the fluoro-substituted adenine nucleosides were even better substrates for calf intestine adenosine deaminase than adenosine. These results (Table II) indicate that

TABLE II

SUSCEPTIBILITY OF 9-β-D-PENTOFURANOSYLADENINES TO ADENOSINE DEAMINASE[a]

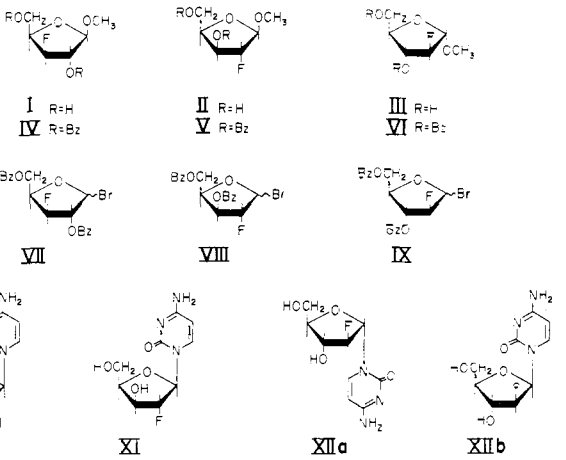
Compound	Configuration at 3′, 2′	Relative velocity
Adenosine	HO, OH	1.0
2′-Deoxyadenosine	HO	1.3
ara-A[b]	OH, HO	0.07
2′-F-*ara*-A	F, HO	1.2
3′-Deoxyadenosine[c]	OH, HO	0.8
xylo-A[d]	OH, F	0.3
3′-F-*xylo*-A	OH, F	1.4

[a] Assay: The reaction mixture contained 0.1 μmol of substrate, 0.15 mmol of Tris buffer (pH 7.4), 0.002 units of calf intestine adenosine deaminase (Sigma Chemical Co., type I) and water to 3.0 ml. Deaminase activity was determined by reading the decrease in optical density at 259 mμ. The reaction products were determined by paper chromatography on Whatman No. 3MM paper, using butanol–water–ammonia 86:14:1, v/v, as the developing solvent. [b] W. W. Lee, A. Benitez, L. Goodman, and B. R. Baker, *J. Amer. Chem. Soc.*, **82**, 2648 (1960). [c] W. W. Lee, A. Benitez, C. D. Anderson, L. Goodman, and B. R. Baker, *ibid.*, **83**, 1906 (1961). [d] O. P. Crews, and L. Goodman, *Syn. Proc. Nucleic Acid Chem.*, **1**, 139 (1968); we are indebted to Dr. R. J. Suhadolnik for a sample of this compound.

9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine (2′-fluoro-*ara*-A) is deaminated about 17 times faster than *ara*-A and at the same rate as 2′-deoxyadenosine. Similarly, 9-(3-deoxy-3-fluoro-β-D-xylofuranosyl)adenine (3′-fluoro-*xylo*-A) is deaminated twice as fast as 3′-deoxyadenosine and about five times faster than *xylo*-A.

(20) G. W. Camiener, *Biochem. Pharmacol.*, **16**, 1691 (1967).

(21) I. Wempen, I. L. Doerr, L. Kaplan, and J. J. Fox, *J. Amer. Chem. Soc.*, **82**, 1624 (1960).



An interesting conclusion follows from a comparison of the arabino and xylo nucleosides with 2′- and 3′-deoxyadenosine. Thus replacement of the 2′-H by OH (*ara*-A) decreases the susceptibility of this nucleoside to deamination, while replacement of the 2′-H by F (2′-fluoro-*ara*-A) does not change its activity as a substrate. A somewhat different situation occurs in the xylo series where the rate of deamination is in the order: 3′-fluoro-*xylo*-A > 3′deoxyadenosine > *xylo*-A. Thus in both the arabino and xylo series, the presence of a 2′- or 3′-OH (respectively) is not necessary for deamination by this enzyme.

In agreement with the results of Cory and Suhadolnik[22] with the same enzyme, we also find that *xylo*-A is deaminated faster than *ara*-A. Others[23] have reported that mouse tumor adenosine deaminase deaminates both compounds at approximately the same rate.

Screening Studies.[24]—Preliminary results reveal that in an L1210 mouse leukemia suspension culture, 2′-F-*ara*-C (XIIb) has a growth inhibitory effect comparable with that of *ara*-C and *ara*-FC [1-β-D-arabinofuranosyl-5-fluorocytosine][25] (5.0 mμg ml for 50% inhibition). Also like *ara*-C, the growth inhibition by 2′-F-*ara*-C could be reversed by 2′-deoxycytidine. 2′-F-*xylo*-C (XI) and 3′-F-*xylo*-C (X) were considerably less effective than 2′-F-*ara*-C as growth inhibitors of these cells since more than 2000 times the concentration was required for 50% inhibition.

Experimental Section[26]

Melting points were measured using a Hoover-Thomas capillary apparatus, and are corrected. Tlc was performed on microscope slides coated with silica gel GF 254 (Merck) and preparative tlc on 40 × 20 cm glass plates coated to a thickness of 2 mm with silica gel PF 254. Column chromatography was carried out using the technique described by Hunt and Rigby,[27] with silica gel G as adsorbent. All evaporations were carried out *in vacuo*. Nmr spectra were determined using a Varian A-60 instrument, with TMS or DSS as standard, and were consistent with the proposed structures. Ir and uv spectra were measured on Perkin-Elmer 221 and Unicam SP 800 instruments, respec-

(22) J. G. Cory and R. J. Suhadolnik, *Biochemistry*, **4**, 1729 (1965).

(23) G. A. LePage and I. G. Junga, *Cancer Res.*, **25**, 46 (1965).

(24) The authors are indebted to Dr. Dorris J. Hutchison and Mariann R. Bjerregaard of this Institute for these preliminary data.

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

(26) Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. Elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich., and by Galbraith Laboratories, Knoxville, Tenn.

(27) B. J. Hunt and W. Rigby, *Chem. Ind.* (London), 1868 (1967).

tively; pK values were measured spectrometrically and are accurate to ±0.05 unit. Cotton effects were observed on a Cary Model 60 spectropolarimeter operating in the circular dichroism mode. Bis(trimethylsilyl)cytosine[11] and 2,5-di-*O*-benzoyl-3-deoxy-3-fluoro-D-xylofuranosyl bromide (VII)[5] were prepared as described in the literature.

Methyl 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-xylofuranoside and -α-D-arabinofuranoside V and VI.—The same procedure for benzoylation was used for both isomers. To a solution of glycoside II (4.11 g) in pyridine (55 ml) at 0°, PhCOCl (14 ml) was added. After stirring 5 hr at 0°, ice (10–20 g) was added, and stirring continued a further 30 min. The solution was diluted with $CHCl_3$ (250 ml) and washed [saturated $NaHCO_3$ solution (three times with 100 ml), H_2O (100 ml)], then evaporated. Repeated evaporation of a solution of the residue in aqueous EtOH served to free it from pyridine, leaving the product V as a viscous, pale yellow sirup (7.5 g, 81%) suitable for the next step. A sample for analysis was purified by preparative tlc using a single elution with EtOAc–C_6H_6 (1:4). Compound V possessed $[\alpha]^{24}D$ −51° (*c* 1.4, EtOH). *Anal.* ($C_{20}H_{19}O_6F$) C, H, F. Compound VI, similarly prepared from glycoside III, showed $[\alpha]^{24}D$ +108° (*c* 1.8, EtOH). *Anal.* ($C_{20}H_{19}O_6F$) C, H, F.

3,5-Di-*O*-benzoyl-2-deoxy-2-fluoro-D-xylo- and -arabinofuranosyl Bromides VIII and IX.—Both isomers were prepared using the same procedure, illustrated here for the *xylo* isomer VIII.

To the glycoside V (2.0 g) ice-cold 30% HBr in glacial AcOH (25 ml) was added, and the solution was allowed to reach room temperature with stirring. After 1 hr, during which the product partially crystallized out, the solution was evaporated (bath temperature <35°), then several aliquots of toluene were evaporated from the residue to remove traces of AcOH. The semisolid residue was dissolved in anhydrous MeCN (10 ml) and used without delay for the next step.

Condensation of Bromo Sugars VII, VIII, and IX with Bis(trimethylsilyl)cytosine.—The procedure was standardized, and is illustrated here for the 2-deoxy-2-fluoroarabinose isomers. Bis(trimethylsilyl)cytosine[11] (2.4 g) was stirred in anhydrous MeCN (22 ml) until partial solution occurred. To the resulting suspension, bromo sugar VIII (dissolved in 6 ml of MeCN; freshly prepared from 1.85 g of V) was added with stirring. The suspension cleared within 5–15 min, and stirring was continued 4 days. MeOH (5 ml) was added, and the mixture was stirred for 20 min to hydrolyze the silyl groups, then Celite (1 g) was added and the mixture filtered to remove cytosine. The filter pad was washed with a small volume of MeCN, and filtrate and washings were evaporated, leaving the reaction mixture as a yellow amorphous solid (2.30 g). On tlc (MeOH–$CHCl_3$, 1:6), many components were visible, including the α and β nucleosidic products of R_f 0.80 and 0.85, respectively. These were separated from the rest of the mixture and from each other by preparative tlc on ten 40 × 20 cm plates, using three elutions with MeOH–$CHCl_3$ (1:14), to give the α anomer, mp 168–170° (617 mg), and the β anomer, mp 216–218° (654 mg). These were then deacylated as described below.

In the case of the 3-deoxy-3-fluoroxylo isomer, the crude reaction mixture after filtration was relatively free of nonnucleosidic material, and was deacylated without prior purification.

The 2-deoxy-2-fluoroxylo isomer gave a complex mixture, and on tlc (MeOH–$CHCl_3$, 1:6), the blocked nucleosides possessed R_f values 0.34 and 0.38 and were present in an α:β ratio of ~1:6. Preparative tlc, eluting twice with MeOH–$CHCl_3$ (1:15), effected some separation, but it was found impossible to free the anomers completely from each other.

The same procedure, illustrated here for 2'-deoxy-2'-fluoro-β-D-xylosylcytosine, was used in all cases for the debenzoylation of blocked nucleosides.

1-(2-Deoxy-2-fluoro-β-D-xylofuranosyl)cytosine (XI).—The blocked nucleoside (656 mg) was dissolved in MeOH (50 ml), and to the solution, NaOMe (20 mg) in MeOH (10 ml) was added. After standing 24 hr at room temperature, the soln was neutralized with glacial AcOH and evaporated to dryness. The residue was partitioned between H_2O (100 ml) and $CHCl_3$ (25 ml), and the aqueous layer washed with a further 25-ml portion of $CHCl_3$, then evaporated to 5 ml and placed on a column 12 × 2.2 cm of Dowex AG 50W-X8 resin (100–200 mesh, H^+ form). After washing with H_2O (250 ml), 30% aqueous MeOH (250 ml), and H_2O (250 ml), the column was eluted with 2 *N* aqueous NH_3 (400 ml). The eluate was evaporated to dryness to give nucleoside XI, contaminated with the α anomer, as an amorphous solid (316 mg). This was dissolved in the minimum volume of MeOH, and the solution cooled to 0°, whereupon pure nucleoside XI (108 mg) crystallized as a monomethanolate, mp 208–209°. By dilution of the mother liquor with EtOH and evaporation, a further 254 mg of XI, mp 205–207°, was isolated. Compound XI possessed $[\alpha]D$ +77° (*c* 0.6, water); uv $\lambda_{max}^{H_2O}$ 269 mμ (ε 9100); $\lambda_{max}^{pH\ 1}$ 277 mμ (ε 13,500); $\lambda_{max}^{pH\ 12}$ 269 mμ (ε 9600); pK_a = 4.04 ± 0.05. *Anal.* ($C_9H_{12}O_4FN_3 \cdot CH_3OH$) C, H, F, N. The presence of 1 mol of MeOH was confirmed in the nmr spectrum of a dried sample of crystalline XI.

All other nucleosides were obtained in amorphous form and were characterized as HCl salts by dissolving in a small volume of MeOH at 0°, then saturating the solution with HCl gas. After standing at 0°, the crystals were filtered off, and washed with ice-cold MeOH. Recrystallization from MeOH–EtOH gave pure compounds.

1-(3-Deoxy-3-fluoro-β-D-xylofuranosyl)cytosine (X)·HCl possessed mp 216–218° dec, $[\alpha]^{24}D$ +37° (*c* 0.8, H_2O); uv $\lambda_{max}^{H_2O}$ 271 mμ (ε 9900); $\lambda_{max}^{pH\ 1}$ 280 mμ (ε 14,100); $\lambda_{max}^{pH\ 12}$ 274 mμ (ε 10,100); pK_a = 4.12 ± 0.05. *Anal.* ($C_9H_{12}FN_3O_4 \cdot HCl$), C, H, F, N.

1-(2-Deoxy-2-fluoro-α-D-arabinofuranosyl)cytosine (XIIa)·HCl possessed mp 220–222° dec; $[\alpha]D$ −12° (*c* 0.5, H_2O); uv $\lambda_{max}^{H_2O}$ 269 mμ (ε 9450); $\lambda_{max}^{pH\ 1}$ 277 mμ (ε 13,200); $\lambda_{max}^{pH\ 12}$ 269 mμ, (ε 9900). *Anal.* ($C_9H_{12}FN_3O_4 \cdot HCl$) C, H, F, N.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)cytosine (XIIb)·HCl possessed mp 240–242° dec; $[\alpha]D$ +108 (*c* 0.2, H_2O); uv $\lambda_{max}^{H_2O}$ 269 mμ (ε 9300); $\lambda_{max}^{pH\ 1}$ 277 mμ (ε 13,000); $\lambda_{max}^{pH\ 12}$ 269 mμ, (ε 9700); pK_a = 3.88 ± 0.05. *Anal.* ($C_9H_{12}FN_3O_4 \cdot HCl$) C, H, F, N.

Preparation of Cytidine Deaminase From Pig Kidney.—A modification of the method of Tomchick, *et al.*,[19] was used. All steps were carried out at 5°. Pig kidney acetone powder (3 g; Mann Research Laboratories) was homogenized in 30 ml of 0.15 *M* KCl with a Potter-Elvehjem homogenizer fitted with a Teflon pestle. After centrifugation for 30 min at 20,000*g* the supernatant was separated and heated for 7 min in a 60° water bath. The heat-denatured mixture was cooled and then centrifuged for 10 min at 20,000*g*. The residue was discarded. Solid $(NH_4)_2SO_4$ was added to the supernatant to give 50% saturation. After standing for 1 hr, the mixture was centrifuged for 10 min at 15,000*g*. The precipitate was dissolved in 2.5 ml of 0.02 *M* potassium phosphate buffer (pH 7.5) and the solution was used directly in the deaminase study. This enzyme preparation was stable at 0° for at least 1 week.

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