K_2CO_3 (8.85 g, 64.0 mmoles), and dimethylformamide (20 ml) was heated to 115°, and 1,2-dibromoethane (3.00 g, 16.0 mmoles) was added dropwise. The resultant mixture was heated at 115–120° for 5 hr, allowed to cool somewhat, and poured into water (100 ml). The precipitate that formed was collected, washed with water, and dried *in vacuo*. Recrystallization of this material (0.91 g, mp 132–134°) from ethanol gave 0.67 g (17%) of VII as white needles, mp 144–146°.

B. From 2-Aminoethanol.—A solution of *p*-toluenesulfonyl chloride (860 g, 4.52 moles) in dimethylformamide (800 ml) was added from a dropping funnel to a mechanically stirred mixture of 2-aminoethanol (276 g, 4.52 moles), anhydrous K_2CO_3 (624 g, 4.52 moles), and dimethylformamide (1.6 l.). Throughout the addition period the temperature of the reaction mixture was maintained at 30–35°, moderate external cooling being necessary. Fifteen minutes after the addition had been completed, the continuously stirred mixture was gradually heated to 115–120°. More K_2CO_3 (1.26 kg, 9.03 moles) was added followed by the dropwise addition during about 45 min of 1,2-dibromoethane (424 g, 2.26 moles). The resultant mixture was stirred and heated at 115–120° for 5 hr, allowed to cool, and poured into water (25 l.). The aqueous mixture was allowed to stand overnight at room temperature, and the white crystalline precipitate that separated was collected and washed thoroughly with water. The product, dried *in vacuo* (P_2O_5), was obtained in 15% crude yield (81.0 g) and melted at 139–141°. Recrystallization from ethanol raised the melting point to 144–146°. Further recrystallization from benzene–ligroin (bp 30–60°) gave a sample of VII with mp 146–147°.

C. From Morpholine.—A freshly prepared solution of *p*-toluenesulfonyl chloride (2.86 g, 15.0 mmoles) in dimethylformamide (5 ml) was added dropwise to a magnetically stirred solution of morpholine (2.61 g, 30.0 mmoles) in the same solvent (5 ml) at such a rate that the reaction temperature did not exceed 40°. The mixture was stirred 1 hr longer at room temperature and was then diluted with water (80 ml). The white precipitate that formed was collected, washed with water, and dried *in vacuo* at room temperature [yield 2.74 g (76%), mp 144–145°]. Recrystallization from benzene–ligroin raised the melting point to 147–148° (lit.[14] mp 147°). Melting points of mixtures of this authentic VII with the products described above (A and B) were not depressed, and their infrared spectra were identical.

Detosylation of VII.—When VII (14.1 g) [erroneously identified at the time as III ($n = 2$) on the basis of melting point] was subjected to treatment with boiling 48% HBr (150 ml initially) in the manner described above for conversion of III ($n = 3$–6) to IV ($n = 3$–6), the only product isolated was morpholine hydrobromide (11.21 g, 12% yield), mp 210–212° (recrystallized from ethanol). No effort was made to isolate any products that might have resulted from ether cleavage.

Anal. Calcd for $C_4H_9NO \cdot HBr$: C, 28.60; H, 6.00; Br, 47.57; N, 8.34. Found: C, 28.90; H, 5.88; Br, 47.9; N, 8.28.

An authentic sample of **morpholine hydrobromide**, prepared from the free base and ethanolic HBr solution, is identical with the above-described sample with respect to melting point, mixture melting point, pmr, and infrared spectra.

(14) J. Soc. Soc. Jpn., **34**, 2906 (1961).

Nucleosides. XXXIII. N^4-Acylated 5-Fluorocytosines and a Direct Synthesis of 5-Fluoro-2′-deoxycytidine[1]

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A series of N^4-acylated 5-fluorocytosines was prepared as starting material for nucleoside synthesis and for chemotherapeutic screening. A direct synthesis of 5-fluoro-2′-deoxycytidine (FCDR, V) and its α-anomer (VII) from the monomercury salt of N^4-toluoyl-5-fluorocytosine (II) was achieved whereby N^4-toluoyl-5-fluoro-2′-deoxycytidine (VIII) was isolated as an intermediate. Compounds II and VIII are converted into 5-fluorouracil (FU) and 5-fluoro-2′-deoxyuridine (FUDR, IX), respectively, by treatment with 0.5 *N* HCl at 37°. The labilization of the exocyclic amino group by aroylation suggested utility of II and VIII as releasers of FU and FUDR (IX) in biological systems. The acylated 5-fluorocytosines are relatively nontoxic compounds exhibiting some activity against systemic *Candida albicans* infections in mice. The nucleoside (VIII) is a potent and toxic agent against experimental tumors in mice. The chemotherapeutic data indicate that *in vivo* the acylated 5-fluorocytosines act as releasers of FC (I) and not of FU, while the nucleoside (VIII) acts as releaser of FCDR (V) and/or FUDR (IX).

5-Fluoro-2′-deoxycytidine (FCDR)[2] was first obtained from 5-fluoro-2′-deoxyuridine (FUDR) by a thiation procedure[3] and its biological and chemotherapeutic properties have been reviewed.[3b] It may be added that, as an inhibitor of the incorporation of formate into DNA thymine in a suspension of Ehrlich ascites carcinoma cells, FCDR was found to be the only fluorinated pyrimidine among 35 screened which was more potent than FUDR.[4a] FCDR showed a relatively high chemotherapeutic index against mouse leukemia B82.[4b] In comparative studies with FUDR, FCDR exhibited a different spectrum of activity against a wide

(1) A preliminary account of this work was presented before the Medicinal Chemistry Section at the 145th National Meeting of the American Chemical Society, New York, N. Y., Sept 1963, p 18-O. This investigation was supported in part (to Sloan-Kettering Institute) by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA-08748).

(2) The designations for compounds used in this paper (*i.e.*, FU, FC, FCDR, and FUDR) conform to widely prevalent usage in the chemical and biological literature.

(3) (a) J. J. Fox, I. Wempen, and R. Duschinsky, Abstracts (supplement) of the 4th International Congress of Biochemistry, Vienna, 1958, p 6; (b) I. Wempen, R. Duschinsky, L. Kaplan and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 4755 (1961).

(4) (a) K. L. Mukherjee and C. Heidelberger, *Cancer Res.*, **22**, 815 (1962); (b) J. H. Burchenal, E. A. D. Holmberg, J. J. Fox, S. C. Hemphill, and J. A. Reppert, *ibid.*, **19**, 494 (1959); (c) K. Sugiura, "Progress in Experimental Tumor Research," Vol. 2, Verlag S. Karger, Basel, 1961, p 357; (d) J. W. Cramer, W. H. Prusoff, and A. D. Welch, *Biochem. Pharmacol.*, **8**, 331 (1961); M. Y. Chu and G. A. Fischer, *ibid.*, **11**, 423 (1962); (e) B. Clarkson, C. Young, W. Dierick, P. Kuehn, M. Kim, A. Berret, P. Clapp, and W. Lawrence, Jr., *Cancer*, **15**, 472 (1962).

TABLE I
PHYSICAL PROPERTIES OF N^4-ACYLATED 5-FLUOROCYTOSINES

II, R =	Formula	Calcd, % C	H	N	F	Found, % C	H	N	F	Spectrophotometric data λ_{max}, mμ	$\epsilon \times 10^{-3}$
Acetyl	$C_6H_6FN_3O_2$	42.11	3.53	24.56		42.34	3.42	24.52		216, 240, 305	14.55, 10.41, 6.21[a]
Propionyl	$C_7H_8FN_3O_2$	45.41	4.36	22.70	10.26	45.66	4.64			214, 242, 309	12.55, 9.4, 4.8[b]
Pivaloyl	$C_9H_{12}FN_3O_2$	50.70	5.67	19.71	8.91	50.45	5.81			214, 242, 313	12.05, 6.8, 5.6[b]
Benzoyl	$C_{11}H_8FN_3O_2$	56.65	3.46	18.02	8.15	57.29	3.21	18.02	8.36	259, 327	11.29, 14.17[b]
p-Toluoyl	$C_{12}H_{10}FN_3O_2$	58.30	4.08	17.00	7.68	58.43	4.41	17.10	7.51	265, 325	12.7, 17.0[b]
p-Methoxybenzoyl	$C_{12}H_{10}FN_3O_3$	11.79[c]		15.96	7.22	11.78[c]		15.82	7.23	286, 332	10.95, 17.10[b]
p-Chlorobenzoyl	$C_{11}H_7ClFN_3O_2$	13.23[d]		15.70	7.10	13.45[d]		15.70	6.60	265, 332	12.50, 15.30[b]
p-Nitrobenzoyl	$C_{11}H_7FN_4O_4$	47.49	2.54	20.14		47.35	2.37	19.86		270, 344	18.45, 17.78[b]
p-Carboxybenzoyl	$C_{12}H_8FN_3O_4$	51.99	2.91	15.16	6.85	51.84	2.93	15.16	7.03	267, 332	22.50, 26.50[b]

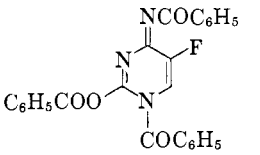
[a] Spectrum taken in water at pH 7.05. [b] Spectrum taken in ethanol. [c] Methoxyl. [d] Chlorine.

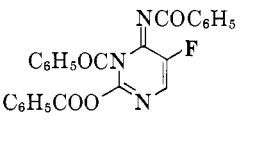
variety of mouse tumors.[4c] Moreover, FCDR proved to be effective against leukemias P815Y and P815 which were resistant to FUDR.[4d]

FCDR had also been prepared[5] enzymatically by a process involving deoxyribose transfer from thymidine to 5-fluorocytosine (FC). This method was not suitable for large-scale preparations. In view of its potential as a useful drug,[4e] a less elaborate synthesis of FCDR was undertaken.

A direct chemical synthesis of FCDR starting from 5-fluorocytosinemercury and a suitably protected 2-deoxy-D-ribofuranosyl chloride was reported in a preliminary communication[6] and a patent.[7] This method gave mixtures of anomers which were difficult to separate. The present paper describes an improved procedure which, like the method described by Fox, *et al.*, for the synthesis of cytidine[8a] and 2′-deoxycytidine,[8b] uses an N^4-acylated cytosine as starting material. Whereas the former studies[8] were limited to N^4-acetylcytosine, in the fluorinated series described herein, we investigated the utility of higher aliphatic acyl groups and aroyl groups as blocking agents for the exocyclic amino function of 5-fluorocytosine. This was undertaken not only for the purpose of obtaining more stable and well-crystallized intermediates suitable for the separation of nucleoside anomers, but also for a study of N^4-acyl-5-fluorocytosines themselves (Table I) as potential antimicrobial and cancerostatic agents (*vide infra*).

Brown, Todd, and Varadarajan[9] have described various acylated cytosines. In general agreement with their findings, 5-fluorocytosine reacted with acid chlorides or anhydrides in the presence of pyridine to yield mainly mono-N^4-acylated FC. The only exception was the action of benzoyl chloride on FC which produced (depending on the amount of benzoyl chloride used and the duration of the reaction) a dibenzoyl- and/or a tribenzoyl-FC and practically no monobenzoyl-FC. Monobenzoyl-FC may be obtained by partial debenzoylation of tribenzoyl-FC with sodium methoxide in methanol. This tribenzoyl-FC derivative probably contains one of its benzoyl groups in ester linkage on the 2 position since its infrared spectrum shows bands at 1746 (C=O) and 1235 (COC) cm^{-1}. Plausible structures for tribenzoyl-FC are shown. Of





the N^4-acyl-5-fluorocytosines prepared (Table I), the N^4-*p*-toluoyl derivative (II, see Scheme I) gave the more easily reproducible results in the subsequent condensation reaction to FCDR. When 5-FC (I) was refluxed with a 10% excess of *p*-toluoyl chloride, an 80% yield of II was obtained. When a 33% excess of acyl halide was used in this reaction, a small amount of a di-*p*-toluoyl-5-fluorocytosine was obtained as a by-product along with II. Conversion of II to N^4-*p*-toluoyl-5-fluorocytosinemercury (III) was accomplished in excellent yield. It had been demonstrated that mercury pyrimidines in which the pyrimidine:mercury ratio is 1:1 are relatively more reactive in condensation reactions with halogenoses than monochloromercuripyrimidines or dipyrimidylmercury derivatives.[8,10]

Condensation of III with 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-ribofuranosyl chloride in hot toluene yielded an anomeric mixture of nucleosides from which the β-anomer (IV) was isolated in 40% yield. Treatment of IV with hot alcoholic ammonia or with hot sodium methoxide in methanol afforded a good yield of FCDR (V) which was identical with that obtained previously[3b] *via* the thiation procedure.

The α-anomer (VI) formed from the condensation of III with halogenose seems to remain largely in a mercury–iodide complex. Isolation of this anomer was accomplished only after repeated and exhaustive washing of the toluene layer with aqueous potassium iodide. "α-FCDR" (VII) was obtained as a levorotatory crystalline product by alcoholysis of VI or of the mercury complex.

It is noteworthy that the 1-(2-deoxy-D-ribofuranosyl)-5-fluorocytosines (V and VII) do not obey Hudson's rules of isorotation, a phenomenon previously observed with other pyrimidine nucleoside anomers.[6,8b,11]

When the tri-*p*-toluoyl derivative (IV) was treated in the cold with sodium methoxide in methanol, only the sugar-blocking groups were removed and N^4-*p*-toluoyl-FCDR (VIII) was obtained. This N-aroylated nucleoside was converted to FUDR (IX) in 93% yield after treatment with 0.5 *N* hydrochloric acid at 37° for 60 hr. Similarly N^4-*p*-toluoyl-5-fluorocytosine (II) was converted in 63% yield to 5-fluorouracil (FU) under these reaction conditions. Under identical conditions, 5-fluorocytosine (I) was found to

(5) J. Malbica, L. Sello, B. Tabenkin, J. Berger, E. Grunberg, J. H. Burchenal, J. J. Fox, I. Wempen, T. Gabriel, and R. Duschinsky, *Federation Proc.*, **21**, 384 (1962).

(6) M. Hoffer, R. Duschinsky, J. J. Fox, and N. Yung, *J. Am. Chem. Soc.*, **81**, 4112 (1959).

(7) R. Duschinsky, U. S. Patent 3,040,026 (1962).

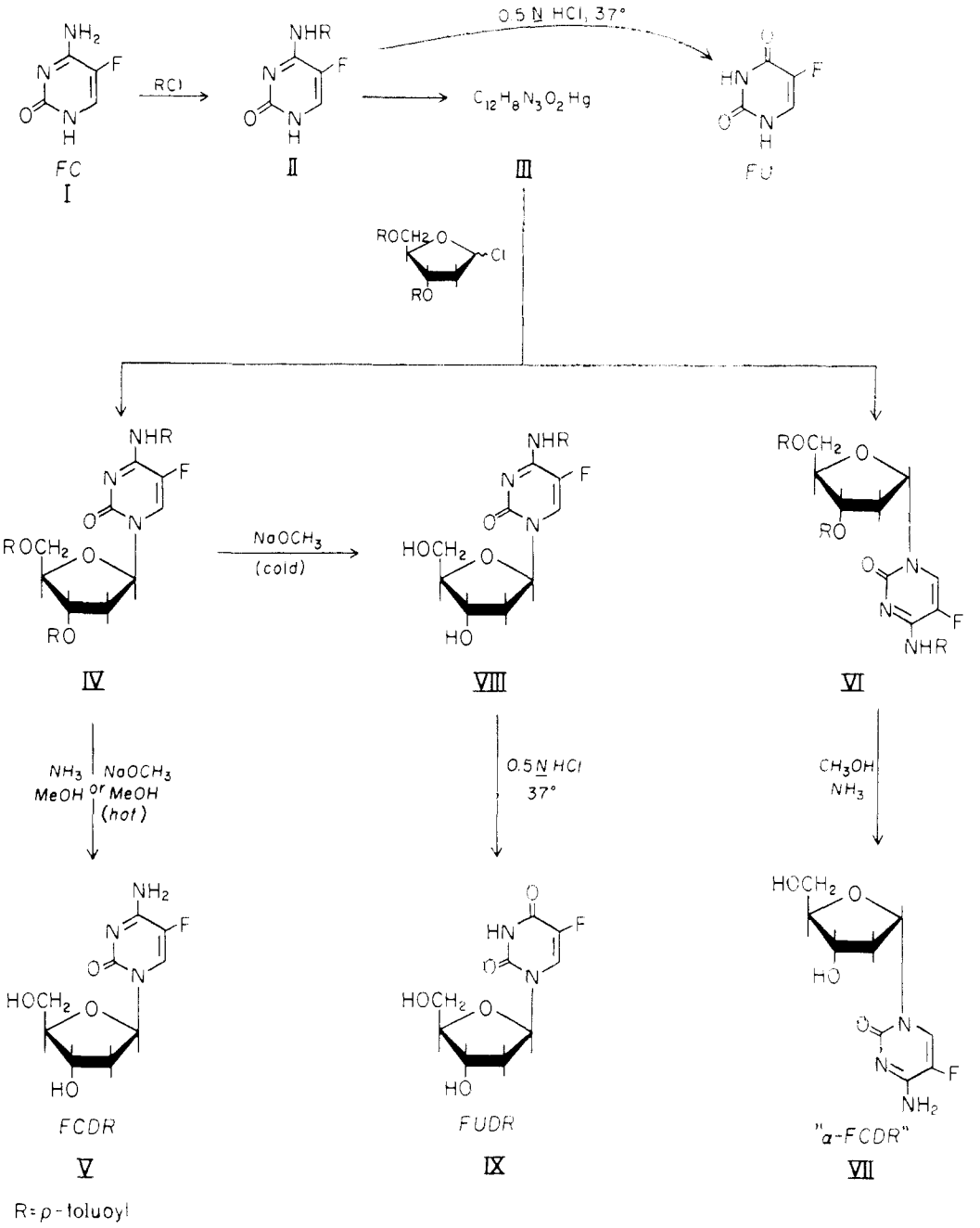
(8) (a) J. J. Fox, N. C. Yung, I. Wempen, and I. L. Doerr, *J. Am. Chem. Soc.*, **79**, 5060 (1957); (b) J. J. Fox, N. C. Yung, I. Wempen, and M. Hoffer, *ibid.*, **83**, 4066 (1961).

(9) D. M. Brown, A. Todd, and S. Varadarajan, *J. Chem. Soc.*, 2384 (1956).

(10) M. Hoffer, *Ber.*, **93**, 2777 (1960).

(11) J. J. Fox and I. Wempen, *Advan. Carbohydrate Chem.*, **14**, 283 (1959); J. Farkaš, L. Kaplan, and J. J. Fox, *J. Org. Chem.*, **29**, 1469 (1964).

SCHEME 1



be stable while N^4-acetyl-5-fluorocytosine was converted to FC in 92% yield and only 8% to 5-fluorouracil.[12]

Biological Aspects.—The chemical data suggested that the labilization of the exocyclic amino group by acylation, and especially aroylation, may have utility in biological systems where *p*-toluoyl-FC (II) or *p*-toluoyl-FCDR (VIII) may act as releasers of FU or FUDR, respectively. On the other hand, biological deacylation would make the N^4-acylated 5-fluorocytosines or N^4-*p*-toluoyl-FCDR act as releasers of FC or FCDR, respectively.

A. Antimicrobial Activity *in Vitro.*—In accord with previous observations,[5] FC_1 in contrast to FU, FUDR, and FCDR, was inactive *in vitro* against bacteria, but it was very effective against the four fungi listed in Table II. When N^4-*p*-toluoyl-FC was tested against the same organisms, it likewise showed no antibacterial activity but it did exhibit moderate antifungal activity. On the other hand, the nucleoside, N^4-*p*-toluoyl-FCDR (VIII), exhibited antibacterial activity of the same order as FCDR or FUDR, but compound VIII was essentially inactive against yeasts and fungi. α-FCDR (VII), when tested at 0.4 μmole/ml, was completely inactive against all the organisms listed in Table II. It is noteworthy that α-FCDR is neither cleaved nor deaminated by extracts of *Escherichia coli*.[13]

B. Antimicrobial Activity *in Vivo.*—The results of intraperitoneal, oral, or subcutaneous treatment with FC and its N^4-acylated derivatives in mice infected

(12) These results appear to parallel those of Brown, *et al.*[9] They found that treatment of N^4-acetylcytosine with 80% acetic acid for 1 hr at 100° yielded uracil and cytosine in approximately equal amounts. N^4-Benzoylcytosine yielded uracil and cytosine in a ratio of *ca.* 33:1 when heated in 80% acetic acid at 100° for 2 hr.

(13) The authors are indebted to J. Malbica of Hoffmann-La Roche for these results.

TABLE II

THE *in Vitro* ANTIMICROBIAL ACTIVITY OF SOME FLUORINATED PYRIMIDINES[a]

	Diameter of inhibition zones[c], mm						
			FC (I)		N^4-Toluoyl-FC[d] (II)		N^4-Toluoyl-FCDR (VIII)
Test organism[b]	FU 0.77 μmole/ml	FUDR (IX) 0.4 μmole/ml	0.88 μmole/ml	0.034 μmole/ml	0.4 μmole/ml	FCDR (V) 0.4 μmole/ml	0.4 μmole/ml
Escherichia coli	21	11	0	0	0	16	0
Aerobacter aerogenes	16^{23}	0	0	0	0	16	0
Bodenheimer's bacillus	23 ha	0	0	0	0	0	0
Proteus vulgaris	13^{29}	19	0	0	0	16^{24}	16
Pseudomonas aeruginosa	42 ha	0	0	0	0	0	0
Mycobacterium phlei	30	12	0	0	0	0	27
Streptomyces cellulosae	34 ha	0	0	0	0	0	0
Bacillus simplex	28	21	0	0	0	16^{25}	25
Sarcina lutea	>50 (32)[e]	>50 (25)[e]	0	0	0	24^{50} (26)[e,f]	17^{21}
Bacillus E	17 ha	20	0	0	0	0	0
Bacillus subtilis	24	0	0	0	0	19^{26}	16^{19}
Staphylococcus aureus	43^{48}	15^{37}	0	0	0	19^{40}	19^{36}
Paecilomyces varioti	40 ha	35	34	19	23	27	14
Penicillium digitatum	0	36	39	18	26	37	0
Candida albicans	0	18	30	13	0	0	0
Saccharomyces cerevisiae	20^{31}	0	37	23	12	0	0

[a] The familiar penicillin-type cup–plate agar diffusion assay (D. C. Grove and W. A. Randall, "Assay Methods of Antibiotics. A Laboratory Manual," Medical Encyclopedia Inc., New York, N. Y., 1955, pp 7–16) was employed, the upper 4-ml layer of agar being seeded with various microorganisms. [b] The bacteria (the first five gram-negative and the next seven gram-positive organisms listed) were grown at 35° (except *Bacillus* E at 42°) on a pH 6.2 nutrient agar containing 0.15% beef extract, 0.3% yeast autolysate, 0.4% casein hydrolysate (N-Z-Amine A), 0.6% peptone, and 0.1% glucose. The yeasts and fungi were grown on a semisynthetic vitamin–salts–amino acid hydrolysate medium, pH 5.5; *P. digitatum* at 26°, the others at 35°. [c] The superscript numbers indicate the diameters of secondary faint but definite zones of growth inhibition which extend beyond the primary clearer inhibition zone; the symbol "ha" refers to definite but hazy inhibition zones. [d] Other N^4-acyl-FC derivatives (Table I) were screened against these bacteria; none of them showed any significant activity. [e] Values in parentheses are for 0.0077 μmole of FU and 0.004 μmole of FCDR and FUDR. [f] Refers to secondary zone only; the primary zone had disappeared.

TABLE III

TREATMENT OF SYSTEMIC *Candida albicans* INFECTION OF MICE[a]

	Toxicity, LD_{50}, mg/kg			Therapeutic response					
				Dose, mg/kg	Kidney		Dose, mg/kg	Kidney	
Drug	sc	ip	*po*	ip	Lesions[b]	Cultures[b]	*po*	Lesions[b]	Cultures[b]
FC	>2000	>500	>2000	100	14/20	10/20[c]	500	14/19	10/19[d]
				50	6/9	3/9	200	5/9	4/9
							100	1/9	4/9
N^4-Acetyl-FC				200	8/8	3/8			
N^4-Propionyl-FC	>500	>500	>500	200[e]	5/9	2/9	200	1/8	0/8
N^4-Pivaloyl-FC		>500	>1000	50	6/10	1/10			
N^4-Benzoyl-FC				200	8/9	3/10			
N^4-*p*-Toluoyl-FC	>2000	>500	>2000	100	4/10	0/10	500	7/9	2/9
N^4-*p*-Methoxybenzoyl-FC	>2000	>500	>2000	100	7/14	1/14			
				50	2/7	0/7			
N^4-*p*-Chlorobenzoyl-FC		>500	>500	100	5/5	1/5			
N^4-*p*-Nitrobenzoyl-FC	>2000	>500	>2000	100	0/7	0/7			
N^4-Di-*p*-toluoyl-FC	>2000		>2000	200	9/10	2/10			
Tribenzoyl-FC		>500	>500	200	8/8	2/8	500	6/10	2/10
				100	0/7	0/7			

[a] Swiss albino mice weighing 18–20 g were conditioned with cortisone by the intramuscular administration of 100 mg/kg daily in two divided doses on the day before, the day of, and the day after infection. Infection was accomplished by the intravenous injection of 0.5 ml of a suspension of *C. albicans* containing 100,000 cells/ml. Treatment was begun on the day of infection and continued daily for 28 days or until the animals succumbed. Upon the death of the mice, the kidneys were examined for the presence of gross lesions. In addition, contact cultures of the bisected kidneys were made on Sabouraud's agar plates. [b] Number negative/total animals treated. [c] CD_{50} = 87 mg/kg ip. [d] CD_{50} = 302 mg/kg *po*. [e] Drug administered subcutaneously.

intravenously with *Candida albicans* according to a technique similar to that described by Grunberg, *et al.*,[14a] are shown in Table III. FC exerted an appreciable activity when treatment was administered intraperitoneally or orally and when effectiveness was evaluated by the criteria of negative cultures and negative kidney lesions. The remaining compounds were less effective than FC when evaluated by the same criteria, but these results indicated the presence of some anticandida activity with the N^4-acylated-FC derivatives.[14b] FU, FUDR, and FCDR showed little or no effect against the systemic candida infection when evaluated by the same technique. In addition, FC and the majority of its acylated derivatives were found to be

(14) (a) E. Grunberg, E. Titsworth, and M. Bennet, *Antimicrobial Agents Chemotherapy*, 566 (1963). (b) Using the average survival time of mice infected with *Candida albicans* as the criterion for the evaluations of chemotherapeutic activity, Dr. H. Scholer, Hoffmann-La Roche, Basel, obtained essentially the same results as those described in the present paper: the acylated 5-fluorocytosines tested were found to be 10–20 times less effective than fluorocytosine itself. We thank Dr. Scholer for permission to use this summary of his data which will be published in detail elsewhere.

TABLE IV: ANTITUMOR ACTIVITY OF FLUORINATED PYRIMIDINES

Compd[a]	Daily dose, mg/kg ip	Tumor[b]	Wt change, g T/C	Tumor growth T/C
FC	500 × 8	S180	+4.5/+5.1	0.87
N[3]-*p*-Toluoyl-FC[c]	200 × 8	S180	−1.1/+1.7	1.18
N[4]-*p*-Chlorobenzoyl-FC	200 × 8	S180	+1.6/+1.7	1.10
N[4]-*p*-Nitrobenzoyl-FC	200 × 8	S180	+0.1/+0.3	1.26
FU[c]	18 × 7	S180	+0.1/−2.1	0.22
N[4]-*p*-Toluoyl-FC[c]	75 × 7	S180	−1.5/−2.1	0.86
FCDR[c]	33 × 7	S180	−2.5/−1.0	0.06
N[4]-*p*-Toluoyl-FCDR[c]	50 × 7	S180	−2.5/−1.0	0.05
FCDR[c]	33 × 7	Ca755	+1.5/+2.3	0.52[d]
N[4]-*p*-Toluoyl-FCDR[c]	50 × 7	Ca755	−1.4/+2.3	0.39[d]
FUDR	24.5 × 10	B82T	−0.4/+1.2	0.07
FCDR	24.5 × 10	B82T	−0.3/+1.2	0.03
N[4]-*p*-Toluoyl-FCDR[e]	4.4 × 10	B82T	−0.9/+2.7	0.06
N[4]-*p*-Toluoyl-FCDR[e]	2.6 × 10	B82T	+0.1/+2.7	0.17
N[4]-*p*-Toluoyl-FCDR[e]	1.6 × 10	B82T	+1.6/+2.7	0.48
FC	13.0 × 10	B82T	−0.3/+2.0	0.02
FU	6.5 × 10	B82T	+0.8/+2.0	0.15
N[4]-Acetyl-FC	43.0 × 10	B82T	+1.5/+2.0	0.97
N[4]-*p*-Toluoyl-FC	109.0 × 10	B82T	+2.1/+2.0	0.94
FCDR	12.2 × 10	B82T	−2.3/+2.0	0.03
FCDR	6.1 × 10	B82T	+0.1/+2.0	0.19
N[4]-*p*-Toluoyl-FCDR	6.7 × 10	B82T	−3.0/+2.0	0[f]
N[4]-*p*-Toluoyl-FCDR	3.35 × 10	B82T	−0.3/+2.0	0.23
N[4]-*p*-Toluoyl-FCDR	1.7 × 10	B82T	+1.1/+2.0	0.54

[a] Bracketed compounds were tested simultaneously. [b] S180 = Sarcoma 180; Ca755 = Adenocarcinoma 755; B82T = leukemia B82T (solid form). [c] The authors are indebted to Dr. C. Heidelberger of the University of Wisconsin for these results. [d] Average survival time (days) of mice was 24.5 (FCDR), 31 (toluoyl-FCDR), 23.8 (controls). [e] LD_{50} (single intraperitoneal injection) = 130 mg/kg. [f] 9/10 mice died early from toxicity.

inactive against the following experimental infections in mice: (1) systemic *Streptococcus pyogenes, Staphylococcus aureus, Proteus vulgaris,* and *Pseudomonas aeruginosa;* (2) systemic *Histoplasma capsulatum;* (3) Col SK and influenza A viruses; (4) local *Trichomonas vaginalis;* (5) *Syphacia obvelata* and *Hymenolepis fraterna.*

C. Antitumor Activity.—Table IV summarizes results obtained with various transplantable mouse tumors. Like FC, and in contrast to FU, FUDR, and $FCDR_1$ the acylated 5-fluorocytosines are essentially inactive against tumors.[15] N[4]-*p*-Toluoyl-FCDR (VIII) however proved to be a very potent and toxic cancerostatic agent with a narrow therapeutic range. On a molar basis, VIII was found to be more effective against most tumors but also more toxic toward the host than FUDR and FCDR. Compound VIII exhibited teratogenic activity in the chick embryo in the LD_{50} range of FUDR and FCDR.[16]

Discussion

5-Fluorocytosine, in contrast to FU, FUDR, or FCDR, is not active against bacteria and it is neither toxic nor cancerostatic in mice.[5] In fact, fluorocytosine (FC) is not metabolized in the rat or in man.[17] However, FC is effective against fungi (*e.g., Candida albicans*) *in vitro* and *in vivo*.[5,14] The screening data showed that N[4]-*p*-toluoyl-FC (as well as the other N[4]-acylated-FC derivatives) were not bacteriostatic but they did exhibit moderate antifungal activity *in vitro* (Table II) and *in vivo* (Table III). The N[4]-acylated-FC derivatives were essentially nontoxic in mice and showed no antitumor activity. These data indicate that the N[4]-acylated-5-fluorocytosines function as releasers of FC but not of FU.

It is difficult to make a definitive statement concerning nucleoside VIII. Compound VIII exhibited general antibacterial activity *in vitro* as did FUDR and FCDR. On a molar basis the toxicity and antitumor activity in mice was higher than FUDR and FCDR. One can assume that like N[4]-*p*-toluoyl-FC the nucleoside (VIII) undergoes deacylation to become biologically active.[18] Accordingly, VIII would act as a releaser of FCDR, but, the latter compound could undergo concomitant deamination to FUDR. Since it is known that FUDR is considerably more potent when administered to man[19a] and to mice[19b] by slow infusion rather than by quick injection, the high potency of toluoyl-FCDR (VIII) could be explained by the slow release of FUDR *in vivo*. However, too little is known about the behavior of FCDR on slow administration, and insufficient data have been collected thus far on the biological properties of VIII to decide whether this N[4]-acylated nucleoside functions as a releaser of FCDR or of FUDR.

Experimental Section[20]

5-Fluorocytosine.[5,21]—5-Fluorocytosine was prepared in almost quantitative yield by the method previously described

(15) Occasional positive results obtained with a high dosage of compounds could be explained by the presence of FU as contaminant.

(16) The authors are indebted to Dr. David A. Karnofsky of Sloan-Kettering Institute for these data.

(17) B. A. Koechlin, F. Rubio, S. Palmer, T. Gabriel, and R. Duschinsky, *Biochem. Pharmacol.*, **15**, 435 (1966).

(18) The compound was neither cleaved nor deaminated by extracts of *Escherichia coli*.[13]

(19) (a) R. D. Sullivan, C. W. Young, E. Miller, N. Glaistein, D. Clarkson, and J. H. Burchenal, *Cancer Chemotherapy Rept.*, **8**, 77 (1960); (b) Dr. Margaret S. Lyman, Sloan-Kettering Institute, personal communication.

(20) Melting points were taken on a Thomas–Hoover apparatus and are uncorrected.

(21) R. Duschinsky, E. Pleven, and C. Heidelberger, *J. Am. Chem. Soc.*, **79**, 4559 (1957).

involving hydrolysis with hot concentrated HCl of 2-chloro-4-amino-5-fluoropyrimidine.[7,22] To avoid further hydrolysis of FC to FU the reaction was monitored spectrophotometrically. Ultraviolet absorption data are for 2-chloro-4-amino-5-fluoropyrimidine, $\lambda_{max}^{0.1NHCl}$ 235, 269–270 mμ (ε 7410, 6100), for 5-fluorocytosine, $\lambda_{max}^{0.1NHCl}$ 286 mμ (ε 9100), $\lambda_{max}^{0.1NNaOH}$ 292 mμ (ε 7600). The progress of the reaction was followed by the change from 0.1 to 0.85 in the 300/270 extinction ratio determined in 0.1 *N* HCl. When the higher ratio was reached, the reaction was stopped.

N⁴-Acetyl-5-fluorocytosine.—5-Fluorocytosine (12.9 g) was heated to 110–115° with 60 ml of acetic acid and 12 ml of acetic anhydride until solution was complete (5 min). The product crystallized from the cooled solution and was separated by filtration and dried to constant weight at 80°. The yield was quantitative (17.1 g), mp 235–237° dec.

N⁴-Propionyl-5-fluorocytosine.—A mixture of 5-fluorocytosine (12.9 g) in dimethylformamide (50 ml) and propionic anhydride (13 g) was heated with stirring at 130–140° for *ca.* 15 min, whereupon a clear solution resulted. The product which crystallized from the cooled solution was collected by filtration and washed successively with a small volume of water and alcohol, mp 237–238° dec, 16.5 g (90% yield).

N⁴-Pivaloyl-5-fluorocytosine.—A mixture containing 13 g of 5-fluorocytosine, 26 ml of dimethylformamide, and 19 g of pivalic anhydride was heated with stirring at 110–120° for 15 min. The reaction was cooled and the crystalline product was filtered and recrystallized from methanol to afford a 56% yield of pure product, mp 227–229° dec.

Tribenzoyl-5-fluorocytosine.—To a suspension of 13 g (0.1 mole) of FC in 100 ml of pyridine, 100 ml of benzoyl chloride (0.87 mole) was added dropwise with vigorous stirring over 45 min. The temperature rose spontaneously to 50° and a clear purple solution was obtained which gradually deposited crystals. After stirring overnight, the crystals of pyridinium chloride were separated by filtration and the filtrate was evaporated *in vacuo* to a syrup which became crystalline upon cooling. The purple solid was collected by filtration, washed with pyridine, and slurried with 100 ml of 6 *N* HCl, whereupon it became colorless. After slurrying with 100 ml of ether, the precipitate was collected by filtration and washed with water. After drying *in vacuo*, 20 g of product melting at 127–129° was obtained. [The mother liquor (A) was kept for further work-up]. After two recrystallizations from 100 to 200 ml of ethanol, 12.44 g of a compound melting at 140°, λ_{max}^{EtOH} 240 mμ (ε 35,700), was obtained. The infrared spectrum in chloroform showed bands at 1746 (ester), 1704 (amide), 1235 (COC=O) cm⁻¹.

Anal. Calcd for $C_{25}H_{16}FN_3O_4$: C, 67.87; H, 3.87; F, 4.29; N, 9.50. Found: C, 68.33; H, 3.64; F, 3.84; N, 9.44.

Treatment of the original pyridine-containing mother liquor with 100 ml of concentrated HCl produced an oil which was washed with 100 ml of water and extracted four times with 100 ml of ether. The ether extract was washed with $NaHCO_3$ solution and water and dried (Na_2SO_4). Upon addition of 500 ml of petroleum ether (bp 30–60°), 15.0 g of product, mp 131–133°, was obtained. This material gave upon crystallization from 250 ml of ethanol 12.9 g of product, mp 139.5–140.5°.

Mother liquor (A) (*vide supra*) was extracted three times with 300 ml of ether, the ether extract was washed with $NaHCO_3$ solution, and the product was precipitated from the ether with petroleum ether. The yield of product was 3.8 g, mp 138–139.5°. An additional 4.9 g of product melting at 139.5–140.5° was recovered from the alcoholic recrystallization mother liquors. The total yield of tribenzoyl-FC was *ca.* 75%.

N⁴-Benzoyl-5-fluorocytosine.—To an ice-cooled stirred suspension of 2.21 g of tribenzoyl-FC (0.005 mole) in 50 ml of methanol was added 2.25 ml of 4.44 *N* sodium methoxide solution. A clear solution was obtained in about 10 min and was allowed to stand at 2° for 16 hr. After neutralization with alcoholic HCl the solution was evaporated *in vacuo* and the resulting white solid was slurried with 20 ml of water and 20 ml of ether, filtered, and washed with water and ether (until disappearance of methyl benzoate odor and chloride ions). The air-dried material was recrystallized from 85 ml of ethano , yield 0.52 g (44%), mp 256–257°. For analysis, the product was recrystallized from 6.5 ml of hot DMF to which was added gradually 10 ml of water. After filtration and washing with methanol and ether, 0.45 g of product, mp 256–257°, was obtained.

Dibenzoyl-5-fluorocytosine.—To a suspension of 2.58 g (0.02 mole) of FC in 100 ml of pyridine, 28.1 g (0.2 mole) of benzoyl chloride was added within 5 min. The mixture became warm spontaneously and was maintained at room temperature for 45 min with stirring. Crystallized pyridinium chloride was filtered off. The filtrate formed two phases upon addition of 120 ml of concentrated HCl. The mixture was extracted twice with 100 ml of ether and the ether layer was washed five times with 100 ml of 5% $NaHCO_3$ solution and then dried (Na_2SO_4). The ether solution was treated with 500 ml of petroleum ether and cooled, whereupon 1.3 g of product (19%) was obtained. After recrystallization from 20 ml of ethanol, the melting point was 158–160°. It resolidified and melted again at 203° with gas evolution; λ_{max}^{EtOH} 236, 275–276, 331 mμ (ε 21,600, 13,000, 2850); λ_{min}^{EtOH} 255, 305 mμ (ε 7400, 2590).

Anal. Calcd for $C_{18}H_{12}FN_3O_3$: C, 64.09; H, 3.59; F, 5.63; N, 12.46. Found: C, 64.23; H, 3.69; F, 5.54; N, 12.66.

Acid hydrolysis of dibenzoyl-5-fluorocytosine gave, in addition to unchanged starting material, 14% of FC and 36% of FU.

N⁴-*p*-Toluoyl-5-fluorocytosine (II).—To a suspension of 12.9 g (0.1 mole) of FC in 100 ml of pyridine was added 17.0 g (14 ml, 0.11 mole) of *p*-toluoyl chloride. The temperature rose spontaneously to 45° and complete solution occurred. After refluxing for 5 hr, the mixture was allowed to crystallize. Excess toluoyl chloride was destroyed by stirring the suspension for 20 min with 50 ml of ethanol. The crystals were filtered and washed free of Cl^- with ethanol, then with ether, and dried *in vacuo* at 60°. The yield was 18.3 g, mp 250–251° dec. After evaporation of the mother liquor to dryness, trituration of the residue with ethanol, and reevaporation and final suspension in 20 ml of ether and 20 ml of water, a second crop, 1.6 g, was obtained. The total yield was 20 g (80%). For analysis, a sample was recrystallized from *ca.* 300 vol of 90% ethanol, mp 257–258° dec.

Di-*p*-toluoyl-5-fluorocytosine.—In a similar run with 9.85 g of 5-fluorocytosine, a 33% excess of *p*-toluoyl chloride (15.74 g) was added in the course of 1.5-hr reflux period. The yield of II was 14.7 g (78%). The alcoholic mother liquor gave, upon evaporation, a residue which was extracted with 250 ml of boiling 2-propanol. Upon addition of 140 ml of water to the extract, the ditoluoyl derivative (2.25 g) crystallized as needles. After recrystallization from 2-propanol–water (2:1) it melted at 165–166°; λ_{max}^{EtOH} 244, 278, 329 mμ (ε 29,800, 22,100, 5210); λ_{min}^{EtOH} 221, 262, 307 mμ (ε 13,540, 16,510, 4400).

Anal. Calcd for $C_{20}H_{16}FN_3O_3$: C, 65.75; H, 4.41; N, 11.50; F, 5.20. Found: C, 65.52; H, 4.45; N, 11.60; F, 5.63.

The following compounds were obtained as described for the preparation of N⁴-*p*-toluoyl-FC using a 10% excess of aroyl chloride: **N⁴-*p*-methoxybenzoyl-FC,** mp 260–261° dec, yield 71%; **N⁴-*p*-chlorobenzoyl-FC,** mp 238–239° dec, yield 60%; **N⁴-*p*-nitrobenzoyl-FC,** mp 280° dec, yield 80%.

N⁴-(*p*-Carboxybenzoyl)-5-fluorocytosine.—A suspension of 1.29 g (0.01 mole) of 5-fluorocytosine and 2.03 g (0.011 mole) of *p*-chloroformylbenzoic acid in 20 ml of anhydrous pyridine was heated to reflux. The initial suspension cleared rapidly. After 5 hr of refluxing the solution was cooled to room temperature, 75 ml of methanol was added, and the solid was filtered and washed with methanol. The filtrate was concentrated to dryness, and the residue was slurried with 20 ml of water and filtered. Crystals separated from the filtrate after several days at room temperature, and the solid was filtered and washed with water, methanol, and ether. The crude N-(4-*p*-carboxybenzoyl)-FC (0.50 g, 18% yield) melted at 270° dec. For recrystallization, the material was dissolved in 7 ml of hot DMF, and 16 ml of methanol and 20 ml of water were added to the hot solution. The resulting suspension was cooled to room temperature, and the solid was filtered and washed with water, methanol, and ether. After drying at 110° *in vacuo* to constant weight, the crystalline N-(*p*-carboxybenzoyl)-5-fluorocytosine melted at 271–272° dec.

N-*p*-Toluoyl-5-fluorocytosinemercury (III).—A solution of 8.97 g (0.028 mole) of mercuric acetate in 75 ml of boiling ethanol was added to a solution of 6.95 g of II in 75 ml of hot (100°) DMF. The resulting milky solid was completely precipitated by the addition of 800 ml of ether. It was centrifuged, washed with ether, and dried at 35° *in vacuo* to give 11.8 g (94%) of almost white mercury salt.

Anal. Calcd for $C_{12}H_8N_3O_2Hg$: F, 4.26; N, 9.43. Found: F, 4.07; N, 9.13.

(22) G. G. Durr, *J. Med. Chem.*, **8**, 253 (1965).

In further experiments the mercury salt was precipitated quantitatively with cyclohexane and used (without being analyzed) for the next step.

1-[2-Deoxy-3,5-di-(*O*-*p*-toluoyl)-β-D-ribofuranosyl]-N⁴-*p*-toluoyl-5-fluorocytosine (IV).—A suspension of 36.7 g (0.081 mole) of monomercury N-*p*-toluoyl-5-fluorocytosine (III) in 850 ml of toluene was dried azeotropically by distillation of 100 ml of toluene. The suspension was cooled to room temperature and 62.6 g (0.161 mole) of 3,5-di-(*O*-*p*-toluoyl)-2-deoxy-D-ribofuranosyl chloride (95% pure) was added with stirring. The temperature rose slightly and before the suspended material dissolved completely, crystallization began. After 45 min of stirring, 200 ml of 30% aqueous KI solution was added and stirring was continued for 12 min. The resulting dense crystals were washed subsequently with 50 ml of water, 80 ml of ether, twice with a mixture of 50 ml of 30% KI solution and 50 ml of ethanol, and finally with ethanol and ether. The yield was 14.9 g, mp 212–215°. The toluene layer was washed once with 300 ml and twice with 75 ml of 30% KI solution and finally with eight 250-ml portions of water. [If these repeated washings with KI solution are omitted, a mercury iodide nucleoside complex which consists of mostly the α-anomer (VI) remains intact. Though product (VII) can be obtained from this complex, the procedure is very laborious.] After standing overnight, a second crop (3.1 g) of IV, mp 213–216° was obtained from the toluene mother liquor. Additional crops of IV were obtained by repeated washing of the toluene mother liquors with KI solution and concentration of the toluene layer to a syrup which was crystallized from boiling methanol. The total yield of crude IV was *ca.* 20 g (44%). Recrystallization of the crude material from 70 to 100 vol of butyl acetate afforded 17 g of pure IV: mp 233–234°; $\lambda_{max}^{CH_2Cl_2}$ 243, 332–333 mμ (ε 37,500, 26,500); $\lambda_{min}^{CH_2Cl_2}$ 290 mμ (ε 8490); $[\alpha]^{25}D$ +5.5° (*c* 1.0, DMF).

Anal. Calcd for $C_{33}H_{30}FN_3O_7$: C, 66.10; H, 5.04; F, 3.17; N, 7.01. Found: C, 66.16; H, 5.03; F, 3.01; N, 6.90.

1-[2-Deoxy-3,5-di(*O*-*p*-toluoyl)-α-D-ribofuranosyl]-N⁴-*p*-toluoyl-5-fluorocytosine (VI).—The methanolic mother liquor remaining from the isolation of IV (*vide supra*) was evaporated to dryness and the residue was dissolved in 80 ml of CCl_4. The waxy crystals which formed were triturated with 75 ml of ether and filtered. The product was purified by boiling with 75 ml of ether, decantation of the cooled mixture, and finally by treatment with 225 ml of boiling methanol. Clustered needles (7.5 g) were obtained, which melted at 152–153°. After a recrystallization from 80 vol of ethanol, the product melted at 152–153°; $[\alpha]^{24}D$ −133° (*c* 0.5, DMF); $\lambda_{max}^{CH_2Cl_2}$ 243, 334 mμ (ε 37,500, 28,200); $\lambda_{min}^{CH_2Cl_2}$ 291 mμ (ε 9200).

Anal. Calcd for $C_{33}H_{30}FN_3O_7$: C, 66.10; H, 5.04; F, 3.17; N, 7.01. Found: C, 66.54; H, 5.33; F, 3.27; N, 6.42.

N⁴-*p*-Toluoyl-5-fluoro-2′-deoxycytidine (VIII).—To a suspension of 5.61 g (0.01 mole) of IV in 125 ml of methanol was added gradually 17.3 ml of 1 *N* sodium methoxide. Solution occurred after 30 min of shaking. The solution was stored in the refrigerator for 1 hr. Neutralization with methanolic HCl produced crystals, which were filtered and washed with ether, yield 1.55 g. The mother liquor and washings were evaporated to dryness, and the residue was triturated with 50 ml of ether and 10 ml of water. The resulting crystals were filtered and washed first with a mixture of 100 ml of water and 20 ml of ethanol, then with ethanol, and finally with ether. A second crop of 0.82 g was thus obtained. Both products melted at 193–195°, resolidified, and then melted with decomposition around 215°. The crude product was dissolved in a boiling mixture of 230 ml of water and 46 ml of ethanol, the solution was clarified by filtration and cooled. Pure product, 2.17 g (60%), crystallized as long needles: mp 214–216° dec; λ_{max}^{EtOH} 264–265, 329–330 mμ (ε 15,670, 18,400); λ_{min}^{EtOH} 234, 292 (ε 8170, 8690); $[\alpha]^{25}D$ +90.3° (*c* 0.4, DMF).

Anal. Calcd for $C_{17}H_{18}FN_3O_5$: C, 56.19; H, 4.99; F, 5.23; N, 11.56. Found: C, 56.11; H, 4.74; F, 4.84; N, 11.32.

5-Fluoro-2′-deoxycytidine (FCDR, V). A. Ammonia Method.—Compound IV (10.71 g, 19.2 mmoles) was suspended in 170 ml of ethanol containing 15% NH_3 and stirred at room temperature for 16 hr. Since this treatment resulted in incomplete reaction, 330 ml of methanol was added. The stirred mixture was cooled, saturated with NH_3, and allowed to stand 20 hr longer. The resulting solution was clarified with Celite and Norit and evaporated to dryness. The resulting crystalline powder, after triturating with 80 ml of boiling ethanol, cooling, and adding of 80 ml of ether, yielded 2.33 g of FCDR, mp 193.5–195°. Addition of ether to the mother liquor followed by evaporation to dryness and trituration of the residue with 100 ml of boiling butyl acetate afforded additional lower melting crops of FCDR; total yield 4.21 g (89%).

For analysis, pooled samples were slurried with *ca.* 10 vol of boiling ethanol, the mixture was cooled, and the crystals were filtered; $[\alpha]^{25}D$ +71.7° (*c* 1, water), mp 196.5–197°.

Anal. Calcd for $C_9H_{12}FN_3O_4$: C, 44.08; H, 4.93; F, 7.75; N, 17.14. Found: C, 44.33; H, 5.02; F, 7.52; N, 16.73.

B. Sodium Methoxide Method.—A suspension of 2.24 g of IV (0.004 mole) in 25 ml of methanol (containing some phenolphthalein) was refluxed. Dropwise addition of 2.7 ml of 0.85 *N* sodium methoxide within 15 min maintained the alkalinity and produced complete solution. Refluxing was continued and the progress of the detoluoylation was monitored by the disappearance of the absorption maximum at 320 mμ in 0.1 *N* NaOH. After 1 hr of total refluxing time, the solution was neutralized with 2.7 ml of 0.85 *N* alcoholic HCl and evaporated *in vacuo* to a syrup which crystallized. The crystals were dissolved in 1-butanol and reevaporated and dried *in vacuo* at 60°. The yellowish solid was taken up in 80 ml of boiling butanol and the resulting cloudy solution was filtered through Celite and Norit A. After cooling and filtration, 0.43 g of crystals melting at 194–196° was obtained. The mother liquor was evaporated, and the residue was treated with toluene, evaporated *in vacuo*, and crystallized from butanol; yield 0.23 g, mp 196–197° (total yield, 66%). The product was chromatographically and spectrophotometrically pure and showed no melting point depression when admixed with 5-fluoro-2′-deoxycytidine prepared by the thiation procedure.[36] The mother liquor yielded 0.12 g of less pure product.

1-(2-Deoxy-α-D-ribofuranosyl)-5-fluorocytosine (α-FCDR, VII).—A solution of 559 mg of VI in 25 ml of 6 *N* methanolic NH_3 was allowed to stand at room temperature for 24 hr after which time the absorption at 320 mμ had practically disappeared. The solution was evaporated to dryness *in vacuo*, and the residue was refluxed in 30 ml of chloroform for a few minutes and allowed to stand at room temperature overnight. The product was filtered; yield 214 mg (84%), mp 182–183°. After recrystallization from 5 ml of butanol, followed by trituration with hot ethanol, it melted at 186–187°, $[\alpha]^{25}D$ −92° (*c* 1, H_2O), $\lambda_{max}^{0.1\,N\,HCl}$ 291 mμ (ε 12,000), $\lambda_{min}^{0.1\,N\,HCl}$ 247 mμ (ε 1330).

Anal. Calcd for $C_9H_{12}FN_3O_4$: C, 44.08; H, 4.93; F, 7.75. Found: C, 44.51; H, 5.26; F, 7.57.

Hydrolysis of N⁴-Acylated 5-Fluorocytosines.—A suspension of 50 μmoles of the N⁴-acylated 5-fluorocytosines in a mixture of 0.5 ml of ethanol and 0.5 ml of 1 *N* HCl was shaken for 60 hr in a thermostat held at 37°. A clear solution was obtained, 20 μl (1 μmole) of which was submitted to descending paper (Whatmann No. 1, previously washed by extraction with boiling methanol) chromatography in 1-butanol–water (88:14). The spots were located by inspection with ultraviolet light, excised, and extracted with 5 ml of 0.1 *N* HCl. Reference samples with R_f values FU (0.37), FC (0.12), acetyl-FC (0.25 fluorescent), *p*-toluoyl-FC (fluorescent streak from 0.06 to 0.63), FUDR (0.39), FCDR (0.15), *p*-toluoyl-FCDR (0.63, fluorescent), *p*-toluic acid (0.78) were run concomitantly. The extracted products were identified and evaluated quantitatively by ultraviolet spectrophotometry in 0.1 *N* HCl and in 0.1 *N* or 1 *N* alkali solution (see Table I). The ultraviolet data of FU and FUDR are as follows: FU, $\lambda_{max}^{0.1\,N\,HCl}$ 266 mμ (ε 7330), $\lambda_{max}^{0.1\,N\,KOH}$ 282–283 mμ (ε 5420), $\lambda_{max}^{1\,N\,KOH}$ 284 mμ (ε 7050); FUDR, $\lambda_{max}^{0.1\,N\,HCl}$ 268–269 mμ (ε 8980), $\lambda_{max}^{0.1\,N\,KOH}$ 268–269 mμ (ε 6960), $\lambda_{max}^{1\,N\,KOH}$ 269 mμ (ε 7090).

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