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Thiation of Nucleosides. IV. The Synthesis of 5-Fluoro-2'-deoxycytidine and Related Compounds[1,2]

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Thiation of suitably-protected 5-fluoro-2'-deoxyuridine followed by removal of the blocking groups and treatment of the thiated intermediate with various amines led to the synthesis of 5-fluoro-2'-deoxycytidine and a series of analogs with alkylated exocyclic amino functions. 5-Fluorocytidine and its N-methyl analog were also prepared, together with certain N-alkyl derivatives of cytidine and 2'-deoxycytidine. The effect of the presence of a 5-fluorine atom on the ultraviolet absorption spectra and apparent pK_a values of the cytosine nucleosides is described, as is the extent to which replacement of the exocyclic amino hydrogens by alkyl groups affects the basic dissociation and spectral behavior. Exocyclic N-alkylation of cytosine nucleosides (including the 5-methyl and 5-fluoro derivatives) results in a marked reduction in the susceptibility of these compounds to deamination by nucleoside deaminase preparations derived from *E. coli* B. A preliminary report on the effect of the N-alkyl derivatives of 5-fluoro-2'-deoxycytidine against transplanted mouse leukemia B82 is given.

Introduction.[3]—With the successful synthesis of 5-fluorouracil (FU)[4], 5-fluorouridine (FUR)[5,6] and 5-fluoro-2'-deoxyuridine (FUDR),[5,7] an important new series of chemotherapeutic agents became available. It was soon demonstrated that these fluorinated derivatives possess anti-tumor activity of a relatively high order.[8-10] A considerable amount of study has been devoted to an elucidation of the mechanisms of biochemical and metabolic action[8-12] of these fluorinated derivatives in mammalian tumor systems. The anti-cancer activity of the 5-fluorouracil derivatives spurred the investigation of other closely related 5-fluoropyrimidine analogs. Despite the fact that 5-fluorocytosine (FC),[4] in contrast with FU, shows little tumor inhibitory capacity,[9] it should be noted that both fluorouracil nucleosides (FUR and FUDR) have been found to be more potent[10] than the parent base against a variety of tumor transplants. Syntheses of the 5-fluorocytosine nucleosides, 5-fluorocytidine (FCR) and 5-fluoro-2'-deoxycytidine (FCDR) were therefore undertaken, and studies of their action have appeared.[11b-c,13]

It was demonstrated[12,14] with bacterial nucleosidase(s) (*Escherichia coli* B.) that 5-fluorouracil is liberated readily from FUR and FUDR. Previous studies[14-16] on the enzymic action of *E. coli B.* on certain cytosine nucleoside substrates[14] have shown that deamination occurred *prior* to glycosyl cleavage by the nucleosidase(s), and the products were the corresponding 2,4-diketopyrimidines. In chemotherapeutic application the 5-fluorocytosine nucleosides could thus be converted to 5-fluorouracil nucleosides which could in turn be cleaved to 5-fluorouracil. On the other hand, the possibility of the participation of a deoxycytidine derivative as a primary acceptor for a single carbon unit in the biosynthesis of deoxyribonucleic acid (DNA) thymine has been postulated.[17] In the latter case it is conceivable that the 5-fluorocytosine nucleosides, or their biosynthetically-formed 5'-nucleotides, might themselves be important in the mechanism of inhibition of this enzymic methylation step,

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, United States Public Health Service (Grant No. CY-3190, CY-3192).

(2) Preliminary reports of this work have appeared: J. J. Fox, I. Wempen and R. Duschinsky, "Abstr. (Suppl.) Fourth Intl. Congr. of Biochem. (Vienna)," p. 6 (1958); J. J. Fox, N. Yung, I. Wempen, R. Duschinsky and L. Kaplan, "Abstr. Intl. Union Pure and Applied Chemistry (Symposium on Natural Products)," Australia, 1960, p. 66.

(3) The designations for compounds used in this paper (*i.e.*, FUDR, FCDR, FUR, FCR) conform to widely-prevalent usage in the chemical and biological literature pertaining to this class of compounds. The authors are aware that these designations are not strictly correct since they refer to compounds as "ribosides" rather than ribosyl derivatives.

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

(5) R. Duschinsky, E. Pleven, J. Malbica and C. Heidelberger, Abstr., 132nd Meeting Am. Chem. Soc., 1957, p. 19C.

(6) N. C. Yung, J. H. Burchenal, R. Fecher, R. Duschinsky and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 4060 (1961).

(7) M. Hoffer, R. Duschinsky, J. J. Fox and N. Yung, *ibid.*, **81**, 4112 (1959).

(8) C. Heidelberger, N. K. Chaudhuri, P. Dannenberg, D. Mooren, L. Griesbach, R. Duschinsky, R. J. Schnitzer, E. Pleven, and J. Scheiner, *Nature*, **179**, 663 (1957).

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

(10) C. Heidelberger, L. Griesbach, O. Cruz, R. J. Schnitzer and E. Grunberg, *Proc. Soc. Exp. Biol. Med.*, **97**, 470 (1958).

(11) (a) C. Heidelberger, L. Bosch, E. Harbers, P. B. Dannenberg and N. K. Chaudhuri, Abstr. 132nd Meeting, Am. Chem. Soc., 1957, p. 20C; N. K. Chaudhuri, B. J. Montag and C. Heidelberger, *Cancer Research*, **18**, 318 (1958); P. B. Dannenberg, B. J. Montag and C. Heidelberger, *ibid.*, **18**, 329 (1958); L. Bosch, E. Harbers and C. Heidelberger, *ibid.*, **18**, 335 (1958); M. A. Rich, J. L. Bolaffi, J. E. Knoll, L. Cheong and M. L. Eidinoff, *ibid.*, **18**, 730 (1958); N. K. Chaudhuri, K. L. Mukherjee and C. Heidelberger, *Biochem. Pharm.*, **1**, 328 (1958); M. L. Eidinoff and M. A. Rich, *Cancer Research*, **19**, 521 (1959); K. L. Mukherjee and C. Heidelberger, *J. Biol. Chem.*, **235**, 433 (1960); C. Heidelberger, A. B. Sunthankar, L. Griesbach and S. Randerson, *Proc. Soc. Exp. Biol. Med.*, **104**, 127 (1960). (b) J. H. Burchenal, E. A. D. Holmberg, J. J. Fox, S. C. Hemphill and J. A. Reppert, *Cancer Research*, **19**, 494 (1959). (c) E. Harbers, N. K. Chaudhuri and C. Heidelberger, *J. Biol. Chem.*, **234**, 1255 (1959); L. Cheong, M. A. Rich and M. L. Eidinoff, *Cancer Research*, **20**, 1602 (1960); R. E. Handschumacher and A. D. Welch in "The Nucleic Acids," Vol. III, Chargaff and Davidson, eds., Academic Press, Inc., New York, N. Y., 1960, p. 498.

(12) S. S. Cohen, J. G. Flaks, H. D. Barner, M. R. Loeb and J. Lichtenstein, *Proc. Natl. Acad. Sci.*, **44**, 1004 (1958).

(13) M. L. Eidinoff, M. A. Rich and A. G. Perez, *Cancer Research*, **19**, 638 (1959); J. Lichtenstein, H. D. Barner and S. S. Cohen, *J. Biol. Chem.*, **235**, 457 (1960).

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

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

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

(17) W. H. Prusoff, *ibid.*, **231**, 873 (1958); J. G. Flaks and S. S. Cohen, *Biochem. Biophys. Acta*, **25**, 667 (1959).

and not merely as precursors of the 5-fluorouracil analogs. Preservation of the cytosine structure might be of possible chemotherapeutic value, and, if the initial deamination could be prevented or decreased in rate, the subsequent glycosyl cleavage might also be retarded. The undegraded cytosine derivative then could have a longer period to act as an antimetabolite. Replacement of one of the 4-amino hydrogen atoms by an alkyl group might decrease the susceptibility of the nucleoside to enzymic deamination. This hypothesis is supported by the fact that 1-(2-deoxy-β-D-ribofuranosyl)-4-methylamino-5-methyl-2(1H)-pyrimidinone,[18] unlike 5-methyl-2′-deoxycytidine, is neither deaminated nor cleaved to any appreciable extent at its glycosyl bond by the *E. coli* B. enzyme. It was thus of interest to compare the susceptibility of N-alkylated 5-fluorocytosine nucleosides to enzymic systems.

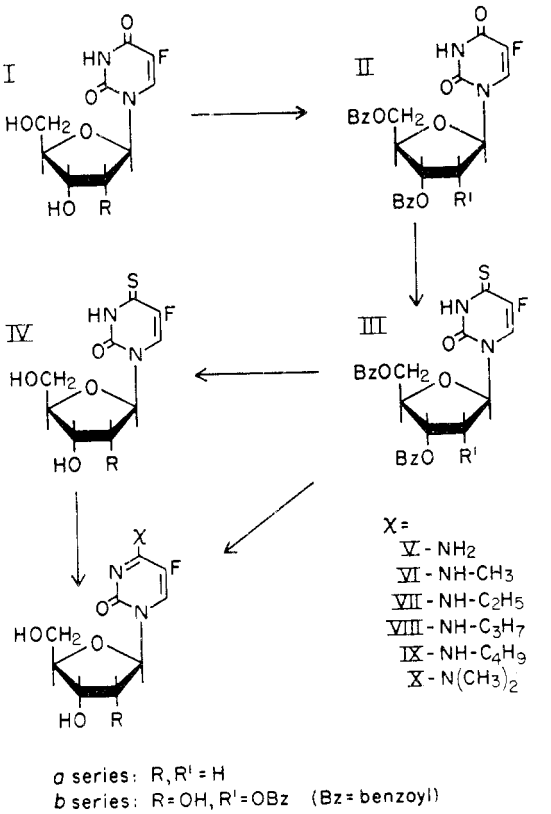
Previous studies[18] have demonstrated the chemical conversion of a preformed uracil nucleoside, such as uridine or thymidine (5-methyl-2′-deoxyuridine), into its cytosine counterpart, *i.e.*, cytidine or 5-methyl-2′-deoxycytidine, by direct thiation of the suitably protected nucleoside with phosphorus pentasulfide, and subsequent treatment of the 4-thio intermediate with alcoholic ammonia. The 4-thio group was replaced by an amino function and the blocking groups were also removed from the sugar moiety. Similar transformations of 5-fluorouracil nucleosides FUR and FUDR into their respective 5-fluorocytosine analogs 5-fluorocytidine (FCR) and 5-fluoro-2′-deoxycytidine (FCDR)[19] were undertaken.

Syntheses are presented for 5-fluorocytidine (FCR) and 5-fluoro-2′-deoxycytidine (FCDR), together with some N-alkyl analogs. Syntheses of the N-methyl- and N,N-dimethyl derivatives of 2′-deoxycytidine are also presented in order to define more closely the physico-chemical properties attributable to the presence of a fluorine atom in these compounds.

Results and Discussion

Treatment of FUDR (Ia) (see flow sheet) in pyridine solution with *exactly* two moles of benzoyl chloride[20] afforded an 86% yield of the 3′,5′-di-*O*-benzoyl derivative (IIa) of FUDR. The dibenzoate was thiated by phosphorus pentasulfide in pyridine to the 4-thio analog IIIa in *ca.* 85% yield. The extent of conversion of IIa to IIIa (>85%) was determined conveniently from the ultraviolet absorption spectrum in alcohol solution at 334 mμ (the band here due essentially to the presence of the 4-thio function). Treatment of the crude 4-thio-di-benzoate IIIa with alcoholic ammonia in a sealed tube replaced the thio-group by an amino function and concurrently debenzoylated the compound. On examination of the product, however, it was apparent that a number of side reactions involving the loss or replacement of the 5-fluorine atom had occurred. Together with some starting material, the major by-products, after separation by ion-exchange chromatography, were identified as FUDR and 2′-deoxycytidine. Two other substances, identified but not isolated, were 5-amino- and 5-ethoxy-2′-deoxycytidine. In addition, some unidentified compounds were also present in trace amounts. The presence of FUDR was due to the use of the incompletely thiated intermediate IIIa. Recrystallization of IIIa proved to be difficult because of its very poor solubility. It was found, however, that if removal of the benzoyl groups was carried out prior to the amination step, pure crystalline 4-thio-FUDR (IVa) readily was isolated, thus eliminating the carry-over of FUDR to the amination step.

FLOW CHART



a series: R,R′ = H
b series: R = OH, R′ = OBz (Bz = benzoyl)

The presence of 2′-deoxycytidine appeared to arise from the loss of the 5-fluorine atom through the reducing action of the sulfides released in the amination reaction.[22] This side reaction could be

(18) J. J. Fox, D. Van Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich and G. B. Brown, *J. Am. Chem. Soc.*, **81**, 178 (1959).

(19) Preliminary studies on an alternate synthesis of FCDR by the mercuri procedure have been reported.[7] These studies are being continued in order to develop a direct synthesis of FCDR from 2-deoxy-D-ribose and the results will be reported later.

(20) An excess of benzoyl chloride resulted in the formation of some tri-*O*-benzoylated product whose presence in the subsequent thiation step resulted in somewhat poorer yields. A similar situation has been noted previously in other thiation reactions.[18,21]

(21) J. J. Fox, I. Wempen, A. Hampton and I. L. Doerr, *J. Am. Chem. Soc.*, **80**, 1669 (1958).

(22) The replacement of a 5-halogeno group by hydrogen by treatment with hot ammonia solutions has been noted previously in the case of the 5-*iodo* derivatives of cytosine, uracil, 2-ethylthio-4-hydroxypyrimidine [T. B. Johnson and C. O. Johns, *J. Biol. Chem.*, **1**, 305 (1906)] and 2,4-diamino-6-hydroxypyrimidine [A. Bendich and G. C. Clements, *Biochim. Biophys. Acta*, **12**, 462 (1953)]. It is noteworthy that with the 5-fluoro-4-thionucleosides, the reductive removal of the 5-fluoro group is prevented by the presence of one equivalent of copper acetate even though a large excess of ammonia is present in the reaction tube.

prevented by the addition of a molar equivalent of copper acetate to bind the sulfide ions responsible for the reductive elimination of the fluorine. The presence of the copper, however, appeared to complicate further the still-necessary chromatographic purification of the product and this additive was therefore abandoned. Contamination of FCDR by 5-amino-2′-deoxycytidine was expected to occur to some extent under the reaction conditions used.[23]

The synthesis of FCDR finally was accomplished in better yields (50%), and with a minimum of by-products, by treatment of IVa with liquid ammonia at *ca.* 60°, followed by purification on a Dowex 50 (NH_4^+) column. All attempts to induce the replacement of the 4-thio group of IVa by treatment with ammonia under such mild conditions as to eliminate nearly all of the side reactions (and thereby the necessity for final purification by ion-exchange chromatography) gave only a small amount of FCDR. The failure of ammonia to displace the thio group except under conditions of heat and pressure is a direct reflection of this reagent's weakly basic nature, since, as will be shown below, the mono-alkylamines, being stronger bases, readily react with 4-thiopyrimidine ribonucleosides at room temperature.

5-Fluorocytidine (FCR, Vb) was synthesized in a manner somewhat similar to that used at first for the preparation of FCDR. Since 5-fluorouridine[5,6] had been prepared from 5-fluorouracil and 2,3,5-tri-*O*-benzoyl-D-ribosyl chloride *via* the mercuri procedure,[24,25] a suitably blocked intermediate (IIb) was already available for the thiation step. Treatment of IIb with phosphorus pentasulfide in pyridine afforded the 4-thio-tri-*O*-benzoylated derivative IIIb in 72% yield. Upon treatment of IIIb with alcoholic ammonia in a sealed tube,[26] both debenzoylation and amination occurred and the resulting amino compound was subsequently isolated as the hydrochloride salt from the same reaction mixture. The crude hydrochloride was purified by ion-exchange chromatography and a 33% yield of FCR (Vb) based on IIIb was obtained.

In the synthesis of the respective N-alkyl analogs of FCDR and FCR, advantage was taken of the more nucleophilic nature of the monoalkylamine reagents as compared to ammonia to carry out the replacement of the 4-thio group under extremely mild conditions. The products obtained were uncontaminated by the previously-discussed by-products. Progress of the conversion was followed readily by the disappearance of the ultraviolet absorption maximum (at 340 mμ) attributed to the presence of the 4-thio function, and the concurrent rise of a new maximum (*ca.* 290 mμ). The N-alkyl-FCDR and -FCR derivatives were easily purified by recrystallization, obviating the use of column chromatography.

4-Thio-FUDR (IVa), treated with a solution of methylamine in methanol and allowed to stand at room temperature until the reaction was complete, afforded a 62% yield of N-methyl-FCDR (VIa). Similarly, N-ethyl-FCDR (VIIa) was prepared by treatment of IVa with an ethanolic solution of ethylamine at room temperature. The crystalline product was isolated in 73% yield. N-Propyl- and N-*n*-butyl-FCDR (VIIIa and IXa) were synthesized in good yield by dissolving 4-thio-FUDR (IVa) in an excess of the respective alkylamine and allowing the reaction to stand at room temperature until the conversion was completed.

N-Methyl-FCR (VIb) was prepared from IVb by treatment with a methanolic solution of methylamine in a manner analogous to that used for the preparation of VIa above. The N,N-dimethyl derivatives of FCDR and FCR could not be prepared under the same conditions as those used for the monoalkyl analogs. This failure of dimethylamine to react probably is due to a certain amount of steric hindrance, since the pK_a of this substituted amine (10.72) is approximately the same as that of methylamine (10.70) or ethylamine (10.75). The probability of steric effect[27] here is further substantiated by the fact that isopropylamine (pK_a 10.73) likewise failed to displace the 4-thio group under similar reaction conditions. N,N-Dimethyl-FCDR was prepared, finally, by a method similar to that used for preparation of FCDR itself. Treatment of IVa with liquid dimethylamine at 60° afforded the desired product in 60% yield.

Some of the corresponding N-substituted analogs of 2′-deoxycytidine were prepared starting from 2′-deoxyuridine, which, on treatment with two moles of benzoyl chloride in pyridine, afforded the dibenzoate in excellent yield. The product was thiated with phosphorus pentasulfide to the 4-thio-dibenzoate derivative. Treatment of this intermediate in a sealed tube with an alcoholic solution of ammonia or of the appropriate amine afforded 2′-deoxycytidine, N-methyl-2′-deoxycytidine or N,N-dimethyl-2′-deoxycytidine in good yields.

Spectral Properties.—The ultraviolet absorption spectra of FCDR (Va), N-methyl-FCDR (VIa) and N,N-dimethyl-FCDR (Xa) as a function of *p*H are given in Fig. 1. The introduction of a fluorine atom into the 5-position of a cytosine derivative (see Table I) results in the expected bathochromic shift (*ca.* 10 mμ)[28] in the position of the absorption maximum and also a decrease in the intensity of that maximum (hypochromic effect). Spectral data available for other 5-halogenocytosine derivatives, such as 5-bromo- and 5-chlorocytidine[23] and their corresponding 2′-deoxynucleosides,[29] demonstrate that the magnitude of both the bathochromic and hypochromic shifts, in comparison to the un-

(23) Somewhat analogous to the preparation of 5-aminocytidine by the nucleophilic displacement of the 5-halogeno atom of 5-bromocytidine [T. K. Fukuhara and D. W. Visser, *J. Am. Chem. Soc.*, **77**, 2393 (1955)].

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

(25) See J. J. Fox and I. Wempen, *Adv. in Carbohydrate Chem.*, **14**, 283 (1959), for a review of this procedure.

(26) It is recommended that the sealed tube reaction be avoided by the use of the same procedure outlined in the FCDR synthesis (*i.e.*, prior debenzoylation of II and subsequent amination by means of liquid ammonia).

(27) Steric inhibition of the reaction of secondary alkylamines with certain 4-thio-5-substituted pyrimidines has been reported previously (P. B. Russell, G. B. Elion, E. A. Falco and G. H. Hitchings, *J. Am. Chem. Soc.*, **71**, 2279 (1949)).

(28) A. Albert in "Heterocyclic Chemistry," Essential Books, Fair Lawn, N. J., 1959, p. 302.

(29) D. M. Frisch and D. W. Visser, *J. Am. Chem. Soc.*, **81**, 1756 (1959).

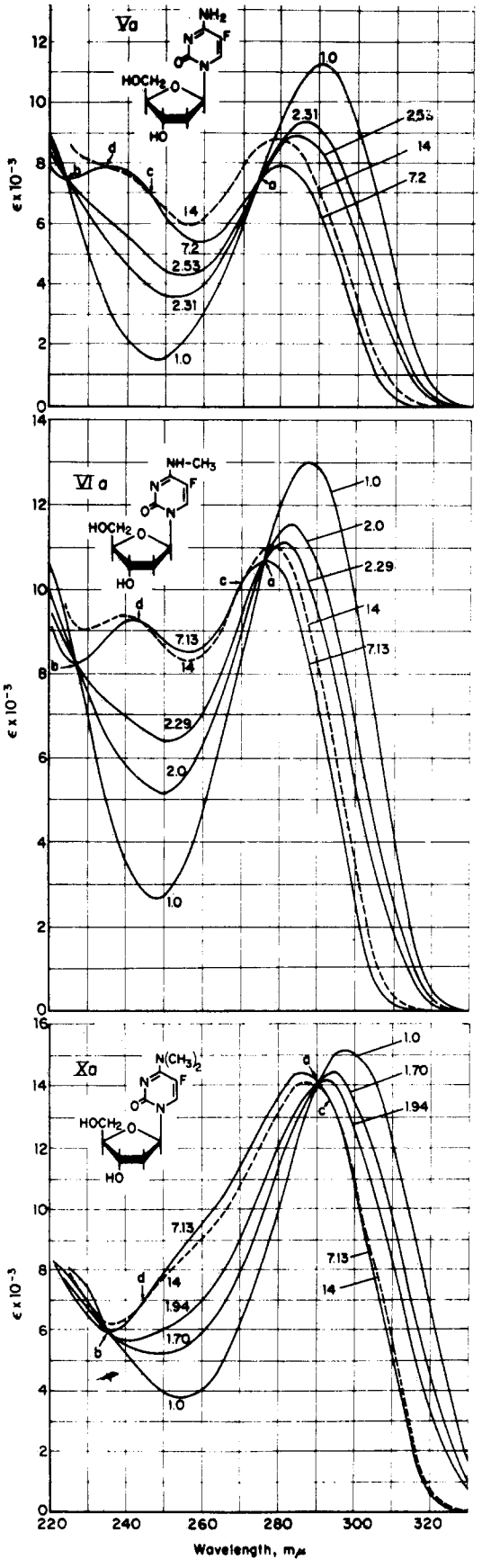


Fig. 1.

halogenated analogs,[30] appears to be correlated with the size of the halogen substituent, the bromine atom having the greatest effect and the fluorine the least. The over-all spectral pattern, as a function of *p*H, exhibited by the 5-fluorocytosine nucleosides, however, is similar to that shown by cytidine[30] and 2′-deoxycytidine.[30] The inflection in the *p*H range 7–12 (neutral species) at *ca.* 230 mμ, characteristic of all cytosine nucleosides so far studied,[30] is present in the fluorocytosine nucleosides and their monoalkylated analogs to an even more pronounced degree, although the over-all bathochromic shift has altered the position of this maximum. It is interesting to note that the spectrum of N,N-dimethyl-FCDR (see Fig. 1) and that of the corresponding CDR analog shows an almost total disappearance of this 230 mμ absorption peak.

TABLE I

Compound	*p*H	$m\mu_{max}$	$\Delta m\mu_{max}$	$\epsilon_{(max)} \times 10^{-3}$	$\Delta\epsilon_{(max)}$
Cytidine[a]	7	271		9.10	−1.97
5-Bromo-[b]	7	289	+18	7.13	−1.85
5-Chloro-[b]	7	287	+16	7.25	−1.04
5-Fluoro- (Vb)	7	281	+10	8.06	
2′-Deoxycytidine[a]	2	280		13.2	−3.60
5-Bromo-[c]	2	300	+20	9.60	−3.22
5-Chloro-[c]	2	295	+15	9.98	−2.7
5-Fluoro- (Va)	2	288	+ 8	10.50	

[a] Data taken from ref. 30. [b] Data taken from ref. 23. [c] Data taken from ref. 29.

A second dissociation in the high alkaline region (*p*H 12–14) is indicated in the spectra shown in Fig. 1 by the *p*H 14 curves which do not pass through the isosbestic points[31] a and b belonging to the cationic dissociation. This alkaline dissociation is characteristic of the pyrimidine nucleosides so far examined and is attributed to the effect of ionization of the carbohydrate moiety on the pyrimidine ring.[30] It is interesting that in the case of 5-fluorocytidine and 5-fluoro-2′-deoxycytidine, the extent of the hyperchromic shift of the maximum of the *p*H 14 curve (at 278 mμ) with respect to the limiting curve of the neutral species (*p*H 7.2–12) is considerably greater than that found with their non-fluorinated analogs.

The presence of an alkyl group on the exocyclic amino group of FCDR, FCR or CDR gives rise to a pronounced hyperchromic shift (increase) in the intensity of the ultraviolet maximum and an almost insignificant hypsochromic shift of this maximum (toward the shorter wave-length region). The higher homologs of N-methyl-FCDR (*i.e.*, N-ethyl, N-propyl, N-*n*-butyl derivatives) demonstrate a further gradual increase in the absorption maximum with the addition of each —CH_2— group. The N,N-dimethyl derivative of FCDR or CDR shows not only this hyperchromic effect (the absorption value being slightly greater than that of the N-*n*-

(30) J. J. Fox and D. Shugar, *Biochim. Biophys. Acta*, **9**, 369 (1952).

(31) For a discussion of the significance of isosbestic points, see ref. 30 and 32. See also A. Bendich in "The Nucleic Acids," Vol. 1, Chargaff and Davidson, eds., Academic Press, Inc., New York, N. Y., 1955, p. 81.

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

(33) D. J. Brown, E. Hoerger and S. F. Mason, *J. Chem. Soc.*, 4035 (1955).

butyl analog) but also a pronounced shift of the entire spectrum toward the longer wave lengths, consistent with the bathochromic effect noted previously for other dimethylaminopyrimidines.[33]

The spectra of the thiated derivatives used as intermediates in the syntheses of the cytosine nucleosides show the usual strongly bathochromic shift of the maxima to a region in which neither the uracil precursors nor the cytosine derivatives have absorption.

Dissociation Constants.—The presence of a fluorine atom on position 5 of uracil and its nucleosides has a pronounced acid-strengthening effect (see the dissociations listed in part A, Table II). The pK_a values of the 5-fluoro nucleosides average 1.6 pK_a units below those of the corresponding uracil nucleosides. The lowering of the pK_a is a reflection of the electronegativity of the 5-fluoro atom in these compounds.

TABLE II

EFFECT OF THE PRESENCE OF A FLUORINE ATOM ON THE "APPARENT" pK_a VALUES[a]

	pK_a	ΔpK_a[b]
A. Some uracil nucleosides and related compounds		
Uracil	9.5[c]	
5-Fluorouracil	7.98[d]	−1.5
Uridine	9.25[e]	
5-Fluorouridine (Ib) (FUR)	7.57	−1.68
4-Thio-5-fluorouridine (IVb)	6.72	
2′-Deoxyuridine	9.3[e]	
5-Fluoro-2′-deoxyuridine (Ia) (FUDR)	7.66[d]	−1.6
4-Thio-5-fluoro-2′-deoxyuridine (IVa)	6.77	
B. Some cytosine nucleosides and related compounds		
Cytosine	4.61[f]	
5-Fluoro-	2.90[d]	−1.71
N-Methyl-	4.55 ± 0.01[g]	
N-Methyl-5-fluoro-	2.66	−1.89
Cytidine	4.1[e]	
5-Fluoro- (Vb) (FCR)	2.26	−1.8
N-Methyl-[h]	3.92	
N-Methyl-5-fluoro- (VIb)	2.05	−1.87
2′-Deoxycytidine	4.3[e]	
5-Fluoro- (Va) (FCDR)	2.39	−1.9
N-Methyl-	4.01	
N-Methyl-5-fluoro- (VIa)	2.14	−1.87
N,N-Dimethyl-	3.79	
N,N-Dimethyl-5-fluoro- (Xa)	1.89	−1.90

[a] The pK_a values are spectrophotometrically determined and are accurate to ±0.05 pH unit unless otherwise indicated. The pK_a value in part A of this table refers to ionization of the pyrimidine from the 3-NH to the enolate form at position 4.[30] The pK_a value in the high alkaline range was not determined. [b] Decrease in the pK_a values due to presence of 5-fluorine atom. [c] This value taken from ref. 32. [d] Data furnished by Dr. A. Motchane, Hoffmann LaRoche, Inc. [e] This value taken from ref. 30. [f] This value redetermined as the pK values reported in the literature differ: 4.45 in ref. 32, 4.60 in ref. 38. [g] This value potentiometrically determined, see ref. 36. [h] For synthesis, see ref. 17.

The sulfur-containing derivatives IVa,b are, as expected,[34,35] more acidic than their oxygen analogs as shown by the decrease of *ca.* 0.9 pK_a unit from the pK_a values given for the latter compounds.

Introduction of the fluorine atom into cytosine and its nucleosides (part B, Table II) markedly decreases the basic strength *ca.* 1.8–1.9 units below the pK_a values of the non-fluorinated analogs. This base-weakening effect is likewise attributed to the electronegative nature of the fluorine atom. A similar effect, due to the influence of a strongly electronegative group, has been observed previously in the case of 5-nitrocytosine and 5-nitrocytidine (cationic pK_a values, <3[36] and 1.1,[37] respectively) as compared to the corresponding values for the unsubstituted cytosine (4.60)[38] and cytidine (4.1).[30]

The presence of a monoalkyl group on the exocyclic amino group of the cytosine nucleosides (FCDR, FCR and CDR) decreases the basic strength as shown by the lowering of the cationic pK_a values by *ca.* 0.2–0.3 unit (see Table III),

TABLE III

EFFECT OF ALKYLATION OF THE EXOCYCLIC AMINO GROUP ON THE "APPARENT" pK_a VALUE OF THE PARENT CYTOSINE COMPOUND

Compound	pK_a[a]	ΔpK_a
5-Fluoro-2′-deoxycytidine (FCDR) (Va)	2.39	
N-Methyl- (VIa)	2.14	−0.25
N-Ethyl- (VIIa)	2.21	− .18
N-Propyl- (VIIIa)	2.19	− .20
N-*n*-Butyl- (IXa)	2.21	− .18
N,N-Dimethyl- (Xa)	1.89	− .50
5-Fluorocytidine (FCR) (Vb)	2.26	
N-Methyl- (VIb)	2.05	− .21
Cytidine	4.1[b]	
N-Methyl-	3.92	− .18
N,N-Dimethyl-	3.62	− .48
2′-Deoxycytidine	4.3[b]	
N-Methyl-	4.01	− .29
N,N-Dimethyl-	3.79	− .51
Cytosine	4.61[c]	
N-Methyl-	4.55 ± 0.01[d]	− .06
N-Ethyl-	4.58 ± .02[d]	− .03
N-*n*-Butyl-	4.69 ± .01[d]	+ .08
N,N-Dimethyl-	4.25	− .36
1-Methyl	4.55[b]	
N,1-Dimethyl-	4.47[e]	− .08
N,N,1-Trimethyl-	4.20[e]	− .35
5-Fluorocytosine (FC)	2.90	
N-Methyl-5-fluoro-	2.66	− .24

[a] The values are spectrophotometrically determined and are accurate to ±0.05 pH unit unless otherwise indicated. [b] Value taken from ref. 30. [c] This value, given as 4.45 in ref. 32, was redetermined and agreed with that taken from ref. 38. [d] Value taken from ref. 36 (potentiometrically determined). [e] Potentiometrically determined values from G. W. Kenner, C. B. Reese and Sir A. R. Todd, *J. Chem. Soc.*, 855 (1955).

while the dimethylamino analogs demonstrate a drop in pK_a values of *ca.* 0.4 unit. Although this base-weakening effect of the alkyl groups would appear to constitute a contradiction of their usual electropositive behavior,[39] the same phenomenon

(34) J. R. Marshall and J. Walker, *J. Chem. Soc.*, 1004 (1951).
(35) D. Shugar and J. J. Fox, *Bull. soc. chim. Belg.*, **61**, 293 (1952).
(36) D. J. Brown, *J. Appl. Chem.*, **9**, 203 (1959).
(37) J. J. Fox and D. Van Praag, *J. Org. Chem.*, **26**, 526 (1961).
(38) P. A. Levene, *J. Biol. Chem.*, **70**, 229 (1926).
(39) C. K. Ingold in "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p. 70.

has been observed previously in the case of 5-methyl-2′-deoxycytidine[18] and its N-methyl analog, 1-(2-deoxy-β-D-ribofuranosyl)-4-methylamino-5-methyl-2(1H)-pyrimidinone,[18] for which the cationic pK_a values are 4.40 and 4.04, respectively.

In this regard, it is interesting to compare the cationic pK_a values of cytosine and its exocyclic N-alkyl derivatives with those of the corresponding analogs of 1-methylcytosine (see Table III). Monomethylation of cytosine itself, whether on the N1 nuclear atom (1-methylcytosine) or on the exocyclic amino function (N-methylcytosine), has an almost insignificant base-weakening effect on the basic pK_a value. An N-ethyl group has approximately the same effect on the basic pK_a value of cytosine as the N-methyl, while the N-*n*-butyl group shows a slight base-strengthening effect. Substitution of a methyl group for one amino hydrogen in 1-methylcytosine leads to a small decrease in the basic pK_a value. (Data for the higher monoalkyl homologs of 1-methylcytosine are not available for comparison.) In contrast, methylation of the exocyclic amino group of 5-fluorocytosine results in an appreciable weakening of the basic strength (*ca.* 0.17 pK_a unit) of the parent compound, the decrease being of the same order of magnitude as that already observed in the case of the mono-methylated cytosine nucleosides. In all of the above examples, substitution of both the hydrogens on the exocyclic amino function leads to a larger decrease of basic strength than that noted for the monoalkyl derivatives.

There appears to be no single interpretation for the above data since several factors could conceivably contribute a share to the over-all picture. Some steric hindrance to protonation (of the basic center) introduced by the alkyl substituent may result in weakening the basicity; or alternatively, the presence of alkyl groups on the exocyclic amino group may influence adversely the degree of hydrogen bonding with the solvent, thus opposing the inductive effect.[40] These hypotheses would be particularly applicable in the case of the dimethylamino derivatives. The effect of the presence of a monoalkyl group on an exocyclic amino group in a cytosine derivative in which the N1 nuclear nitrogen already bears a substituent may be to alter the center of basicity. This new basic center may be one of lowered electron density which would exhibit a lower pK_a.

In the course of determining spectrophotometrically the pK_a values for the new cytosine derivatives listed in Tables II and III, a second apparent cationic dissociation was observed to occur in the strongly acidic region from 1 *N* to 12 *N* hydrochloric acid, probably attributable to protonation of the heterocyclic ring system.[41] A similar effect has been noted previously[35] in the case of 2-methylthio-3,6-dimethylpyrimidone-4; the pK_a value (0.9) obtained for this compound, which has no dissociable substituents, represents the protonation of the pyrimidine ring. Likewise, Cohn[42] has noted the presence of this dissociation in the strongly acid region in the case of uracil, thymine and their respective ribosyl nucleosides. This phenomenon was observed both in the case of the cytosine bases, FC and N-methyl-FC, and also in the spectra of all of the cytosine nucleosides which were studied in this region, *i.e.*, FCDR, N-methyl- and N,N-dimethyl-FCDR, cytidine[43] and N-methylcytidine. No attempt has been made to determine the apparent pK_a value for this dissociation.

Enzymic Studies.—Previous studies from this[14] and other laboratories[15,16,44] have demonstrated some of the structural requirements with regard to the sugar moiety of nucleosides needed for susceptibility to deaminase. Thus, while 1-β-D-xylofuranosylcytosine is not deaminated by resting cell suspensions of *Escherichia coli* B., cytidine is readily converted to uracil (deamination plus glycosyl cleavage). Using cell-free extracts of *E. coli* B., Pizer and Cohen demonstrated[44] that 1-β-D-arabinosylcytosine can serve as a substrate for conversion to 1-β-D-arabinosyluracil although the rate of deamination is approximately one-fifth that of deoxycytidine. It has been shown also that 5-methyl-2′-deoxycytidine is converted to thymidine by these extracts[16] and to thymine by resting cell suspensions of *E. coli* B.[14] It is noteworthy that 5′-fluoro-5′-deoxycytidine is converted by the latter system to the uridine analog without glycosyl cleavage. There is, then, ample evidence which points to the high degree of structural specificity required by nucleoside deaminase(s) with regard to the sugar portion of cytosine nucleosides.

The availability of a number of exocyclic N-alkylated cytosine nucleosides affords the opportunity to investigate the effect of alteration at the 4-position of the pyrimidine moiety on the susceptibility of such compounds to enzymic deamination. The result of the action of nucleoside deaminase(s) in cell-free extracts on these ribofuranosyl- and deoxyribofuranosylcytosines is shown in Table IV. It is clear from the data that N-alkylation of cytidine, 5-methylcytidine, 2′-deoxycytidine and 5-methyl-2′-deoxycytidine results in derivatives which, in contrast to their naturally-occurring parent nucleosides, are not susceptible to deamination[45] by nucleoside deaminase(s). A similar situation is encountered when 5-fluorocytidine is compared to N-methyl-5-fluorocytidine. Although N-alkylation of 5-fluoro-2′-deoxycytidine (FCDR) greatly lowers its susceptibility to enzymic deamination, this decrease in susceptibility is not as marked as that observed with the aforementioned derivatives. Finally, increase in the size of the alkyl chain on the exocyclic nitrogen atom (*i.e.*, ethyl) or dialkylation (*i.e.*, N,N-dimethyl) gives sub-

(40) R. P. Bell in "The Proton in Chemistry," Cornell University Press, Ithaca, N. Y., 1959, p. 176.

(41) See ref. 28, p. 49.

(42) W. E. Cohn in "The Nucleic Acids," E. Chargaff and J. N. Davidson, eds., Academic Press, Inc., New York, N. Y., 1955, Vol. I, p. 217.

(43) It should be noted that after standing 48 hr. at room temperature, the spectral solution of cytidine in 3 *N* hydrochloric acid gave an unchanged extinction value, while that for the 12 *N* solution did show a slight bathochromic shift due probably to a partial cleavage of the glycosyl bond. It is presumed that this acid hydrolysis occurs to a greater or less extent with all of the nucleosides studied in this highly acidic region.

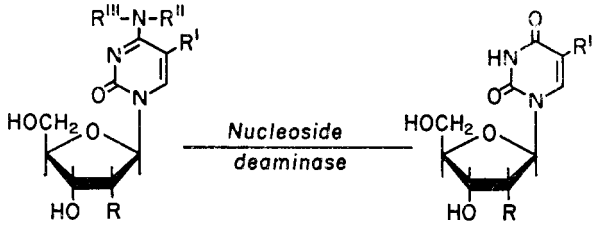
(44) L. I. Pizer and S. S. Cohen, *J. Biol. Chem.*, **235**, 2387 (1960).

(45) In the case of the alkyl-substituted amines (for example, N-methyl-FCDR, etc.), deamination implies removal of the entire group and replacement by hydroxyl. The route by which this process would occur is not established by these experiments.

strates which are essentially inert in this enzymic system. It would be desirable to test these compounds with deaminase derived from mammalian sources.

TABLE IV

ACTION OF NUCLEOSIDE DEAMINASE ON CYTOSINE NUCLEOSIDES



[A] [B]

Compound A	R	R′	R″	R‴	% conversion[a] to B
2′-Deoxycytidine	H	H	H	H	85
N-Methyl-	H	H	CH_3	H	10
5-Methyl-	H	CH_3	H	H	82
N-Methyl-5-methyl-	H	CH_3	CH_3	H	None[b,c]
5-Fluoro-	H	F	H	H	87
N-Methyl-5-fluoro-	H	F	CH_3	H	34
N-Ethyl-5-fluoro-	H	F	C_2H_5	H	None[c]
Cytidine	OH	H	H	H	87
N-Methyl-	OH	H	CH_3	H	5
5-Methyl-	OH	CH_3	H	H	71
N-Methyl-5-methyl-	OH	CH_3	CH_3	H	None[c]
N,N-Dimethyl-	OH	H	CH_3	CH_3	None[c]
5-Fluoro-	OH	F	H	H	79
N-Methyl-5-fluoro-	OH	F	CH_3	H	None[c]

[a] The % values represent an average of several runs for each compound. The values are accurate to 5%. [b] Incubations carried out for as long as three hours did not result in measurable conversion of A to B. [c] Product was not detected by paper chromatographic and spectrophotometric methods used. A 5% yield of product is detectable by the methods employed.

Screening Studies.[46]—In studies on transplanted mouse leukemia B82 injected subcutaneously, the N-alkyl derivatives of FCDR all showed 75% or greater tumor inhibition, but only at doses which usually produced a significant degree of toxicity as shown by weight loss. In this particular system, unsubstituted FCDR had a better chemotherapeutic index than any of the N-alkyl derivatives tested, and produced tumor inhibition at doses which caused no toxicity.[11b] FCDR itself is currently undergoing clinical trial.

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Experimental[47]

1-(3,5-Di-*O*-benzoyl-2-deoxy-β-D-ribosyl)-5-fluorouracil (IIa).—A solution of 40 g. (0.156 mole) of 5-fluoro-2′-deoxyuridine (Ia) in *ca.* 1300 ml. of pyridine (redistilled over barium oxide) was treated with 36 ml. (0.312 mole) of benzoyl chloride and maintained at 40–45° overnight. During this time, the optical rotation of the pyridine solution which was initially positive had shifted to a negative value and had become constant. The pale-yellow solution was dripped into *ca.* 8 l. of a vigorously-stirred water–ice slurry. After addition was complete, the suspension was stirred for an additional 0.5 hr., adding more ice when necessary. The suspension plus ice was filtered on a large Büchner funnel; the precipitate was returned to the stirring setup with fresh ice-water and stirred for an additional 0.5 hr. After filtration, the white solid was triturated thoroughly with cold alcohol, filtered, washed with ether and dried; yield of crude product, 61 g. (86%), m.p. 233–237°. An aliquot, recrystallized from methanol for analysis, gave m.p. 237.5–238.5°, $[\alpha]^{28}D$ −28° (*c* 0.3 in methylene chloride); ultraviolet absorption data: in ethanol, maxima at 229 and 267 mμ, ϵ_{max} 30,780 and 10,960, respectively; minima at 222 and 250 mμ, ϵ_{min} 30,710 and 8,290, respectively.

Anal. Calcd. for $C_{23}H_{19}N_2O_7F$: C, 60.79; H, 4.21; N, 6.17; F, 4.18. Found: C, 61.13; H, 4.19; N, 6.20; F, 4.15.

3′,5′-Di-*O*-benzoyl-2′-deoxyuridine.—Ten grams (0.044 mole) of 2′-deoxyuridine was dissolved in *ca.* 300 ml. of pyridine (distilled over barium oxide) and treated with 10.1 ml. (0.088 mole) of benzoyl chloride. The reaction mixture was maintained at 55–60° overnight (or until no further change in the optical rotation value occurred). The red solution was poured slowly into a well-stirred slurry of *ca.* 1500 ml. of water-ice. An immediate precipitation of a pale pink solid occurred. The suspension was stirred 20 min., filtered, the precipitate resuspended in fresh ice-water and stirred for an additional 10 min. After filtration, the solid was washed with cold alcohol–ether mixture (50:50), then with ether alone and air-dried; yield of crude product 17.6 g. (92%), m.p. 215–219°. For analytical work, an aliquot was recrystallized twice from ethanol (1 g./200 ml.); m.p. 219–220°, $[\alpha]^{28}D$ −43° (*c* 1.2 in methylene chloride); ultraviolet absorption data: in ethanol, maxima at 230 and 259 mμ, ϵ_{max} 29,040 and 11,750, respectively; minimum at 249 mμ, ϵ_{min} 10,690.

Anal. Calcd. for $C_{23}H_{20}N_2O_7$: C, 63.30; H, 4.62; N, 6.42. Found: C, 63.43; H, 4.54; N, 6.07.

1-(3,5-Di-*O*-benzoyl-2-deoxy-β-D-ribosyl)-4-thio-5-fluorouracil (IIIa).—A vigorously stirred solution of 61 g. (0.135 mole) of 3′,5′-di-*O*-benzoyl-2′-deoxy-β-D-ribofuranosyl)-5-fluorouracil (IIa) and 136 g. (0.673 mole) of phosphorus pentasulfide in *ca.* 3500 ml. of reagent grade pyridine was treated *dropwise* with 3 ml. of water,[48] refluxed for 7 hr. and allowed to stand at room temperature overnight. The pyridine was decanted from the solid, evaporated *in vacuo* at less than 50° to a thin sirup which was added, together with the original solid, to *ca.* 9 l. of vigorously stirred water warmed to 40°. The stirring was continued until the sticky precipitate became granular. The solid was then filtered, washed thoroughly with fresh water, taken up in warm methylene chloride (*ca.* 2 l.) and filtered to remove any insoluble material. The organic solution was washed twice with tepid water, dried briefly with sodium sulfate at room temperature, and evaporated to a thick slurry. Ethanol (*ca.* 250 ml.) was added to this suspension which was then reconcentrated to a slurry. This treatment was repeated a total of three times until all of the methylene chloride had been replaced by ethanol. The suspension then was chilled thoroughly, filtered and washed well with ether. While still ether-damp, the precipitate was transferred to a tared *brown* bottle and the ether removed in a vacuum desiccator. (In the dry state, the

(46) The authors are indebted to Dr. J. H. Burchenal of the Leukemia Section of the Division of Experimental Chemotherapy of the Sloan-Kettering Institute for these preliminary data.

(47) All melting points were taken on a Thomas–Hoover capillary melting point apparatus. Microanalyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.; Spang Microanalytical Laboratory, Ann Arbor, Mich.; and by Dr. Al Steyermark of Hoffmann LaRoche, Inc., Nutley, N. J. Melting points are corrected.

(48) If water is omitted from this reaction, the contents darken considerably within 30 minutes, isolation of the desired product becomes more laborious and the yields are usually lower. Enough water should be added dropwise so that the reaction takes on an orange turbid appearance which remains permanent. Attempts to correlate (stoichiometrically) the amount of water needed to produce the turbid, orange appearance with starting materials have been unsuccessful, although in many cases the molar amount of water added amounts to *ca.* 60–65% of the molar quantity of phosphorus pentasulfide used.

product acquires an electrostatic charge which makes handling quite difficult; the product is also somewhat light sensitive.) The yield of crude IIIa was 51 g. (81%), m.p. 208–212. This crude product was found to be 88% pure[49] as determined by its ultraviolet absorption maximum in alcohol at 334 mμ. A sample recrystallized from ethanol for analysis gave m.p. 214–215°, $[\alpha]^{28}D$ −2° (*c* 1.1 in methylene chloride); ultraviolet absorption data: in ethanol, maxima at 231, 274, 282 and 334 mμ, ϵ_{max} 29,300, 4,090, 3,980 and 19,600, respectively; minima at 260, 278 and 288 mμ, ϵ_{min} 3,200, 3,800 and 3,190, respectively.

Anal. Calcd. for $C_{23}H_{19}N_2O_6FS$: C, 58.71; H, 4.07; N, 5.95; F, 4.04; S, 6.82. Found: C, 58.84; H, 4.05; N, 5.82; F, 4.33; S, 6.86.

1-(2,3,5,-Tri-*O*-benzoyl-β-D-ribosyl)-4-thio-5-fluorouracil (IIIb).—A well-stirred mixture of 21.4 g. (0.037 mole) of 2′,3′,5′-tri-*O*-benzoyl-5-fluorouridine (IIb) and 31.2 g. (0.14 mole) of phosphorus pentasulfide in *ca.* 850 ml. of pyridine was treated *dropwise* with 0.9 ml. of water,[48] refluxed for 6.5 hr. and allowed to stand at room temperature overnight. The reaction mixture was poured into a well-stirred water–ice slurry and stirred until complete solidification had occurred. After filtration and washing with water, the precipitate was dissolved in methylene chloride (*ca.* 600 ml.), filtered to remove any insolubles and the organic solution washed twice with water, dried over sodium sulfate at room temperature and evaporated to dryness. The product was recrystallized from ethanol with considerable difficulty; yield 15.7 g. (72%), m.p. 182–184°. Recrystallization of a small aliquot for analytical purposes raised the melting point to 183.5–184°, sinters 181°; spectral properties: in ethanol, maxima at 230, 273 and 333 mμ, inflection at 282 mμ; minima at 257 and 292 mμ.

Anal. Calcd. for $C_{30}H_{23}N_2O_8FS$: C, 61.01; H, 3.93; N, 4.74; S, 5.43. Found: C, 61.23; H, 4.23; N, 4.54; S, 5.79.

1-(3,5-Di-*O*-benzoyl-2-deoxy-β-D-ribosyl)-4-thiouracil.—A well-stirred mixture of 3′,5′-di-*O*-benzoyl-2′-deoxyuridine (15 g., 0.034 mole) and phosphorus pentasulfide (38.3 g., 0.172 mole) in *ca.* 1 liter of pyridine was treated, *dropwise*, with 1 ml. of water.[48] The orange turbid solution was refluxed for 6 hr. and allowed to stand overnight at room temperature. The pyridine was decanted from the semi-solid residue and evaporated *in vacuo* to a thin sirup which, together with the original residue, was added slowly to 2 l. of vigorously stirred water. Stirring was continued for 1 hr. during which time the precipitate gradually became granular. The solid was filtered and dissolved in methylene chloride, the solution was filtered to remove insolubles and was washed twice with water. The organic layer was dried briefly over sodium sulfate and evaporated *in vacuo* to dryness. The residue was reconcentrated with ethanol from which a yellow solid was obtained. The crude solid was recrystallized from 500 ml. of ethanol from which the product precipitated as long yellow needles. After filtration, the product was dried for 1 hr. in an oven at 80°, 11.5 g. (74%), m.p. 137–139° with softening at 135°, $[\alpha]^{28}D$ −17° (*c* 0.6 in methylene chloride); ultraviolet absorption data: in ethanol, maxima at 230, 273, 282 and 329 mμ, ϵ_{max} 28,670, 4,120, 4,120 and 19,550, respectively; minima at 271, 278 and 287 mμ, ϵ_{min} 4,030, 3730 and 3,770, respectively.

Anal. Calcd. for $C_{23}H_{20}N_2O_6S$: C, 61.05; H, 4.46; N, 6.19; S, 7.09. Found: C, 60.82; H, 4.39; N, 5.88; S, 7.16.

1-(2-Deoxy-β-D-ribofuranosyl)-4-thio-5-fluorouracil (4-Thio-FUDR) (IVa).—A suspension of 10 g. (0.021 mole) of 3′,5′-dibenzoyl-4-thio-FUDR (IIIa) in 100 ml. of ethanol was treated with 50 ml. of 2 *N* sodium hydroxide solution and the reaction mixture was shaken 1 hr. at room temperature. The brown solution was diluted with 100 ml. of water, made acid to congo red paper with *ca.* 7.2 ml. of concentrated HCl and evaporated *in vacuo* <40° until heavy precipitation resulted. The precipitate was filtered (mostly benzoic acid), taken up in ether and a small amount of insoluble 4-thio-FUDR isolated by filtration. The original filtrate was again evaporated until heavy precipitation of a bright yellow solid occurred. The precipitate was filtered and washed separately with ether to remove any occluded benzoic acid. The acidic filtrate was extracted with ether to remove traces of benzoic acid. The acidic filtrate was then neutralized with ammonium hydroxide and evaporated to dryness *in vacuo*. The solid residue was leached several times with boiling acetone until no more color was extracted from the salts. The acetone was removed *in vacuo* and the residue, combined with the first two crops of IVa, was recrystallized from 1-butanol which afforded a shining yellow crystalline product, 5.44 g. (75%), m.p. 160–163°, $[\alpha]^{28}D$ +98° (*c* 1.4 in water). The product appeared to be pure when examined by paper chromatography in five systems (see Table V); ultraviolet absorption data: at *p*H 1, maxima at 236, 268 and 340 mμ, ϵ_{max} 3,310, 2,520 and 21,710, respectively; minima at 254 and 282 mμ, ϵ_{min} 2,320 and 2,280, respectively; at *p*H 12, maximum at 325 mμ, ϵ_{max} 20,075; minimum at 254 mμ, ϵ_{min} 1,470.

Anal. Calcd. for $C_9H_{11}N_2O_4FS$: C, 41.22; H, 4.23; N, 10.68; F, 7.24; S, 12.22. Found: C, 41.47; H, 4.23; N, 10.81; F, 7.50; S, 12.30.

1-(β-D-Ribofuranosyl)-4-thio-5-fluorouracil (4-Thio-FUR) (IVb).—A solution of 7.3 g. (0.12 mole) of 2′,3′,5′-tri-*O*-benzoyl-4-thio-5-fluorouridine (IIIb) in 100 ml. of ethanol containing 70 ml. of *N* potassium hydroxide was shaken 1 hr. at room temperature. The bright yellow solution was treated slowly with 6 *N* hydrochloric acid until acid to congo red paper (required about 15.5 ml.). The solution was evaporated *in vacuo* at <40° until heavy precipitation occurred. The suspension was then chilled briefly, filtered and the precipitate (benzoic acid) washed with ice-water until colorless. The aqueous filtrate was extracted twice with ether to remove the last traces of benzoic acid. The aqueous phase was neutralized with ammonium hydroxide and evaporated *in vacuo* at <45°. The yellow sirup was reconcentrated several times with ethanol, and the resulting semi-solid residue was leached repeatedly with hot acetone until no more color was extracted from the salts. The acetone solution was evaporated *in vacuo* to a slurry which was treated with 100 ml. of 1-butanol and reconcentrated until the acetone had been removed. The suspension was then dissolved in hot 1-butanol, filtered, and cooled slowly. The product precipitated as a bright yellow solid, 2.57 g. (75%), m.p. 173–176°. An aliquot was recrystallized from ethanol for analysis, m.p. 180–181°, $[\alpha]^{26}D$ +64° (*c* 1.2 in water) (see Table V for R_f values); ultraviolet absorption data: at *p*H 1, maxima at 238, 266–270 and 340 mμ, ϵ_{max} 3,660, 2,410 and 20,730, respectively; minima at 225, 257 and 282 mμ, ϵ_{min} 3,140, 2,340 and 2,260, respectively; at *p*H 12, maximum at 325 mμ, ϵ_{max} 19,570; minimum at 256 mμ, ϵ_{min} 1,790.

Anal. Calcd. for $C_9H_{11}N_2O_5FS$: C, 38.85; H, 3.99; N, 10.07; F, 6.83; S, 11.52. Found: C, 39.07; H, 4.15; N, 10.03; F, 6.98; S, 11.71.

5-Fluoro-2′-deoxycytidine (FCDR) (Va).—A solution of 10 g. (0.038 mole) of 4-thio-FUDR (IVa) in 150 ml. of liquid ammonia was heated under nitrogen in a glass-lined autoclave for 24 hr. at 58°. After evaporation of the ammonia, a tan residue was obtained which was taken up in 80 ml. of hot ethanol. Upon cooling the solution, 4.43 g. (48%) of crystalline material, m.p. 180–182°, was obtained. The mother liquor was evaporated *in vacuo* and the residue taken up in 20 ml. of water. An insoluble product (0.2 g., containing 50% sulfur) was filtered and the filtrate clarified with charcoal. Both the crystalline product and the filtrate were found to be contaminated with unreacted starting material as determined from the ultraviolet absorption at 340 mμ. In addition, examination by paper chromatography revealed the presence of other impurities. Therefore, the crystals were combined with the aqueous filtrate, the resulting solution rendered acid to congo red paper with 2.5 ml. of 10 *N* formic acid and submitted to chromatography on a Dowex 50-X8 (NH_4^+) 100–200 mesh column (4 × 48 cm.). Fractions of 130 ml. were taken at intervals of 30 minutes. Fractions 1–35 were eluted with water; from fraction 36–73, the elution was performed with 0.1 *N* ammonium hydroxide. Fractions showing more or less identical spectrophotometric and paper chromatographic properties were combined. The bulk of the FCDR was found in fractions 14–68; other fractions contained various substances, some unidentified and some, such as FUDR, starting material, and 2′-deoxycytidine, were identified by ultraviolet absorption data and by paper chromatography

(49) It was found that product of higher purity (95–98%) could be obtained if the preparation were made on a scale one-half as large.

in comparison with authentic samples. Another by-product obtained upon further elution with ammonia has been tentatively identified as 5-amino-2'-deoxycytidine. The combined fractions (14–68) containing the FCDR were evaporated *in vacuo* to a yellow residue which was taken up in 250 ml. of boiling ethanol, filtered to remove insoluble material, and cooled. Colorless crystals deposited which were washed with cold ethanol and ether; yield 3.50 g. (38%), m.p. 195–196.5°, $[\alpha]^{26}D$ +66° (*c* 2.5 in water). Evaporation of the alcoholic mother liquor produced a second crop (1.09 g., 12%) which was only slightly less pure than the higher melting material. The ultraviolet absorption spectrum is shown in Fig. 1; spectral ratios in 0.1 *N* HCl: 280/260 = 3.01, 300/280 = 0.97.

Anal. Calcd. for $C_9H_{12}N_3O_4F$: C, 44.08; H, 4.93; N, 17.14; F, 7.75. Found: C, 44.39; H, 5.10; N, 16.97; F, 7.54.

The hydrochloride of FCDR was obtained by dissolving 0.25 g. of the base in 12 ml. of hot ethanol, cooling the solution to room temperature and adding 0.15 ml. of ethanol saturated with gaseous hydrogen chloride, followed by 25 ml. of ether; yield of the salt, 0.25 g. (85%), decomposing *ca.* 140°.

Anal. Calcd. for $C_9H_{12}N_3O_4F$. HCl: C, 38.37; H, 4.65; N, 14.92; F, 6.75. Found: C, 38.64; H, 4.96; N, 15.24; F, 7.05.

5-Fluorocytidine (FCR) (Vb).—Twelve grams (0.02 mole) of 2',3',5'-tri-*O*-benzoyl-4-thio-5-fluorouridine (IIIb) were treated with *ca.* 130 ml. of methanol, previously saturated at 0° with ammonia, and heated in sealed tubes at 85° for 15 hr. The cooled tubes were opened and the greenish-yellow solutions filtered. The combined filtrates were evaporated *in vacuo*, water was added to the residue and the precipitated benzamide filtered. The aqueous filtrate was concentrated *in vacuo* and the methyl benzoate was removed by repeated co-distillation with water. The aqueous solution was extracted three times with chloroform to remove traces of benzamide. The water layer was decolorized with Norite, filtered and concentrated *in vacuo* to a sirup which was azeotroped twice with toluene to remove water. Absolute ethanol was added to the sirup and anhydrous hydrogen chloride was passed in slowly. The sirup gradually dissolved and some cooling became necessary. After a sufficient amount of gas had been added, precipitation of a cream-colored solid began. The suspension was chilled overnight, filtered and washed free of acid with anhydrous ether; yield 4.4 g. (74%), m.p. 173–174°. An additional 0.5 g., m.p. 172–173°, was obtained from the mother liquor bringing the total yield to 4.9 g. (82%).

A solution of 3.03 g. of the crude hydrochloride was purified by chromatographing on a 2.2 × 40 cm. column of Dowex 50-X2 (NH_4^+) 100–200 mesh resin. Elution was performed with water taking 20-ml. fractions at 30-minute intervals. The fractions (10–142) which showed an ultraviolet absorption ratio 300/280 mμ of 0.96–0.99 were combined and evaporated to dryness *in vacuo*.[50] The glass-like residue was extracted with *ca.* 50 ml. of hot ethanol and the extract filtered to remove insoluble material. On cooling, the alcoholic solution deposited crystals which were filtered and washed with cold alcohol and ether. Evaporation of the alcoholic mother liquor afforded a second crop; total yield of FCR, 1.03 g. (33%). Recrystallization from ethanol gave an analytically pure compound, m.p. 193–193.5°; ultraviolet absorption properties: in 0.1 *N* HCl, maximum at 290 mμ, ϵ_{max} 11,770; minimum at 248 mμ, ϵ_{min} 1,730; at *p*H 5–12, maxima at 237 and 281 mμ, ϵ_{max} 8,140 and 8,060, respectively; minima at 226 and 259 mμ, ϵ_{min} 7,630 and 5,670, respectively; spectral ratios in 0.1 *N* HCl: 280/260 = 3.00, 300/280 = 0.95.

Anal. Calcd. for $C_9H_{12}N_3O_5F$: C, 41.38; H, 4.63; N, 16.08; F, 7.27. Found: C, 41.45; H, 4.90; N, 15.88; F, 7.44.

The hydrochloride was prepared from the free base in a manner similar to that used in the preceding synthesis. The salt decomposed *ca.* 170°.

Anal. Calcd. for $C_9H_{12}N_3O_5F$.HCl: C, 36.31; H, 4.40; N, 14.12. Found: C, 36.29; H, 4.82; N, 13.97.

1-(2-Deoxy-β-D-ribofuranosyl)-4-methylamino-5-fluoro-2(1H)-pyrimidinone (N-Methyl-FCDR) (VIa).—Fifteen grams (0.057 mole) of 4-thio-FUDR (IVa) was dissolved in 500 ml. of dry methanol and cooled to 10°. Anhydrous methylamine was passed slowly through the solution until the volume increased to *ca.* 700 ml. The reaction flask was then fitted with a drying tube and allowed to stand at room temperature until a spectrophotometric aliquot in *N* hydrochloric acid showed the complete disappearance of the characteristic ultraviolet absorption maximum at 340 mμ (due to the 4-thio substituent) and the concurrent rise of a new maximum at 290 mμ (due to the presence of the amine function). The reaction was finished when the ratio of the maxima 340/290 mμ became <0.10. Depending on the environmental conditions, the addition of more methylamine may be necessitated if the rate of decrease of this ratio lessens. The time required for completion of the reaction was 27 hr. (Reactions of smaller magnitude, *ca.* 3 g. of starting material, were usually complete in 12–15 hr.) The green solution was evaporated *in vacuo* at <45° to a yellow sirup which was reconcentrated several times with methanol to remove excess amine. The residue was treated twice with isopropyl alcohol resulting in solidification of the sirup to a granular precipitate which was filtered and triturated with a cold solution of ethyl acetate–isopropyl alcohol (90:10) until all color was removed. The crude solid, 12.57 g. (88%), was recrystallized from 140 ml. of methanol and the cooled solution was treated to incipient turbidity with ether. Crystallization was slow, yielding 8.99 g. (62%) of product, m.p. 189–192° (eff.), resolidifying at *ca.* 198° to diamond-shaped translucent prisms which remelted with decomposition *ca.* 260°.[51] Two further recrystallizations from methanol raised the melting point to 207–208° (no eff.), $[\alpha]^{25}D$ +60° (*c* 1.0 in water). The product was proved to be pure when examined by paper chromatography in five systems (see Table V) and by paper ionophoresis (acetate buffer, *p*H 4, 750 volts, 8 milliamp., 3.5 hr.). The ultraviolet absorption spectrum is shown in Fig. 1, spectral ratios in 0.1 *N* HCl: 280/260 = 2.56, 300/280 = 0.80.

Anal. Calcd. for $C_{10}H_{14}N_3O_4F$: C, 46.33; H, 5.44; N, 16.21; F, 7.33; N-methyl, 5.80. Found: C, 46.65; H, 5.56; N, 16.24; F, 7.42; N-methyl, 5.75.

1-(β-D-Ribofuranosyl)-4-methylamino-5-fluoro-2(1H)-pyrimidinone (N-Methyl-FCR) (VIb).—A solution of 0.8 g. (0.0027 mole) of 4-thio-FUR (IVb) in 15 ml. of methanol was treated with a slow stream of methylamine gas as described in the preparation of VIa. The time required for completion of reaction was *ca.* 35 hr. The solvent and excess amine were removed in usual manner and the residual sirup taken up in hot methanol, filtered, concentrated to dryness *in vacuo*. The hygroscopic solid was triturated thoroughly with ethyl acetate–methanol (95:5) until a granular product was obtained, 0.42 g. (53%). Recrystallization from a minimum amount of hot methanol yielded shining white crystals, m.p. 214.5–216°, $[\alpha]^{26}D$ +43° (*c* 1.2 in water); ultraviolet absorption properties: in 0.1 *N* HCl, maximum at 287 mμ, ϵ_{max} 12,700; minimum at 248 mμ, ϵ_{min} 2,810; at *p*H 5–12, maxima at 243 and 277 mμ, ϵ_{max} 9,310 and 10,510, respectively; minima at 227 and 257 mμ, ϵ_{min} 8,070 and 8,550, respectively; spectral ratios in 0.1 *N* HCl: 280/260 = 2.48, 300/280 = 0.82.

Anal. Calcd. for $C_{10}H_{14}N_3O_5F$: C, 43.64; H, 5.13; N, 15.27; F, 6.90. Found: C, 43.58; H, 5.14; N, 15.13; F, 6.74.

1-(2-Deoxy-β-D-ribofuranosyl)-4-ethylamino-5-fluoro-2-(1H)-pyrimidinone (N-Ethyl-FCDR) (VIIa).—Three grams (0.011 mole) of IVa was dissolved in *ca.* 30 ml. of ethanol and treated with ethylamine gas in a manner similar to that described in method A above. The reaction time required for the spectrophotometric ratio 340/290 mμ in *N* hydrochloric acid to drop to <0.1 was 44 hr. The green solution was evaporated *in vacuo* at <45° to a sirup which was reconcentrated several times with ethanol to remove excess amine. The sirup was then triturated with ethyl

(50) Further elution with 0.02 *N* ammonium hydroxide afforded a second compound (fractions 187–202) which was isolated and found to be fluorine free. Comparison of the ultraviolet spectrum, elementary analysis and mixed melting point with an authentic sample established the identity of this substance with cytidine.

(51) The crystalline end product of the effervescence and resolidification was identified as the free base, N-methyl-5-fluorocytosine, by elemental analysis and by its ultraviolet absorption properties (see below).

acetate containing about 1% ethanol until complete solidification to a granular solid occurred. The crude product was recrystallized from isopropyl alcohol–methanol (3:2) decolorizing if necessary. Crystallization was slow unless seeds were added; yield of shining white prisms, 2.27 g. (73%), m.p. 173–175.5°, $[\alpha]^{26}D$ +58° (*c* 1.54 in water). The product showed only one ultraviolet absorbing spot when examined by paper chromatography in five systems (see Table V) or when subjected to paper ionophoresis in acetate buffer, *p*H 4 (750 volts, 8.4 milliamp., 3.5 hr.); ultraviolet absorption properties: in 0.1 *N* HCl, maximum at 289 mμ, ϵ_{max} 13,690; minimum at 249 mμ, ϵ_{min} 2,690; at *p*H 5–12, maxima at 247 and 278 mμ, ϵ_{max} 9,600 and 11,400, respectively; minima at 229 and 257 mμ, ϵ_{min} 8,000 and 9,200, respectively; spectral ratios in 0.1 *N* HCl: 280/260 = 2.71, 300/280 = 0.92.

Anal. Calcd. for $C_{11}H_{16}N_3O_4F$: C, 48.35; H, 5.90; N, 15.38; F, 6.95. Found: C, 48.44; H, 5.96; N, 15.11; F, 7.05.

1-(2-Deoxy-β-D-ribofuranosyl)-4-propylamino-5-fluoro-2-(1H)-pyrimidinone (N-Propyl-FCDR) (VIIIa).—One gram (0.0038 mole) of IVa was treated with 20 ml. of propylamine with slight cooling and the reaction allowed to stand at room temperature for 45 hr. by which time the spectrophotometric ratio 340/290 mμ had become *ca.* zero. After evaporation of the filtered solution *in vacuo*, the residue was azeotroped several times with benzene and taken up in absolute ethanol; petroleum ether was added resulting in slow crystallization. The product was filtered, 0.80 g. (72%), m.p. 139–141°, $[\alpha]^{22}D$ +56° (*c* 1.0 in water). The product showed only one ultraviolet absorbing spot when examined by paper chromatography in 5 systems (see Table V), or when subjected to paper electrophoresis in acetate buffer, *p*H 4 (750 volts, 8.4 milliamp., 3.5 hr.); ultraviolet absorption properties: in 0.1 *N* HCl, maximum at 289 mμ, ϵ_{max} 14,010; minimum at 249 mμ, ϵ_{min} 2,920; at *p*H 7–12, maxima at 245 and 277.5 mμ, ϵ_{max} 9,700 and 11,720, respectively; minima at 228 and 255 mμ, ϵ_{min} 8,190 and 9,430, respectively; spectral ratios in 0.1 *N* HCl: 280/260 = 2.69, 300/280 = 0.93.

Anal. Calcd. for $C_{12}H_{18}N_3O_4F$: C, 50.17; H, 6.31; N, 14.63; F, 6.61. Found: C, 50.01; H, 6.37; N, 15.09; F, 6.79.

1-(2-Deoxy-β-D-ribofuranosyl)-4-*n*-butylamino-5-fluoro-2(1H)-pyrimidinone (N-*n*-Butyl-FCDR) (IXa).—One gram (0.004 mole) of IVa was dissolved in 5 ml. of 1-butanol, treated with 10 ml. of *n*-butylamine with slight cooling, and allowed to stand at room temperature until the reaction was complete. The reaction mixture was then worked up in a manner similar to that used for the N-ethyl homolog (*vide supra*). The crude product, 0.70 g. (62%), m.p. 154–159°, dissolved in hot isopropyl alcohol–methanol (3:2) and precipitated with acetone, now had m.p. 157–159° $[\alpha]^{21}D$ +55° (*c* 1.1 in water). The product was pure as demonstrated by paper chromatography (see Table V) and by paper electrophoresis in acetate buffer, *p*H 4 (750 volts, 8.4 milliamp., 3.5 hr.); ultraviolet absorption properties: in 0.1 *N* HCl, maximum at 289 mμ, ϵ_{max} 14,310; minimum at 249 mμ, ϵ_{min} 2,950; at *p*H 7–12, maxima at 246 and 277 mμ, ϵ_{max} 9,760 and 12,000, respectively; minima at 227 and 254 mμ, ϵ_{min} 8,040 and 9,610, respectively; spectral ratios in 0.1 *N* HCl: 280/260 = 2.70, 300/280 = 0.92.

Anal. Calcd. for $C_{13}H_{20}N_3O_4F$: C, 51.82; H, 6.69; N, 13.94; F, 6.31. Found: C, 51.64; H, 6.68; N, 13.72; F, 6.19.

1-(2-Deoxy-β-D-ribofuranosyl)-4-dimethylamino-5-fluoro-2(1H)-pyrimidinone (N,N-Dimethyl-FCDR) (Xa).—Conversion of IVa to the dimethylamino derivative under the experimental conditions used in the preceding syntheses proved impracticable since the time required for completion of reaction averaged 2 weeks. Warming the reaction mixture to 50° in a pressure bottle considerably shortened the reaction time, but the product was demonstrated by paper ionophoresis to contain *ca.* 10% of the defluorinated analog. Pure product Xa was obtained as follows: a solution of IVa (4.84 g., 0.018 mole) in 100 ml. of liquid dimethylamine was heated under nitrogen in a glass-lined autoclave for 6 hr. at 60°. After evaporation of the amine, the residue was recrystallized from ethanol, using Norite to remove the color; yield of crystalline product, 2.8 g. (57%), m.p. 173–174°. A second crop was obtained from the mother liquor; 0.15 g. (3%), m.p. 181–184°. A recrystallization of the first crop from 40 ml. of ethanol raised the melting point to 183.5–184.5°, $[\alpha]D$ +52° (*c* 1.0 in water). The purity of the product was established by paper chromatography in five systems (see Table V), and by paper ionophoresis (acetate buffer, *p*H 4, 750 volts, 8.4 milliamp., 3.5 hr.). The ultraviolet absorption spectrum is shown in Fig. 1; spectral ratios in 0.1 *N* HCl: 280/260 = 2.51, 300/280 = 1.49.

Anal. Calcd. for $C_{11}H_{16}N_3O_4F$: C, 48.35; H, 5.90; N, 15.38; F, 6.95. Found: C, 48.51; H, 5.92; N, 15.09; F, 6.88.

2′-Deoxycytidine.—One and one-half grams (0.033 mole) of 1-(3′,5′-di-*O*-benzoyl-2′-deoxy-β-D-ribosyl)-4-thiouracil was treated with *ca.* 60 ml. of alcoholic ammonia (previously saturated at 0°) in a sealed tube at 100° for 12 hr. The cooled tube was opened, the contents filtered and the filtrate concentrated *in vacuo* to a sirup which was reconcentrated twice with water to remove the ethyl benzoate. The aqueous residue was extracted several times with methylene chloride to remove the benzamide and, after treatment with Norite, was evaporated *in vacuo* to dryness. The residue was converted directly to the picrate salt, dec. *ca.* 190°, which proved to be identical in all respects with an authentic sample of 2′-deoxycytidine picrate.[52]

1-(2-Deoxy-β-D-ribofuranosyl)-4-methylamino-2(1-H)-pyrimidinone (N-Methyl-CDR).—A sealed tube containing 2 g. (0.004 mole) of 1-(3,5-di-*O*-benzoyl-2-deoxy-β-D-ribosyl)-4-thiouracil and *ca.* 50 ml. of methanol containing 45% of methylamine (by weight) was heated 12 hr. at 100°. The reaction mixture was treated in a manner analogous to that used in the synthesis of 2′-deoxycytidine. Following concentration of the aqueous phase and two reconcentrations of the residue with methanol, a white solid was obtained which was recrystallized from methanol; 0.75 g. (71%), m.p. 191–193°, $[\alpha]^{25}D$ +48° (*c* 1.2 in water). The product was pure as determined by paper chromatography in five systems (see Table V) and by paper ionophoresis (acetate buffer, *p*H 4, 775 volts, 8 milliamp., 2 hr.); ultraviolet absorption properties: at *p*H 1, maximum at 282 mμ, ϵ_{max} 14,600; minimum at 242 mμ, ϵ_{min} 2,550; at *p*H 6–12, maxima at 236 and 270 mμ, ϵ_{max} 9,050 and 11,700, respectively; minima at 229 and 248 mμ, ϵ_{min} 8,830 and 8,100, respectively; spectral ratios in 0.1 *N* HCl: 280/260 = 1.98, 300/280 = 0.35.

Anal. Calcd. for $C_{10}H_{15}N_3O_4$: C, 49.79; H, 6.27; N, 17.42. Found: C, 49.88; H, 6.31; N, 17.33.

1-(2-Deoxy-β-D-ribofuranosyl)-4-dimethylamino-2(1H)-pyrimidinone (N,N-Dimethyl-CDR).—A sealed tube containing 1.5 g. (0.0033 mole) of 1-(3,5-di-*O*-benzoyl-2-deoxy-β-D-ribosyl)-4-thiouracil and *ca.* 50 ml. of methanol containing 45% dimethylamine (by weight) was heated at 100° for 12 hr. The contents of the tube were treated as in the preparation of 2′-deoxycytidine (*vide supra*). The residue obtained was dissolved in hot isopropyl alcohol and treated with ether to turbidity. Slow precipitation resulted in a granular solid, 0.60 g. (71%), m.p. 176–180°. Recrystallization from isopropyl alcohol–methanol (1:1) raised the m.p. to 179–180.5°, $[\alpha]^{26}D$ +47° (*c* 1.3 in water). Purity of the product was established by paper chromatography in five systems (see Table V), and by paper ionophoresis (acetate buffer, *p*H 4, 750 volts, 8.4 milliamp., 3.5 hr.); ultraviolet absorption data: at *p*H 1, maximum at 287 mμ, ϵ_{max} 16,280; minimum at 245 mμ, ϵ_{min} 2,500; at *p*H 6–12, maximum at 278 mμ, ϵ_{max} 14,050; minimum at 238 mμ, ϵ_{min} 7,050; spectral ratios in 0.1 *N* HCl: 280/260 = 2.76. 300/280 = 0.73.

Anal. Calcd. for $C_{11}H_{17}N_3O_4$: C, 51.76; H, 6.71; N, 16.46. Found: C, 51.79; H, 7.00; N, 16.44.

1-(β-D-Ribofuranosyl)-4-dimethylamino-2(1H)-pyrimidinone (N,N-Dimethylcytidine).—A sealed tube containing 5 g. (0.009 mole) of 1-(2,3,5-tri-*O*-benzoyl-β-D-ribosyl)-4-thiouracil[18] and *ca.* 80 ml. of 45% dimethylamine in methanol was heated at 100° for 12 hr. The contents of the cooled tube were treated in a manner similar to that used in the synthesis of 2′-deoxycytidine. The residual sirup was thoroughly dried *in vacuo* at 50° to a hard glass which was triturated in a warm bath with ethyl acetate containing a little methanol until solidification occurred. The precipi-

(52) The sample of 2′-deoxycytidine picrate was kindly supplied by Dr. O. Schindler of the Pharmazeutische Anstalt der Universität, Basel, Switzerland.

tate, filtered and washed with ether, weighed 2.2 g. (89%), m.p. 148–150°. Recrystallization from ethyl acetate–methanol (5:1) raised the melting point to 158–160°, $[\alpha]^{28}D$ +14° (*c* 1.3 in water). The purity of the product was established by paper chromatography in five systems (see Table V); ultraviolet absorption properties: at *p*H 1, maximum at 287 mμ, ϵ_{max} 16,440; minimum at 246 mμ, ϵ_{min} 2,690; at *p*H 6–12, maximum at 278 mμ, ϵ_{max} 14,440; minimum at 238 mμ, ϵ_{min} 7,200; spectral ratios in 0.1 *N* HCl: 280/260 = 2.76, 300/280 = 0.77.

Anal. Calcd. for $C_{11}H_{17}N_3O_6$: C, 48.70; H, 6.32; N, 15.49. Found: C, 48.64; H, 6.17; N, 15.53.

N-Methyl-5-fluorocytosine from N-Methyl-FCDR (VIa).—An aliquot (0.2 g., 0.008 mole) of N-methyl-FCDR (VIa) was placed in a sublimator and heated *in vacuo* (*ca.* 0.1 mm.) at 200–210° for 0.5 hr. The sublimate was recrystallized from ethanol, decolorizing with Norite. The product crystallized in white needles, 50 mg. (50%), m.p. 267–268° with subl. from *ca.* 258°; ultraviolet absorption data: in 0.1 *N* hydrochloric acid, maximum at 283 mμ, ϵ_{max} 10,840; minimum at 246 mμ, ϵ_{min} 2,170; at *p*H 7.13, maxima at 232 and 272.5 mμ, ϵ_{max} 8,430 and 8,010, respectively; minimum at 256 mμ, ϵ_{min} 6,700; in *N* sodium hydroxide, maximum at 292 mμ, ϵ_{max} 9,460; minimum at 255 mμ, ϵ_{min} 1,590; spectral ratios in 0.1 *N* hydrochloric acid: 280/260 = 2.21; 300/280 = 0.60; pK_a values = 2.66 (cationic) and 11.66 (anionic) (determined spectrophotometrically, accurate to ±0.05 *p*H unit).

Anal. Calcd. for $C_6H_6N_3OF$: C, 41.96; H, 4.22; N, 29.36; F, 13.28. Found: C, 42.12; H, 4.23; N, 29.50; F, 12.94.

N,N-Dimethylcytosine.—4-Ethoxy-2(1H)-pyrimidinone[53] (1.0 g., 0.071 mole) was treated with a methanolic solution of dimethylamine (*ca.* 40% by weight) in a sealed tube at 120° for 16 hr. The tube was cooled, opened, and the white crystalline product filtered; 0.5 g. The filtrate was con centrated to dryness, re-evaporated several times with methanol. The residue was taken up in a minimum of hot ethanol, filtered and the filtrate chilled. The white precipitate was filtered, 0.42 g., and combined with the first product; total yield, 0.92 g. (93%). Recrystallization of the crude solid from isopropyl alcohol–ethanol afforded 0.71 g. (72%), m.p. 254.5–256°, sinter 253.5° (lit.[54] 248–250° hot-stage). The product appeared to be pure as determined by paper chromatography in five systems (see Table V); ultraviolet absorption data: at *p*H 1, maximum at 282 mμ, ϵ_{max} 14,030; minimum at 243 mμ, ϵ_{min} 2,450; at *p*H 7.10, maxima at 245 and 272 mμ, ϵ_{max} 7,090 and 10,880, respectively; minima at 234 and 245–250 mμ, ϵ_{min} 6,930 and 7,120, respectively; in *N* NaOH, maximum at 289 mμ, ϵ_{max} 11,400, slight inflection at 235 mμ; minimum at 258 mμ, ϵ_{min} 2,660 (lit.[54] values: *p*H 1, maximum at 282 mμ, ϵ_{max} 13,460; in water, maximum at 281 mμ, ϵ_{max} 11,130; at *p*H 13, maximum at 284 mμ, ϵ_{max} 9,452[55]; spectrophotometrically determined pK_a values: pK_{a1} = 4.25 ± 0.05, pK_{a2} = 12.30 ± 0.05.

Anal. Calcd. for $C_6H_9N_3O$: C, 51.79; H, 6.52; N, 30.20. Found: C, 52.00; H, 6.50; N, 30.00.

Enzyme Studies.—*E. coli* B. was grown in a minimal medium containing glucose as a sole carbon source. The cells were harvested by centrifugation and a nucleoside deaminase preparation was obtained from cell-free extracts following the procedure described by Cohen and Barner.[16] No detectable nucleoside phosphorylase activity was demonstrable in such preparations.

The incubation mixture contained 2.0 μmoles of nucleoside, 160 μmoles of trishydroxymethylaminomethane buffer, *p*H 8.6, 0.2 ml. of the nucleoside deaminase preparation in a total volume of 2.0 ml. Incubation was carried out for 45 minutes at 37°; the reaction was stopped by immersing the tubes containing the incubation mixture in a boiling water-bath for 3 minutes.

(53) Cyclo Chemical Corp., Los Angeles, Calif.

(54) H. M. Kissman and M. J. Weiss, *J. Am. Chem. Soc.*, **80**, 2575 (1958).

(55) For comparison purposes, a sample of their compound was kindly supplied by Dr. Kissman of the Lederle Laboratories. It proved to be identical with our sample when examined by paper chromatography in five systems and by spectrophotometric analysis. The spectral values quoted in ref. 54 are therefore in error.

TABLE V
PAPER CHROMATOGRAPHY

Compound	R_f values in solvent systems[a] A	B	C	D	E
Uracil	0.33	0.59	0.27	0.73	0.63
5-Fluorouracil	.42	.61	.16	.75	.63
Cytosine	.20	.55	.20	.52	.52
N,N-Dimethyl-	.47	.63	.50	.45	.74
5-Fluoro-	.27	.59	.27	.52	.49
N-Methyl-5-fluoro-	.48	.71	.47	.60	.72
Uridine	.15	.52	.11	.68	.53
5-Fluoro-	.33	.58	.04	.75	.67
4-Thio-5-fluoro- (IVb)	.65	.73	.30	.83	.77
Cytidine	.08	.52	.10	.50	.43
N-Methyl-	.25	.60	.28	.59	.66
N,N-Dimethyl-	.32	.61	.31	.59	.69
5-Fluoro (Vb)	.17	.56	.19	.56	.48
N-Methyl-5-fluoro- (VIb)	.36	.64	.39	.52	.76
2′-Deoxyuridine	.36	.63	.24	.79	.71
5-Fluoro-	.46	.64	.07	.80	.75
4-Thio-5-fluoro-	.75	.80	.33	.92	.85
2′-Deoxycytidine	.17	.61	.23	.61	.63
N-Methyl-	.41	.66	.50	.73	.79
N,N-Dimethyl-	.49	.69	.58	.67	.79
5-Fluoro- (Va)	.29	.63	.32	.66	.63
N-Methyl-5-fluoro- (VIa)	.53	.70	.50	.64	.83
N-Ethyl-5-fluoro- (VIIa)	.67	.79	.67	.73	.89
N-Propyl-5-fluoro- (VIIIa)	.85	.87	.85	.84	.90
N-*n*-Butyl-5-fluoro- (IXa)	.85	.88	.87	.92	.96
N,N-Dimethyl-5-fluoro- (Xa)	.65	.80	.57	.67	.88

[a] Solvent systems: A, 1-butanol–water (86:14); B, 1-butanol–acetic acid–water (5:2:3); C, 1-butanol–*N* ammonium hydroxide (86:14); D, isopropyl alcohol–hydrochloric acid (170:41) diluted to 250 ml. with water: E, ethanol–water (85:15). The values shown were determined by the ascending method using Schleicher and Schuell #597 paper.

A 1-ml. aliquot of the incubation mixture in 15 ml. of water was applied to a column (2.5 × 10 cm.) of Dowex 50 (H^+) resin (200–400 mesh) which was washed with water and the effluent collected (total volume *ca.* 80 ml.). By this treatment, any unreacted substrate was retained on the column while the product of deaminase activity was allowed to pass freely. The effluent was evaporated to dryness *in vacuo* at <40°, the residue was taken up in a suitable volume of *N* sodium hydroxide and the solution analyzed spectrophotometrically for the appropriate deaminated nucleoside. The pertinent ultraviolet absorption data for these respective uracil- or thymine-nucleosides at *p*H 14 (*N* NaOH) are given in Table VI. The optical density values were corrected by values obtained from a blank solution (minus only substrate) treated in manner identical with that outlined. The results are shown in Table IV as the percentage of conversion of the cytosine nucleoside substrate (read spectrophotometrically at time zero) to its deaminated derivative as determined above.

The alkaline solutions were then neutralized by treatment with Dowex 50 (H^+), evaporated to dryness *in vacuo*, and the residue examined by paper chromatography. No trace of any reaction product was detected in those cases in which the extent of deamination (spectrophotometrically calculated) appeared to be less than 5%. In all other instances, only one ultraviolet-absorbing spot was detected and its migration was identical with that of the expected deaminated nucleoside. On elution of the spot followed by spectrophotometric analysis of the eluate, no bathochromic shift of the maximum on passing from acid to basic *p*H was obtained. Thus, the absence of any free base, which could result from cleavage of the product of deaminase activ-

TABLE VI

Compound	mμ (max., pH 14)	ε × 10⁻³ (pH 14)
Uridine	264.5	7.5
5-Fluorouridine	270	7.0
1-β-D-Ribofuranosylthymine[24]	268–269	7.6
2′-Deoxyuridine	263.5	7.86
5-Fluoro-2′-deoxyuridine	269–270	6.94
Thymine	268	7.51

ity by trace contamination by nucleosidase(s) in the extract preparation, was verified.

Spectrophotometric Studies.—Ultraviolet absorption data were determined with a Cary recording spectrophotometer, model 11, using buffers and techniques previously described.[30,32] The curves in the strongly acidic region up to *p*H 3 were measured in HCl solutions; acetate buffers were used for the *p*H range 4–6, phosphate buffers for range *p*H 6–9, glycine buffers *p*H 10–12, and sodium hydroxide solutions from *p*H 12–14. All benzoylated derivatives were dissolved in methylene chloride and aliquots delivered into absolute ethanol; all other compounds were dissolved in water and aliquots delivered into the proper buffer solution. The spectrophotometrically measured pK_a values were determined by methods previously described.[32,56]

Polarimetric Determinations.—Optical rotations were determined using equipment and techniques previously described.[24]

Electrophoretic Experiments.—All studies were made using an E.C. electrophoresis apparatus.[57] Whatman 3MM paper was employed; after completion of the run, the paper was air-dried and the products visualized under ultraviolet light.

Paper Chromatographic Determinations.—The R_f values for some 5-fluoropyrimidine nucleosides and related compounds are shown in Table V. The values shown were determined by the ascending method using Schleicher and Schuell #597 paper. The compounds were visualized on the paper chromatograms under ultraviolet light and were all found to be light absorbing with the exception of the thiated intermediates which showed a characteristic pink fluorescence.

Key to Figures: All the spectra listed were run in aqueous solutions at *p*H values indicated on the curves. The italicized letters refer to isosbestic points.[31]

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

(57) Manufactured by E. C. Apparatus Co., Swarthmore, Pa.

[CONTRIBUTION FROM THE LABORATORY OF NUCLEAR MEDICINE AND RADIATION BIOLOGY OF THE DEPARTMENT OF BIOPHYSICS AND NUCLEAR MEDICINE, SCHOOL OF MEDICINE, UNIVERSITY OF CALIFORNIA AT LOS ANGELES*; INSTITUTE FOR MUSCLE RESEARCH, MARINE BIOLOGICAL LABORATORIES, WOODS HOLE+, AND THE LABORATORY OF THE CHILDREN'S CANCER RESEARCH FOUNDATION AND HARVARD MEDICAL SCHOOL, BOSTON 15, MASS.]

A Conformation-dependent Cotton Effect in α-Helical Polypeptides and Proteins[1,2]

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A Cotton effect is described which is associated with the α-helical conformation of polypeptides and proteins. This Cotton effect appears to be negative, with an inflection point at about 225 mμ. It is suggested that the magnitude of the trough at 233 mμ may be used as an approximate measure of α-helix content. Transformation of the polypeptides to random coil conformations, or denaturation of the proteins, results in the loss of the 225 mμ Cotton effect.

Introduction

Anomalous rotatory dispersion, known as the Cotton effect, occurs in or near optically active absorption bands.[4] The chemical groupings making up polypeptide or protein chains give rise to at least three types of electronic absorption bands: the α-carbons of the constituent amino acids absorb in the far ultraviolet; the peptide chromophore absorbs strongly around 145 and 185 mμ[5]; and aromatic side chains absorb in the region below 300 mμ. These chromophores may exhibit Cotton effects when situated in a disymmetric environment. In particular, a helical conformation of the polypeptide chain,[6] or restricted rotation of side chains, would be expected to induce anomalous rotatory dispersion.

Some rotatory dispersion data have been reported for polypeptides and proteins in the spectral range 240–300 mμ. Examination of the α-helical forms of two synthetic polypeptides containing aromatic side chains, poly-γ-benzyl-L-glutamate and poly-β-benzyl-L-aspartate, did not reveal a Cotton effect in this region.[7] Recently, however, measurements on the protein subunits of tobacco mosaic virus showed the presence of a small inflection point at 293 mμ; this unusual rotatory dispersion might be due to a small Cotton effect from oriented aromatic amino acids.[8] Furthermore, the shape of the dispersion curve below 240 mμ indicated the possible presence of a large Cotton effect in this region. An investigation of model polypeptides and proteins was then undertaken to examine this effect.

In this communication we report the rotatory dispersion of certain polypeptides and α-proteins from 400 to about 220 mμ. In all cases, the α-helical conformation is characterized by what may be described as a large negative Cotton effect with a trough at 233 mμ and an inflection at about 225 mμ. When the helix is destroyed, the effect is lost; thus the rotatory dispersive behavior in this region is conformation-dependent.

Experimental

Materials. Polypeptides. Poly-γ-benzyl-L-glutamate (L-PBG).—This sample, RK-1262, was prepared by Mr. Roy

(1) This is Polypeptides. XXXVI. For the preceding paper in this series see E. R. Simons, G. D. Fasman and E. R. Blout, *J. Biol. Chem.*, **236**, Pc64 (1961).

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