Nucleosides. XXXIX. 2′-Deoxy-2′-fluorocytidine, 1-β-D-Arabinofuranosyl-2-amino-1,4(2H)-4-iminopyrimidine, and Related Derivatives[1]

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Thiation of suitably protected 2′-deoxy-2′-halogenouridines followed by alkylation afforded 1-(2-deoxy-2-halogeno-β-D-ribofuranosyl)-4-methylthio-2-pyrimidinone (**5**, X = F or Cl) in good yields which, by treatment with liquid ammonia, gave 2′-deoxy-2′-halogenocytidines (**6**) along with the halide salts of 1-β-D-arabinofuranosyl-2-amino-1,4(2H)-4-iminopyrimidine (**7**). It is shown that in the above reaction of **5**, "aminoimino" nucleoside **7** formed *via* intermediates **6** and 2,2′-anhydroarabinofuranosylcytosine (**8**). The reaction of various 2,2′-anhydroarabinofuranosyl pyrimidines with liquid ammonia afforded 1-β-D-arabinofuranosyl derivatives of 5-methylisocytosine, 5-fluoroisocytosine, and 4-thioisocytosine. The hydrolytic reactions of 2′-deoxy-2′-halogenocytidines, 2,2′-anhydroarabinosylcytosine, and 2-aminopyrimidine nucleosides are reported and discussed.

The replacement of the 5 hydrogen of pyrimidine nucleosides by a fluorine atom has led to several chemotherapeutically active compounds, among which are 5-fluoro-2′-deoxyuridine[2] and -2′-deoxycytidine.[3] In addition, pyrimidine nucleosides containing the 1-β-D-arabinofuranosyl moiety, such as arabinosylcytosine,[4] arabinosyl-5-fluorouracil,[5] and arabinosyl-5-fluorocytosine[6] have also demonstrated interesting biochemical and chemotherapeutic activity.[7] This paper deals with the synthesis and chemical properties of 2′-halogeno derivatives of 2′-deoxycytidines. In addition, arabinosyl derivatives of isocytosine and 2,4-diaminopyrimidine were prepared and their chemistry studied.

The thiation procedure[8] reported for pyrimidine nucleosides was used for the synthesis of the 2′-halogenocytidines (**6**) from the previously reported 2′-chloro- and 2′-fluoro-2′-deoxyuridine (**1**) (Scheme I).[9] Acetylation of **1** (X = F or Cl, R = H), followed by thiation with phosphorus pentasulfide in pyridine, yielded the 4-thiono di-O-acetates (**3**) in high yield. Deacetylation of **3** was achieved by treatment of these blocked nucleosides with liquid ammonia at room temperature. Under these conditions, the 4-thiono group was not replaced and 2,2′-anhydro nucleoside formation was avoided.[10a] Methylation of **4** afforded the methylthio nucleosides **5** (X = F or Cl).

The use of liquid ammonia at room temperature for the replacement of the 4-methylthio group by an amino function has been found to be extremely useful in this laboratory, as exemplified by the facile conversion of 1-β-D-arabinofuranosyl-4-methylthio-2-pyrimidinone[10b] and its 5-fluoro analog[6] to the corresponding cytosine nucleosides by this reagent. However, more vigorous treatment of the 2′-halogeno-4-methylthio nucleosides (**5**) was needed to effect this reaction. Treatment of **5** (X = F) with liquid ammonia at 55–60° for 2 days yielded 2′-deoxy-2′-fluorocytidine (**6**) in 65% yield. Reaction of **5** (X = Cl) with liquid ammonia for 6 days at room temperature caused the complete replacement of the methylthio group by amine. Paper chromatographic examination of the reaction mixture indicated that two products were formed in *ca.* 1:1 ratio, one of which was the expected 2′-chloro-2′-deoxycytidine. The other component was subsequently shown to be the hydrochloride salt of the aminoiminoarabino nucleoside **7**. 2′-Chloro-2′-deoxycytidine was isolated from this mixture in 18% yield. When the reaction mixture containing **6** and **7** was boiled in anhydrous dioxane for 20 min, complete conversion of **6** to the hydrochloride of 2,2′-anhydro-1-(β-D-arabinofuranosyl)cytosine (**8**)[4,11] occurred. About 10% of the aminoimino nucleoside **7** was also converted to **8** by this treatment. Trituration of the semisolid mixture (now containing **7** and **8**) with ethanol caused solution of **7** and afforded crystalline **8**. The mother liquors (containing **7**) were converted to the acetate salt and isolated as the crystalline picrate of **7**.

Proof of structure of the aminoimino nucleoside **7** rests on the following grounds. The picrate of **7** showed correct combustion analyses. The absorption spectrum of the hydrochloride of **7** at pH 1, 7, and ~14 was

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA 08748).

(2) R. Duschinsky, E. Pleven, J. Malbica, and C. Heidelberger, 132nd National Meeting of the American Chemical Society, New York, N. Y., Sept 1957, p 19C; M. Hoffer, R. Duschinsky, J. J. Fox, and N. Yung, *J. Am. Chem. Soc.*, **81**, 4112 (1959).

(3) I. Wempen, R. Duschinsky, L. Kaplan, and J. J. Fox, *ibid.*, **83**, 4755 (1961).

(4) E. R. Walwick, W. K. Roberts, and C. A. Dekker, *Proc. Chem. Soc.*, 84 (1959).

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

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

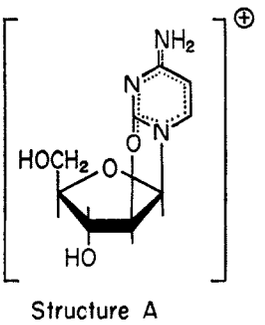
(7) S. S. Cohen, *Progr. Nucleic Acid Res. Mol. Biol.*, **5**, 1 (1966).

(8) 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).

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

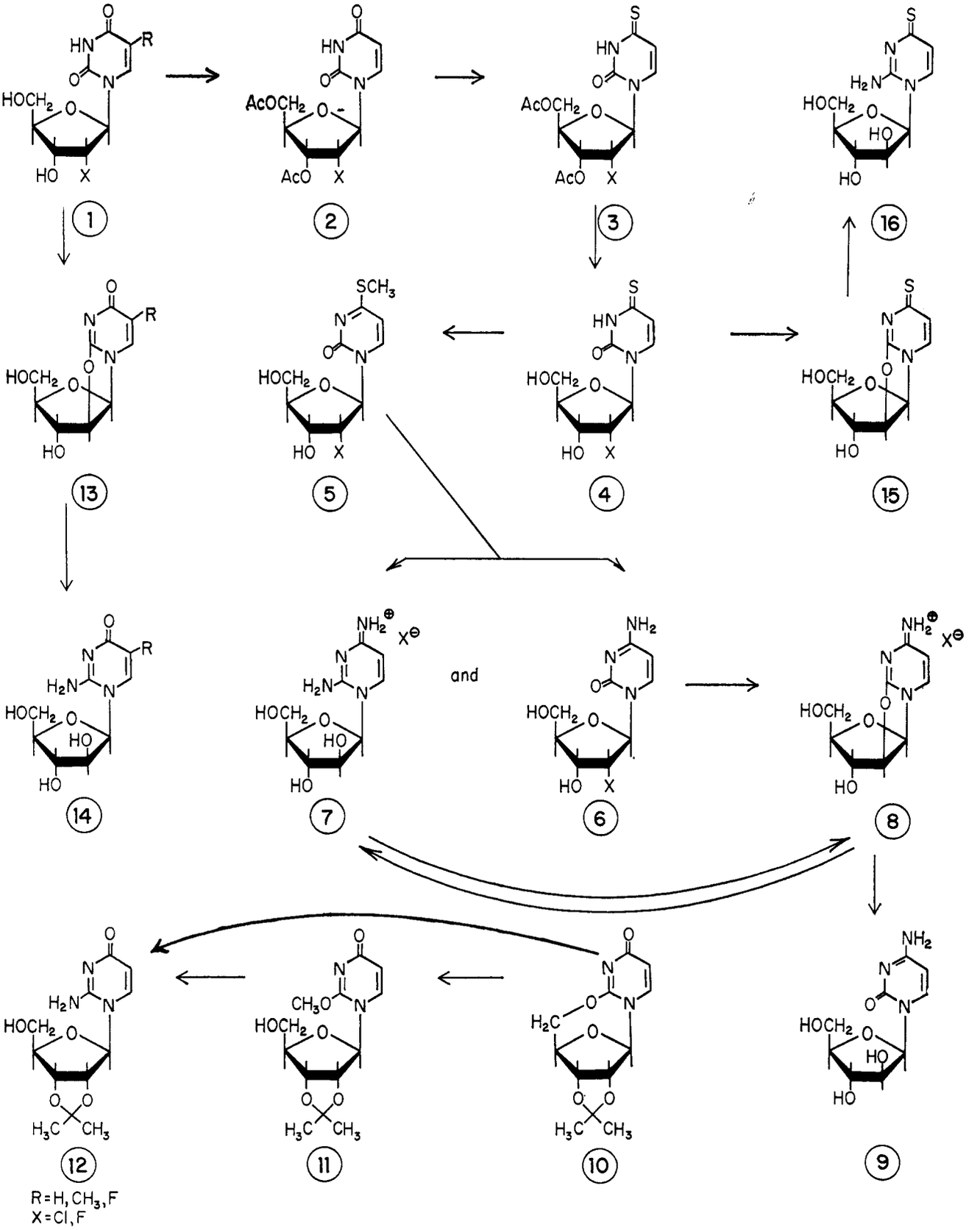
(10) (a) It had been noted previously that aqueous alkali would convert the uracil derivative **1** (X = Cl) to 2,2-anhydroarabinosyluracil (**13**). (b) Naishun C. Miller and J. J. Fox, unpublished data.

(11) In addition to cationic structure **8** given by Walwick, *et al.*,[4] other possible contributing resonance forms may be written. These would include two aromatic Kekulé forms with positive charge on N-1 or a structure with a positive charge on the anhydro bridge oxygen atom. These resonance cations may be represented by structure A.



Structure A

SCHEME I



similar to that reported by Brown and Jacobsen[12a] for 2-amino-4-imino-1,4(2H)-1-methylpyrimidine.[13] The fact that **7** and **8** are interconvertible under proper conditions and, as will be discussed later, that **7** is converted to 1-β-D-arabinofuranosylcytosine (**9**) (probably *via* **8**) establishes the 1-β-D-arabinofuranosyl structure for **7**.

(12) (a) D. J. Brown and N. W. Jacobsen, *J. Chem. Soc.*, 3172 (1962); (b) D. J. Brown and T. Teitei, *ibid.*, 755 (1965).

(13) The true tautomeric form of the neutral species of **7** is unknown. In accord with the rationale used for 2-amino-4-imino-1,4(2H)-1-methylpyrimidine by Brown, *et al.*,[12a] that the *para*-quinonoid form would be expected to be the most stable, the aminoimino nucleoside **7** has been named in this paper as a *para*-quinonoid rather than the tautomeric *ortho*-quinonoid structure. The probable neutral forms of the aminoimino nucleoside **7** are shown as tautomeric structures **17b** (Scheme III).

The formation of **7** by treatment of **5** with liquid ammonia occurs most likely *via* **6** → **8**. Compound **6** would undergo an intramolecular displacement of the 2′-chloro atom by nucleophilic attack of the 2-carbonyl oxygen atom to yield anhydronucleoside **8** which would then be converted to **7** under the ammonolysis conditions employed. In fact, when crystalline **8** hydrochloride was treated with liquid ammonia overnight at room temperature, a quantitative conversion to **7** hydrochloride (obtained as a glass) occurred. Attempts to isolate a crystalline hydroiodide, -bromide, -chloride, -acetate, -nitrate, or -perchlorate of **7** were unsuccessful since these salts were extremely hygroscopic and were very soluble in ethanol. When the glassy hydrogen halide salts of **7** were treated with an excess of hydrogen chloride or hydrogen bromide in ethanol, a slow precipitation of the hydrogen halide salts of anhydro nucleoside **8** occurred. A sulfate salt of **7** was obtained as an amorphous, hygroscopic solid which was insoluble in ethanol but this derivative, too, could not be crystallized. Of all the salts of **7**, only the picrate was obtained in crystalline form from its acetate salt. An alternate approach to the picrate salt of **7** was to treat the picrate of anhydro nucleoside **8** with liquid ammonia.

The ease with which the aminoimino nucleoside **7** was obtained by cleavage of anhydro nucleoside **8** with liquid ammonia prompted us to investigate this reaction with other anhydro nucleosides. Brown, *et al.*,[14a] showed that treatment of 2′,3′-O-isopropylidene-2,5′-anhydrouridine (**10**) with methanolic ammonia for 5 days yielded the isocytidine derivative **12** *via* the 2-O-methyl derivative **11**. Under similar reaction conditions, Brown, *et al.*,[14b] prepared 1-β-D-arabinofuranosylisocytosine (**14**, R = H) from the 2,2′-anhydro nucleoside[14c] **13** (R = H). We have found that treatment of the 2,5′-anhydro nucleoside **10** with liquid ammonia at room temperature overnight gives a nearly quantitative conversion to the isocytidine **12**. Similar treatment of the 2,2′-anhydroarabinofuranosyl nucleoside **13** (R = H) readily formed **14** (R = H). By this liquid-ammonia procedure two new isocytosine nucleosides were prepared. 2,2′-Anhydro-1-(β-D-arabinofuranosyl)thymine was converted in liquid ammonia to 1-β-D-arabinofuranosyl-5-methylisocytosine (**14**, R = CH_3) and similarly **13** (R = F) was converted to the 5-fluoroisocytosine derivative **14** (R = F), nor is this reaction limited to anhydronucleosides of furanosyl sugars since Watanabe and Fox[15] have recently demonstrated the applicability of this method to the conversion of a 2,2′-anhydrohexopyranosyluracil to hexopyranosylisocytosines.

The availability of the 2′-chloro-4-thio nucleoside **4** gave an easy route to 2,2′-anhydro-1-(β-D-arabinofuranosyl)-4-thiouracil (**15**). Boiling the 2′-chloro nucleoside **4** in *n*-butyl alcohol containing 1 equiv of triethylamine yielded **15** which was converted to 1-β-D-arabinofuranosyl-4-thioisocytosine (**16**) with anhydrous ammonia. A 4-thio-2,2′-anhydropyrimidine nucleoside derivative had previously been prepared by thiation of a 2,2′-anhydrouracil nucleoside with phosphorus pentasulfide in pyridine.[16]

Spectral Studies.—The 2′-halogenodeoxycytidines **6** (X = Cl or F) exhibit the same characteristic ultraviolet absorption spectra as does 2′-deoxycytidine apart from a slight hypsochromic shift (2–3 mμ) in both the minimum and maxima (Table I). This hypsochromic shift, due to the presence of the 2′-halogeno group, has also been observed in the 2′-halogeno-2′-deoxyuridines and 2′-halogenothymidines.[9] The 2′-fluoro group in **6** exerts a slight base weakening effect. The apparent pK_a (determined spectrophotometrically[17]) was found to be 3.9 for 2′-deoxy-2′-fluorocytidine compared with 4.3 for 2′-deoxycytidine.

When compared to 1-methylisocytosine,[18] arabinosylisocytosine (**14**) exhibited similar maxima and ε values in 0.1 *N* hydrochloric acid and 0.1 *N* sodium hydroxide (Table I).[19] An examination of the spectra of **14** (R = H, CH_3) revealed one dissociation which occurred in acid medium and was due to protonation of the aglycon. The cationic (pH 1) and neutral (pH 7) species spectra of 1-β-D-arabinofuranosyl-5-fluoroisocytosine (**14**, R = F) were similar to those for **14** (R = H or CH_3); however, in alkali, the spectrum of **14** (R = F) was *rapidly* altered with time.

The spectra of nucleosides **14** in alkali are of interest. The small shift in the maxima (pH 7–13) of **14** (R = H) from 257 to 260 mμ and of **14** (R = CH_3) from 260 to 262 mμ might be ascribed to dissociation either of the sugar moiety (previously observed with other pyrimidine nucleosides)[20a] and/or of the isocytosine moiety.[20b] In addition, both of these isocytosine nucleosides (**14**, R = H or CH_3) undergo a *slow* alteration of their spectra which, as will be described later in the hydrolysis experiments, is due to their slow conversion to the corresponding uracil analogs.

The alkaline instability of the 5-fluoro analog of **14** is different from that observed with the other (R = H, CH_3) analogs. With the 5-fluoro analog (in 1 *N* alkali at room temperature) all selective absorption in the ultraviolet was lost within 90 min. Selective absorption was not recovered by acidification of the alkaline solution. Recently, Fox, Miller, and Cushley[21] showed that, in 0.1 *N* sodium hydroxide (60–70°, 30 min) (see Scheme II), 1-β-D-arabinofuranosyl-5-fluorouracil (FUA) and its cytosine analog (FCA) are converted to the open-chain ureide (B) with a 6 to 2′ anhydro linkage. The mechanism proposed for this alkaline degradation of FUA and FCA involves an intramolecular nucleophilic attack by the up 2′-hydroxyl anion on C-6 to form the 6,2′-anhydro nucleoside which then undergoes cleavage at the 3,4 position to form B

(14) (a) D. M. Brown, A. Todd, and S. Varadarajan, *J. Chem. Soc.*, 868 (1957). (b) D. M. Brown, D. B. Parihar, A. Todd, and S. Varadarajan, *ibid.*, 3028 (1958); (c) D. M. Brown, A. Todd, and S. Varadarajan, *ibid.*, 2388 (1956).

(15) K. A. Watanabe and J. J. Fox, *J. Org. Chem.*, **31**, 211 (1966).

(16) N. C. Yung and J. J. Fox, *ibid.*, **27**, 1477 (1962).

(17) (a) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952). (b) J. J. Fox and D. Shugar, *Bull. Soc., Chim. Belges*, **61**, 44 (1952).

(18) R. B. Angier and W. V. Curran, *J. Org. Chem.*, **28**, 1871 (1961).

(19) From our own spectral data and those reported by Brown,[14a] the 0.1 *N* NaOH spectrum of isopropylideneisocytidine (**12**) [λ_{max} 223–224 mμ (ε 16,500) is very different from that of **14** or of 1-methylisocytosine (Table I).

(20) (a) J. J. Fox, L. F. Cavalieri, and N. Chang, *J. Am. Chem. Soc.*, **75**, 4315 (1953); J. J. Fox, J. F. Codington, N. C. Yung, L. Kaplan, and J. O. Lampen, *ibid.*, **80**, 5155 (1958). (b) In 7 *N* KOH, the spectrum of arabinosylisocytosine (**14**, R = H) shows maxima at 288 and 228 mμ, minimum at 265 mμ; **14** (R = CH_3) shows maxima at 291 and 231 mμ, minimum at 267 mμ. These curves differ appreciably from their 0.1 *N* NaOH spectra (Table I) and suggest that dissociation of the isocytosine moieties occur in strong alkali. This matter is under investigation.

(21) J. J. Fox, N. C. Miller, R. J. Cushley, *Tetrahedron Letters*, 4927 (1966).

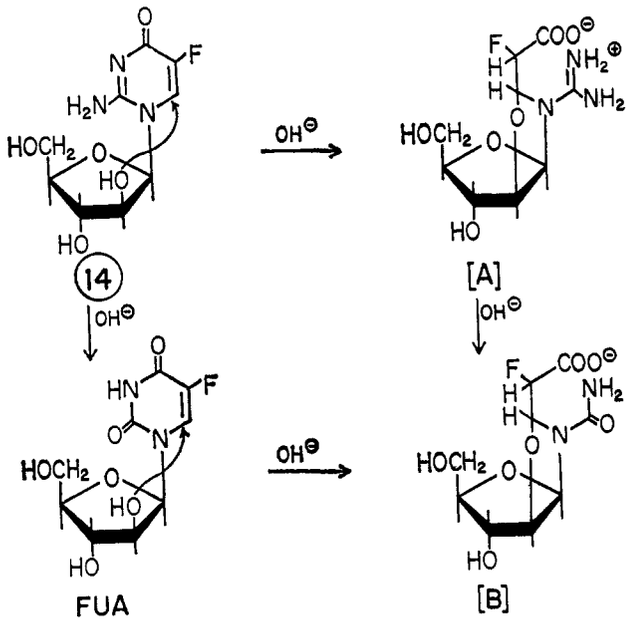
TABLE I

SPECTROPHOTOMETRIC DATA[a]

	pH	λ_{max}, mμ	ε	λ_{min}, mμ	ε
2′-Deoxy-2′-fluorocytidine (6)[b]	1	211, 277	9,550, 12,910	239	1,840
	7.3	227–230*s*, 268	7,780, 8,710	248	6,440
	14	271	9,110	248	5,730
2′-Deoxy-2′-chlorocytidine (6)[c]	1	210, 276.5	9,600, 13,080	239	1,510
	Water	230*s*, 268	7,540, 8,000	248	6,140
2′-Deoxycytidine[d]	1	212.5, 280	10,200, 13,200	241	1,500
	7–12	271	4,000	250	6,130
	14	272.5	9,280	248.5	5,700
Arabinosylisocytosine (14)[c,e]	0	219, 257	9,480, 7,820	238	5,000
	Water	205, 257, *225*	26,400, 6,260	247	5,950
	13	260, *225*	6,060	250	5,800
1-Methylisocytosine[f]	1	217, 260	9,250, 7,620	...	...
	13	260	5,500	...	...
Arabinosyl-5-methylisocytosine (14)[c]	0	225, 260	9,130, 8,300	244	6,430
	Water	204, 260, *214*, *228*	20,600, 6,740	246	5,910
	13	262, *225*	7,050	247	5,900
Arabinosyl-5-fluoroisocytosine (14)[c]	0	222, 262	7,000, 5,700	210, 243	6,500, 3,920
	Water	200, 261, *215*, *230*	14,700, 4,750	248	4,260
Arabinosyl-4-thioisocytosine (16)[c]	0	260, 325	5,060, 16,800	229, 280	2,150, 4,650
	6.9	248, 317	5,380, 20,600	235, 265	3,860, 4,530
2,2′-Anhydroarabinosyl-4-thiouracil (15)[c]	1–7	215, 242, 266, 327	18,400, 3,960, 2,690, 20,600	226, 250, 275	3,110, 2,540, 2,600
Arabinosyl-2-amino-1,4(2H)-4-iminopyrimidine (7)[c,g]	1	210, 233*s*, 266.5	24,200, 11,000, 7,500	254	6,900
	14	230, 291.5	21,900, 3,620	272	2,940
2-Amino-1,4(2H)-4-imino-1-methylpyrimidine (17a)[h]	9	235, 270	10,470, 6,760	...	...
	14.8	297	2,750	...	...
2,4-Diaminopyrimidine (18)[i]	1	206, 265, *225*	24,700, 5,100	253	4,370
	13	281	6,200	254	1,560

[a] Italicized numbers refer to inflections, the letter *s* after the wavelength value refers to a shoulder. [b] An apparent pK_a value of 3.9 (±0.05) was determined spectrophotometrically (*cf.* 2′-deoxycytidine, pK_a = 4.3). [c] Unstable in basic solutions. [d] J. J. Fox and D. Shugar, *Biochim. Biophys. Acta*, 9, 369 (1952). [e] The spectral data for this compound show some significant differences from those reported by Brown, *et al.*[14a] [f] Reference 18. [g] At pH 14, 7 is predominantly in the neutral form. [h] Data taken from ref 12a (pK_a = 12.9). The absence of a maximum in the 210-mμ region (at pH 9) may be due to the buffer used by these workers. [i] Reference 12b reported a similar spectra (pK_a = 7.26 in ref 23). However, they did not report the maximum at 206 mμ and inflection at pH 1. Sample of 18 supplied by Dr. A. Bendich, Sloan-Kettering Institute.

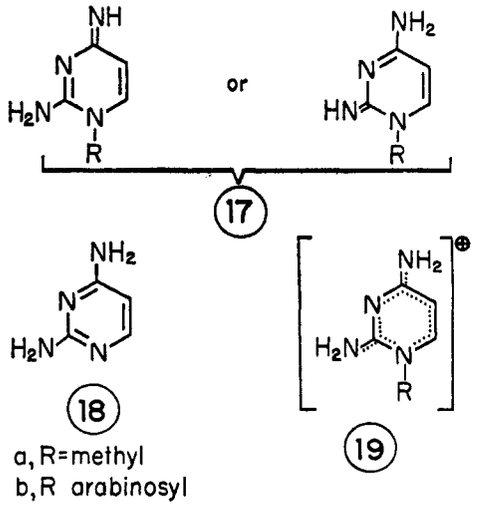
SCHEME II



(Scheme II). During their conversion of FUA or FCA to B, selective absorption in the ultraviolet is lost.[22] Though the product(s) of the reaction of **14** (R = F) with alkali was not investigated, it is quite likely that a similar type transformation occurs to yield A and/or B. The formation of the guanidino derivative (A) would result from attack on C-6 of **14**. If any B formed, this ureide could result by hydrolytic deamination of A or by conversion of **14** (R = F) to FUA followed by attack on C-6. In this latter regard, it is noteworthy that in 0.1 *N* alkali **14** (R = F) is converted slowly to FUA.

Ultraviolet spectral and pK_a studies[12b] support the diamino nature of 2,4-diaminopyrimidine (**18**) (Scheme III). A comparison of the ultraviolet spectra of the

SCHEME III



(22) Treatment of FCA and FUA with 0.1 *N* sodium hydroxide at room temperature for 72 hr produced 90 and 75% loss of extinction, respectively.[21]

TABLE II

HYDROLYSIS OF 1-(β-D-ARABINOFURANOSYL)-2-AMINOPYRIMIDINES

Arabinosyl derivative of	Hydrolytic conditions	Ultraviolet-absorbing product(s)[a]
2,4-Diaminopyrimidine **7** (chloride)	Refluxing, 2 *N* acetic acid, 3 hr	Starting material, anhydro **8** (~33%), **18** (~33%), and traces of **9** and **20** (R = H)
Isocytosine **14** (R = H)	60–70°, dilute acetic acid, 15 min	Anhydro **13** (R = H)
5-Methylisocytosine **14** (R = CH_3)	60–70°, dilute acetic acid, 15 min	Anhydro **13** (R = CH_3)
5-Fluoroisocytosine **14** (R = F)	60–70°, dilute acetic acid, 3.5 hr	Anhydro **13** (R = F)
7 (chloride)	Refluxing, 0.66 *N* H_2SO_4, 1 hr	2,4-Diaminopyrimidine picrate (**18**, 80%)[b] and anhydro **8** (trace)
14 (R = H)	Refluxing 0.66 *N* H_2SO_4, 1 hr	Isocytosine picrate (**21**, 83%),[b] and **20** (R = H)
14 (R = CH_3)	Refluxing, 0.66 *N* H_2SO_4, 1 hr	5-Methylisocytosine picrate[b,c] (**21**, 56%) and **20** (R = CH_3) (trace)
14 (R = F)	Refluxing, 0.66 *N* H_2SO_4, 1 hr	5-Fluoroisocytosine (**21**, 95%)[d]
7 (chloride)	24°, 1 *N* NaOH, 2 days	Arabinosylcytosine (**9**, major component), starting material, and **20** (R = H) (minor components)
14 (R = H)	24°, 1 *N* NaOH, 2 days	Arabinosyluracil (**20**)
14 (R = CH_3)	24°, 1 *N* NaOH, 2 days	Arabinosylthymine (**20**, major component), and starting material (<21%)

[a] Products were determined by paper electrophoresis (acetate buffer) and/or paper chromatography (system 5:1:1). [b] Products isolated. [c] 5-Methylisocytosine picrate (mp 297–299° dec) was analyzed. *Anal.* Calcd: N, 23.70. Found: N, 23.92. [d] The amount of **21** (R = F) was spectrophotometrically determined. The ultraviolet absorption properties of the product were identical with those of an authentic sample of 5-fluoroisocytosine generously furnished by Hoffmann-La Roche, Inc., Nutley, N. J.

neutral species of **18** with the neutral species of the 2(4)-amino-4(2)-imino nucleoside **17b** and of the 1-methyl derivative **17a** shows appreciable dissimilarities (Table I). These dissimilarities are to be expected because of the difference in bond structures in the neutral species of **17** compared with those in **18**.

The most stable cationic structure for the aminoimino derivatives **17** would probably be the resonant cation **19** which is a representation of the possible protonated mesomeric forms. Resonant cation **19** would be formed by protonation of the 2(4)-exocyclic imino group of the neutral species **17**. The strong basic strength of the neutral species **17** supports the large degree of resonance stabilization indicated by structure 19.[23] The similarity of the ultraviolet spectra of the *cationic* species of 2,4-diaminopyrimidine (**18**), of nucleoside **17b**, and the 1-methyl analog **17a** is best explained by the common resonant cation **19**. These data indicate that protonation of **18** occurs at N-1 and that protonation of the 1-substituted derivatives **17a** and **b** probably occurs on the exocyclic imino group rather than on N-3.

It is found that the cationic spectrum of 2,2′-anhydro-β-D-arabinofuranosylcytosine (**8**) (λ_{max} 262 mμ and 231 mμ) is similar to the cationic spectrum of 4-amino-2-methoxypyrimidine (λ_{max} 260.5 mμ and 229.5 mμ).[17a] As suggested by Dekker,[4] protonation of **8** occurred on the exocyclic imino group to give a resonant cation (structure A in ref 11). The same resonant cation is obtained from 4-amino-2-methoxypyrimidine which protonates at N-1.[24] Protonation of the 4-imino group of 3-methylcytidine has also been observed.[25] These data would suggest that protonation of the exocyclic imino group of pyrimidines may be a general rule.

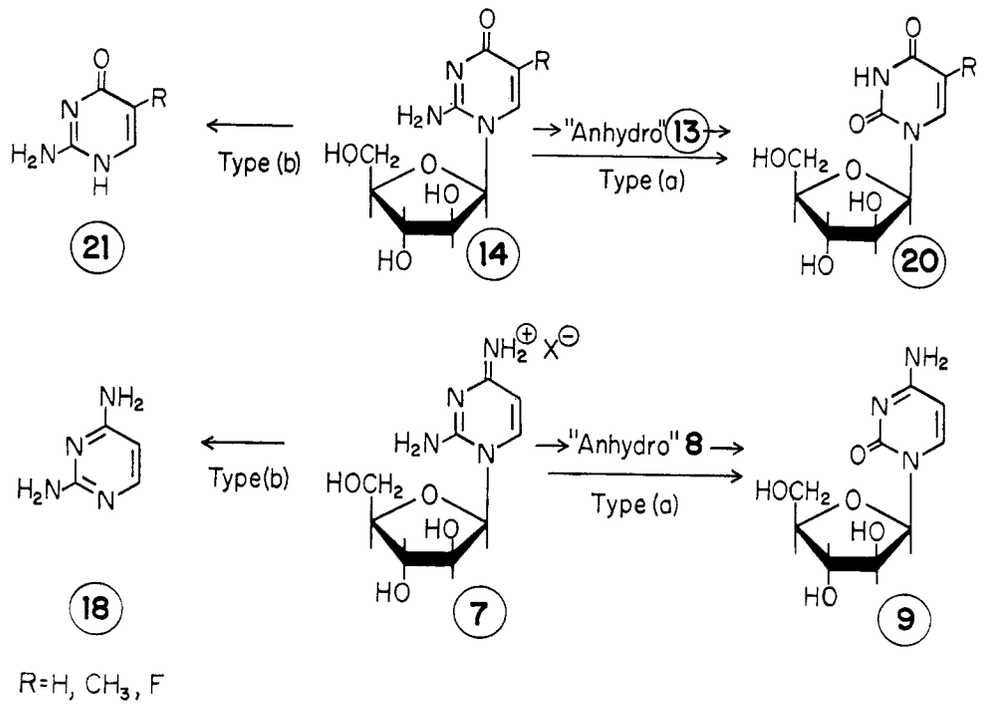
Hydrolytic Experiments on 2-Amino Nucleosides.—As shown in Scheme IV and Table II, 2-aminopyrimidine nucleosides containing an arabinofuranosyl moiety (**7** and **14**) can undergo two main types of reactions: type a, the removal of the 2-amino function; type b, cleavage of the glycosyl bond.

Naito, *et al.*,[26] reported that 1-β-D-arabinofuranosylisocytosine **14** (R = H), 1-β-D-lyxofuranosylisocytosine, and 1-(3′-deoxy-3′-ethylthio-β-D-arabinofuranosyl)isocytosine in acetic acid at 80° for several minutes were cyclized to their corresponding 2,2′-anhydro nucleosides with the concurrent loss of ammonia. We have found that the 5-methylisocytosine and 5-fluoroisocytosine derivatives **14** (R = CH_3 and F) as well as **14** (R = H) were cyclized to 2,2′-anhydro derivatives **13** quantitatively in dilute acetic acid at 60–70°. The mechanism for the removal of the 2-amino group (Scheme IV, type *a*) involves the intramolecular attack of the up 2′-hydroxyl function on C-2. The hydrochloride of the aminoimino nucleoside **7** was stable in 2 *N* acetic acid at room temperature for 24 hr. However, **7** was unstable in hot 2 *N* acetic acid in which it was converted partially (~66%) to equal quantities of 2,4-diaminopyrimidine (**18**) and 2,2′-anhydroarabinosylcytosine (**8**). In this reaction both type a (deamination) and type b (glycosyl cleavage) reactions occurred. In stronger acid, **7** was hydrolyzed quantitatively to 2,4-diaminopyrimidine (**18**). Similar acid lability of the glycosylic bond in arabinosylisocytosines **14** (R = H, CH_3, or F) with the formation of isocytosines **21** (R = H, CH_3, or F) was observed (Scheme IV, type b). In contrast, 1-β-D-arabinofuranosylcytosine (**9**) was stable under these conditions. The acid instability of 2-amino nucleosides **7** and **14** is expected from the unpublished work of Brown[27] who found that, in acid, the glycosylic bond was less stable in isocytidine than in cytidine. A mechanism for the acid hydrolysis of isocytidine nucleosides has been suggested by Dekker.[27]

The hydrolysis of 2-amino or -imino function in a 1-substituted pyrimidine would be expected to occur in alkaline solutions. 1-Methylisocytosine[18] and 4-dimethylamino-1,2-dihydro-2-imino-1-methylpyrimidine[28] are hydrolyzed to the corresponding 2-oxo compounds in 1 *N* sodium hydroxide. We have found that the ultraviolet spectrum of 2′,3′-O-isopropylidene-

(23) A. Albert, R. Goldacre, and J. Phillips, *J. Chem. Soc.*, 2240 (1948).
(24) I. Wempen and J. J. Fox, *J. Am. Chem. Soc.*, **86**, 2474 (1964).
(25) T. Ueda and J. J. Fox, *ibid.*, **85**, 4024 (1963).
(26) T. Naito, M. Hirata, Y. Nakai, T. Kobayashi, and M. Kanao, *Chem. Pharm. Bull.* (Tokyo), **13**, 1258 (1965).
(27) C. A. Dekker, *Ann. Rev. Biochem.*, **29**, 435 (1960).
(28) D. J. Brown and J. S. Harper, *J. Chem. Soc.*, 1276 (1963).

SCHEME IV



isocytidine (12) in 1 *N* sodium hydroxide is converted (at room temperature, 5 days) to a spectrum similar to that of 2′,3′-O-isopropylideneuridine.

Chromatographic and ultraviolet examination of the alkaline hydrolysates of arabinosylisocytosines **14** (R = H or CH_3) showed the corresponding 2-oxo nucleosides **20** (R = H or CH_3) to be the only detectable ultraviolet absorbing components.[29] In 1 *N* sodium hydroxide, **7** was deaminated mainly to 1-β-D-arabinosylcytosine. A minor product of this reaction was 1-β-D-arabinosyluracil (**20**).[30] The deamination of arabinosyl derivatives **14** (R = H or CH_3) and **7** in alkali to **20** and **9** probably occurs either by direct hydrolysis of the 2-amino function or by attack of the 2′- (up) hydroxyl anion on C-2. The 5-fluoroisocytosine derivative **14** (R = F) reacts in 1 *N* sodium hydroxide rapidly and abnormally compared to **14** (R = H or CH_3) to form a 2′,6-anhydro derivative as described above (see Scheme II).

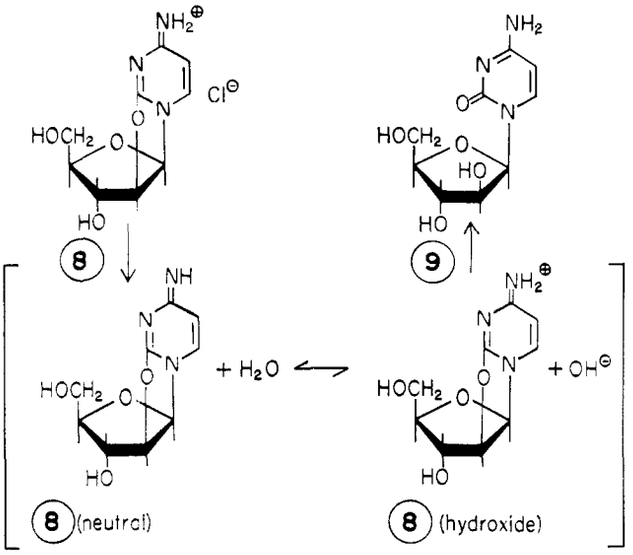
Hydrolytic Experiments on 2′-Halogeno-2′-deoxycytidine.—The formation of 2,2′-anhydro nucleosides from 2′-deoxy-2′-halogenocytidines occurs at a faster rate than from 2′-deoxy-2′-halogenouridines. Halogeno nucleosides **6** (X = F), **1** (X = F), and **1** (X = Cl) were refluxed in water at equal concentrations (0.012 *N*). The liberated hydrogen fluoride or chloride was neutralized periodically with 0.05 *N* sodium carbonate. The pH of the reaction was maintained between 5 and 6 by the use of indicators. The cytosine derivative **6** (X = F) reacted about 29 and 1.3 times faster than the uracil derivatives **1** (X = F) and **1** (X = Cl), respectively. After 92% of the hydrogen fluoride had been liberated (3 hr), the hydrolysate of **6** (X = F) contained one product, arabinofuranosylcytosine **9**. Under the neutralization conditions employed in this reaction, the initial product **8** was readily hydrolyzed to arabinofuranosylcytosine **9**. If an aqueous solution of **6** (X = F) was refluxed for 3.5 hr *without* neutralization, the products were the 2,2′-anhydro nucleoside **8** and arabinosylcytosine **9** in equal quantities. [The ease of hydrolysis of 2,2′-anhydroarabinosylcytosine (neutral form of **8**) is discussed in the next section.] After 20% of the hydrogen fluoride had been liberated (8.4 hr), the neutralized hydrolysate of the uracil derivative **1** (X = F) contained two products, 2,2′-anhydroarabinofuranosyluracil (**13**) and a trace amount of arabinofuranosyluracil **20**. After 92% of the hydrogen chloride had been liberated (4 hr), the neutralized hydrolysate of the uracil derivative **1** (X = Cl) contained 2,2′-anhydro nucleoside **13** (R = H) and a trace amount of **20**. It is interesting to note that the cyclization of 2′-*chloro*-2′-deoxyuridine and 2′-*fluoro*-2′-deoxycytidine proceeded at comparable rates. Therefore, in this latter case the increased nucleophilicity of the cytosinyl compared to the uracilyl moiety somewhat compensates for the decreased reactivity of the 2′-fluoro compared to the 2′-chloro group. As expected 2′-chloro-2′-deoxycytidine was cyclized quantitatively in 0.5 hr to 2,2′-anhydro nucleoside **8** at a faster rate than the 2′-fluoro analog.

The increased nucleophilicity of the cytosinyl *vs.* the uracilyl moiety was also observed under nonaqueous conditions. The cytosine derivative **6** (X = Cl) was converted quantitatively to the **8** hydrochloride by simply boiling it in dioxane for 20 min. The corresponding 2′-fluoro analog **6** (X = F) was cyclized only partially (41%) to the **8** hydrofluoride in boiling dioxane (20 hr). On the other hand, 2′-chloro- and 2′-fluoro-2′-deoxyuridine are stable to the boiling dioxane treatment and are in fact formed from the chloride or fluoride salts of 2,2′-anhydro nucleoside **13** (R = H) under these conditions.[9] The ease of the formation of anhydropentofuranosylcytosines compared with that

(29) The deamination of **14** (R = H or CH_3) with 1 *N* sodium hydroxide was accompanied by an over-all loss of ultraviolet absorbing material of about 50 and 40%, respectively.

(30) 1-β-D-Arabinosyluracil (**20**) probably arose from **9**. The slow hydrolysis of **9** to **20** (R = H) in aqueous alkali has been observed by us.

SCHEME V



of uracil analogs has been observed by other workers.[31,32]

Some Properties of 2,2′-Anhydroarabinofuranosylcytosine.—Although the hydrochloride of 2,2′-anhydro nucleoside **8** was first prepared by Walwick, *et al.*,[4] the properties of this compound have not yet been adequately described.[33] The anhydro linkage of **8**, though stable in water or acid, was unstable in solutions of increased hydroxyl ion concentration.

Whereas 2,2′-anhydroarabinosyluracil (**13**, neutral form) could be isolated easily, 2,2′-anhydroarabinosylcytosine (**8**, neutral form, Scheme V) could not be isolated. Treatment of a solution of the hydrochloride of **8** with 1 equiv of sodium hydroxide rapidly converted the nucleoside to arabinosylcytosine **9**. Our inability to isolate the free 2,2′-anhydroarabinosylcytosine suggests that this compound is a relatively strong base which in water, is autohydrolyzed to arabinosylcytosine.

The increased basicity of 2,2′-anhydroarabinosylcytosine compared to that of 2,2′-anhydroarabinosyluracil may be shown by comparing the pH values of solutions of their respective hydrochlorides before and after partial neutralization with aqueous 1 *N* sodium hydroxide. A 0.05 *N* aqueous solution of the hydrochloride of **8** gave a pH of 4.7 whereas similar solution of the hydrochloride of **13** gave a pH of 1.6. To the 0.05 *N* solution of the **8** hydrochloride (pH 4.7) was added 0.6 equiv of aqueous 1 *N* sodium hydroxide. The resulting solution showed an initial increase in pH to 10.6 which dropped to pH 7.2 in 30 min. As determined by chromatographic and ultraviolet examination, the final solution (pH 6.8) contained only arabinosylcytosine and starting material in amounts of 59 and 41%, respectively. The initial pH of 10.6 and the rapid fall in pH lends support to the base–conjugate acid equilibrium [**8** (neutral) ⟷ **8** (hydroxide)] shown in Scheme V. A plausible explanation for the ease of hydrolysis of 2,2′-anhydroarabinosylcytosine is that the hydroxide ion present in the **8** (hydroxide) ⟷ **8** (neutral) equilibrium attacks at C-2 of the aglycon to form **9**. Such an attack by hydroxide ion would cause a simultaneous decrease in hydroxide concentration. Consistent with this mechanism, a rapid fall in pH (10.6 → 7.2) was observed.

A neutralization experiment similar to that just described was carried out on an aqueous solution of the hydrochloride of 2,2′-anhydroarabinosyluracil (**13**).[9] To an 0.05 *N* solution of the **13** hydrochloride salt (pH 1.6) was added 0.6 equiv of 1 *N* sodium hydroxide. The resulting solution *immediately* gave a pH of 1.9 which did not change with time. The low pH of the unneutralized and partially neutralized solutions of the conjugate acid of **13** indicates that the latter is highly dissociated, as would be expected for the hydrochloride of a very weak base. Chromatographic and ultraviolet examination of the final solution showed that no hydrolysis of the 2,2′-anhydro linkage of **13** had occurred.

In view of our data on the extreme instability of 2,2′-anhydroarabinosylcytosine (**8**, neutral) in water due to the equilibrium shown in Scheme V, it is surprising that Mizuno and Sasaki[34] report the isolation of 2,3′-anhydroxylofuranosylcytosine and its 2′,5′-di-O-trityl derivative in their neutral (unprotonated) forms.

Experimental Section[35]

Paper Chromatographic Determinations.—A 5:1:1 (acetone–chloroform–water) system was used on Schleicher and Schuell paper No. 597 (ascending technique).

Electrophoretic Experiments.—Paper electrophoretic studies were made on an E. C. electrophoresis apparatus (E. C. Apparatus Co., Swarthmore, Pa.) using Whatman No. 3MM paper. Acetate buffer (pH 3.7–3.8) was prepared by combining 250 ml of 2 *N* acetic acid with 34 ml of 2 *N* sodium acetate and dilution to 1 l.

Spectrophotometric Studies.—Ultraviolet absorption data were determined with a Cary recording spectrophotometer, Model 15, using buffers and techniques previously described.[17] Again, 1 *N* NaOH was taken as essentially equal to pH 14, 0.1 *N* NaOH as pH 13, and 0.01 *N* NaOH as pH 12.

1-(Di-O-acetyl-2-chloro-2-deoxy-β-D-ribofuranosyl)uracil (2).—Acetic anhydride (4.4 g, 0.43 mole) was added to a stirred solution of 2′-chloro-2′-deoxyuridine (5.1 g, 0.19 mole) in 50 ml of anhydrous pyridine. The reaction mixture was allowed to stand overnight at room temperature. Ethanol (3 ml) was added to the reaction mixture and the pyridine was evaporated *in vacuo*. Volatile materials were removed by repeated distillation with ethanol–water mixtures (1:1) and then with absolute ethanol. A white solid (6.2 g, 92%) was obtained which on crystallization from ethanol gave colorless cubes, mp 127–130°, $[\alpha]^{25}D$ +6° (*c* 1.5, ethanol).

Anal. Calcd for $C_{13}H_{15}ClN_2O_7$: C, 45.03; H, 4.36; Cl, 10.22; N, 8.08. Found: C, 45.19; H, 4.41; Cl, 10.15; N, 8.16.

1-(Di-O-acetyl-2-chloro-2-deoxy-β-ribofuranosyl)-4-thiouracil (3).—To a stirred pyridine solution (140 ml) containing acetylated nucleoside **2** (X = Cl) (7.1 g, 0.02 mole) was added phosphorus pentasulfide (9.8 g). Within the first 0.5 hr of refluxing, 0.3 ml of water was added. The reaction mixture was refluxed for 3.5 hr.

On cooling the reaction, a yellow hygroscopic solid precipitated from which the product-containing pyridine solution was decanted. The solid was extracted two times with pyridine. The pyridine solutions were evaporated *in vacuo* and the amber syrup was treated with water. After evolution of hydrogen sulfide had subsided, the yellowish solid was filtered and dissolved

(31) V. M. Clark, A. R. Todd, and J. Zussman, *J. Chem. Soc.*, 2952 (1951).

(32) H. P. M. Fromageot and C. B. Reese, *Tetrahedron Letters*, 3499 (1966).

(33) Even though the **8** hydrochloride was stable for 1 month in a refrigerated aqueous solution at 13°, boiling the solution for ~2.3 hr resulted in some hydrolysis to arabinosylcytosine (**9**, 34%) and arabinosyluracil (**20**, 11%). Refluxing of the **8** hydrochloride in 1 *N* sulfuric acid for 2 hr partially hydrolyzed it to **9** (~23%) and **20** (~8%). In a phosphate buffer (pH 7.3) at room temperature, the hydrochloride was hydrolyzed partially (~20%) to **9** in 20 hr.

(34) Y. Mizuno and T. Sasaki, *Tetrahedron Letters*, 4579 (1965).

(35) All melting points were taken on a Thomas–Hoover capillary melting point apparatus and are corrected. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich.

in chloroform. The chloroform solution was dried and the solvent was removed *in vacuo*. An amber glass was obtained which, upon trituration with ethanol, gave a yellow crystalline solid, 6.9 g (95%), mp 125–127°. Recrystallization from ethanol gave yellow needles, mp 127–133°, $[\alpha]^{26}D$ +38° (*c* 0.8 acetone). Ultraviolet absorption properties in 50% ethanol were λ_{max} 327 mμ and 247 mμ, λ_{min} 274 mμ and 221 mμ, ratio 327:247 mμ of 4.2.

Anal. Calcd for $C_{13}H_{15}ClN_2O_6S$: C, 43.04; H, 4.17; Cl, 9.77; N, 7.72; S, 8.84. Found: C, 43.12; H, 4.22; Cl, 9.79; N, 7.64; S, 8.70.

1-(2-Chloro-2-deoxy-β-D-ribofuranosyl)-4-thiouracil (4).—Acetylated nucleoside **3** (X = Cl) (12.1 g, 0.03 mole) was mixed with *ca.* 60 ml of liquid ammonia in a glass-lined steel bomb at room temperature for 18 hr. The bomb was cooled well before opening. The liquid ammonia was driven off by a stream of dry nitrogen. Evaporation *in vacuo* of the residual ammonia gave a yellow glass. Water (100 ml) was added and the mixture was neutralized to ~pH 6 with acetic acid. Yellow crystals, 7.7 g (83%), mp 98–100°, precipitated. Recrystallization from water gave yellow prisms, mp 101–103°, $[\alpha]^{26}D$ +38° (*c* 0.3, ethanol).

Anal. Calcd for $C_9H_{11}ClN_2O_4S$: C, 38.78; H, 3.98; Cl, 12.72; N, 10.05; S, 11.50. Found: C, 38.51; H, 4.43; Cl, 12.71; N, 9.87; S, 11.61.

2,2′-Anhydro-1-(β-D-arabinofuranosyl)-4-thiouracil (15).—To a hot solution of the mercaptonucleoside **4** (X = Cl) (1.0 g, 3.6 mmoles) in 30 ml of 1-butanol was added 0.52 ml (3.8 mmoles) of triethylamine. The solution was boiled for 1 hr during which time the 2,2′-anhydro nucleoside precipitated as yellow rods. The reaction mixture was cooled and the product was filtered and washed with ethanol, then ether (0.66 g, 76%). Recrystallization from 95% methanol gave yellow rods, sintering at 226–250°, 251° dec with effervescence, $[\alpha]^{28}D$ +12° (*c* 0.7 water). Ultraviolet absorption properties are reported in Table I.

Anal. Calcd for $C_9H_{10}N_2O_4S$: C, 44.63; H, 4.16; N, 11.75; S, 13.24. Found: C, 44.79; H, 4.26; N, 11.49; S, 13.26.

1-(2-Chloro-2-deoxy-β-D-ribofuranosyl)-4-methylthio-2-pyrimidinone (5).—To a solution of mercaptonucleoside 4 (X = Cl) (6.2 g, 22.3 mmoles) in 50% ethanol (500 ml) was added methyl iodide (6.3 g) followed by 1 *N* sodium hydroxide (22.3 ml). The ultraviolet absorption properties of the resulting solution in 50% ethanol were as follows: maximum at 301 mμ, shoulder at 270 mμ, and minimum at 246 mμ. The solution was neutralized to pH 6 with acetic acid and the ethanol was evaporated *in vacuo*. White needles, 5.4 g (85%), mp 156–160°, precipitated. Recrystallization from 25% ethanol gave colorless needles, mp 159–162° with effervescence, $[\alpha]^{28}D$ +120° (*c* 0.4 ethanol). The ultraviolet absorption properties in water were as follows: maximum at 300, shoulder at 270, and minimum at 238 mμ.

Anal. Calcd for $C_{10}H_{13}ClN_2O_4S$: C, 41.03; H, 4.48; Cl, 12.11; N, 9.57; S, 10.95. Found: C, 40.94; H, 4.50; Cl, 12.20; N, 9.48; S, 11.13.

2,2′-Anhydro-1-(β-D-arabinofuranosyl)cytosine and 1-β-D-Arabinofuranosyl-2-amino-1,4(2H)-4-iminopyrimidine (8 and 7).—Methylthio nucleoside **5** (X = Cl) (5.3 g, 0.018 mole) was allowed to react with liquid ammonia (75 ml) in a steel bomb for 1 week at room temperature as described in the preparation of **4** (X = Cl). The liquid ammonia was evaporated. The residual ammonia was removed by repeated distillation *in vacuo* at 25° with ethanol–water (1:1), then with ethanol. A white glass was obtained. Paper chromatography (system 5:1:1) showed the presence of two ultraviolet-absorbing components; the hydrochloride of aminoimino nucleoside **7** (55%, R_f 0.23) and 2′-chlorocytidine (**6**) (45%, R_f 0.62).[36] The nucleoside **6** (X = Cl) was cyclized to the hydrochloride **8** by boiling the glass in dioxane for 20–30 min. The nucleoside **6** (X = Cl) was isolated in a subsequent run (*vide infra*). The hot dioxane treatment gave an orange gummy solid (~5.1 g) which contained the hydrochlorides of **8** and **7**. Trituration of the solid with ethanol dissolved the **7** hydrochloride and the **8** hydrochloride (2.7 g, 57%) was obtained in crystalline form. The ethanol filtrate A was saved for isolation of the picrate salt of **7**. The **8** hydrochloride was recrystallized by dissolving in methanol (250 ml), treating with activated charcoal (Norit), and filtering. Methanol (1 ml) containing anhydrous hydrogen chloride was added to the filtrate. On the addition of ether to the filtrate, crystalline needles precipitated, 2.2 g, sintering at 200–250°, 260° dec. Elemental analysis, ultraviolet spectral properties, and the optical rotation agreed with those previously reported for the **8** hydrochloride.[4]

The ethanol filtrate (A) which contained the **7** hydrochloride and a small amount of the **8** hydrochloride was concentrated to dryness and treated with liquid ammonia for 1 day. After the removal of the ammonia, a glass was obtained which was dissolved in 25% ethanol and treated batchwise with Dowex 1 (acetate). A solution of the **7** acetate was brought to ~pH 2 by the addition of a saturated aqueous solution of picric acid. An inorganic picrate (0.1 g) precipitated and was filtered and discarded. The filtrate was evaporated to about 25 ml *in vacuo*. Slow precipitation of the **7** picrate, 2.1 g (25%), mp 165–200° dec, occurred.[37] Recrystallization from 25% ethanol gave yellow rosettes, mp 165–200° dec (ammonia evolved). The optical rotation and infrared data were identical with those of an analytical sample of the **7** picrate (*vide infra*).

2′-Chloro-2′-deoxycytidine (6).—Methylthionucleoside **5** (X = Cl) (0.44 g, 1.7 mmoles) was treated with liquid ammonia for 7 days at room temperature in the manner described in the preparation of **4** (X = Cl). After removal of the ammonia, a white glass was obtained which was a mixture of **6** (X = Cl) and the **7** hydrochloride, 52 and 48%, respectively. The glass was dissolved in ethanol (6 ml) and picric acid (0.24 g) in ethanol (5 ml) was added. The picrate precipitated as yellow needles, 0.15 g (18%), sintering at 165–200°, 230–235° dec. To a methanolic solution of the picrate (37 mg) was added ether until the solution became turbid. White crystals of **6** (X = Cl) (free base) precipitated, 17 mg, sintering at 190–220°, 230–235° dec, $[\alpha]^{25}D$ +33° (*c*, 0.2 g, water). Paper chromatography (system 5:1:1) of compounds **6** (X = Cl), **6** (X = F), and the **8** hydrochloride gave R_f values of 0.65, 0.54, and 0.27, respectively. Paper electrophoresis (acetate buffer, 850–900 v, 20–40 ma, 1 hr) of **6** (X = Cl), **6** (X = F), and the **8** hydrochloride gave cathodic migrations of +3.8, +3.8, and +9.0 cm, respectively. The ultraviolet absorption properties are reported in Table I.

Anal. Calcd for $C_9H_{12}ClO_4N_3 \cdot 2H_2O$: C, 36.32; H, 5.42; N, 14.10. Found: C, 36.18; H, 4.49; N, 14.12.

1-(Di-O-acetyl-2-deoxy-2-fluoro-β-D-ribofuranosyl)uracil (2).—The isolation of the 2′-fluoro nucleoside by acetylation is an improvement of the procedure previously published.[9] Anhydronucleoside **13** (R = H) (1 g, 4.4 mmoles) and anhydrous dioxane (130 ml) were placed in a steel container. Cautiously, liuuid HF (10–12 ml) was added. The container was placed in a bomb which was heated for 18 hr at 107–110°. This temperature is lower than that previously reported (115–120°). Less decomposition occurred at the 110° range. After the work-up of the reaction mixture as previously described,[9] a crude syrup was obtained. The syrup was dried by azeotropic distillation with benzene and then by dessication under high vacuum over phosphorus pentoxide.

The syrup of three 1-g runs (13.8 mmoles) was combined and dissolved in dry pyridine (60 ml). To the solution (0–5°) was added acetic anhydride (5 g). After 18 hr at room temperature, ethanol (2 ml) was added. The reaction mixture was worked up in a manner similar to that used for the preparation of **3** (X = Cl) (*vide supra*). Crystallization of the residue from ethanol gave a white solid, 2.5 g (55%), mp 168–181°. Recrystallization from ethanol (125 ml) gave 2.2 g of colorless needles, mp 173–174°, $[\alpha]^{26}D$ +33° (*c* 0.5, acetone).

Anal. Calcd for $C_{13}H_{15}FO_7N_2$: C, 47.28; H, 4.58; F, 5.75; N, 8.48. Found: C, 47.29; H, 4.57; F, 5.80; N, 8.54.

Deacetylation of **2** (X = F) with liquid ammonia in the manner described for the preparation of **4** (X = Cl) gave a residue. Crystallization of this residue from ethanol afforded white platlets of **1** (X = F, R = H), mp 148–150°, in 80% yield.

1-(Di-O-acetyl-2-deoxy-2-fluoro-β-D-ribofuranosyl)-4-thiouracil (3).—To a stirred solution of acetylated nucleoside **2** (X = F) (1.3 g, 3.9 mmoles) in pyridine (40 ml) was added phosphorus pentasulfide (1.8 g, 7.6 mmoles) and water (0.1 ml). The reaction mixture was refluxed for 3.5 hr. On cooling the orange solution, a yellow hydroscopic solid precipitated. The reaction mixture was worked-up in a manner similar to that used for the preparation of **3** (X = Cl). Crystallization of the crude product from ethanol gave yellow needles, 1 g (74%), mp 108–110°. A sample was recrystallized from ethanol for analysis, mp 109–113°, $[\alpha]^{26}D$ +75° (*c*, 0.5 acetone). Ultraviolet absorption

(36) The relative amounts of products were determined by elution and ultraviolet spectroscopy.

(37) In a subsequent run the yield of the **7** picrate was increased to 35%.

properties in 50% ethanol were λ_{max} 325 mμ and 245 mμ, λ_{min} 274 mμ and 221 mμ, ratio 325:247 mμ of 4.0.

Anal. Calcd for $C_{13}H_{15}N_2SFO_6$: C, 45.09; H, 4.37; F, 5.49. N, 8.09; S, 9.25. Found: C, 45.07; H, 4.61; F, 5.45; N, 8.04; S, 9.17.

1-(2-Deoxy-2-fluoro-β-D-ribofuranosyl)-4-thiouracil (4).—Acetylated thio nucleoside **3** (X = F) (0.9 g, 2.6 mmoles) was treated with liquid ammonia (40 ml) overnight at room temperature. The same procedure as that described for the isolation of **4** (X = Cl) was used. Yellow needles, 0.55 g (81%), mp 159–160° (prior shrinking), $[\alpha]^{26}D$ +105° (*c*, 0.3, ethanol), were obtained from water.

Anal. Calcd for $C_9H_{11}FN_2O_4S \cdot 0.5H_2O$: F, 7.00; N, 10.33. Found: F, 6.19; N, 10.05.

1-(2-Deoxy-2-fluoro-β-D-ribofuranosyl)-4-methylthio-2-pyrimidinone (5).—The thio nucleoside **4** (X = F) (0.055 g, 2.1 mmoles) was dissolved in 35 ml of 50% ethanol. Methyl iodide (4.2 mmoles) and 1 *N* sodium hydroxide (2.1 ml) was added to the solution. The same procedure as that described for the isolation of **5** (X = Cl) was used and gave 0.6 g (96%) of a white solid. Recrystallization from ethanol gave colorless prisms, mp 170–171° with effervescence, $[\alpha]^{26}D$ +156° (*c*, 0.3, ethanol).

Anal. Calcd for $C_{10}H_{13}FN_2O_4S$: C, 43.47; H, 4.75; F, 6.88; N, 10.14; S, 11.60. Found: C, 43.40; H, 4.95; F, 6.80; N, 10.09; S, 11.43.

1-(2-Deoxy-2-fluoro-β-D-ribofuranosyl)cytosine (6).—The methylthionucleoside **5** (X = F) (1.0 g, 3.6 mmoles) was treated with liquid ammonia (20 ml) in a steel bomb at 60–65° for 2 days. After removal of the ammonia, water was added to the residue. Tan needles, 0.57 g (65%), precipitated. (The mother liquor contains aminoimino nucleoside **7**.) Recrystallization from water gave colorless needles, mp 171–173° (prior shrinking), $[\alpha]^{25}D$ +87° (*c* 0.1 water). Ultraviolet absorption properties are reported in Table I.

Anal. Calcd for $C_9H_{12}FN_3O_4$: C, 44.08; H, 4.93; F, 7.75; N, 17.14. Found: C, 43.65; H, 5.15; F, 7.73; N, 16.91.

2,2′-Anhydro-1-(β-D-arabinofuranosyl)cytosine (8) Acetate.[38]—The **8** hydrochloride (0.1 g) was dissolved in 5 ml of water, and the solution was passed through a small Dowex 1 (acetate) column. The ultraviolet-absorbing effluent was evaporated, and the white residue crystallized from methanol. After the addition of ether to the filtrate, cream-colored needles precipitated, 94 mg (91%), sintering at 165–185°, 190–192° dec.

Anal. Calcd for $C_{11}H_{15}N_3O_6$: N, 14.73. Found: N, 14.98.

8 Picrate.—The **8** acetate (0.1 g) in water was treated with aqueous picric acid. A yellow picrate, 0.15 g, mp 215–217°, precipitated. Recrystallization from 25% ethanol gave shiny yellow needles, mp 218–221°.

Anal. Calcd for $C_{15}H_{14}N_6O_{11}$: C, 39.66; H, 3.11; N, 18.50. Found: C, 39.32; H, 2.99; N, 18.35.

1-(β-D-Arabinofuranosyl)-2-amino-1,4(2H)-4-iminopyrimidine (7) Picrate.—The **8** picrate (0.11 g) was left at room temperature in liquid ammonia (10 ml) overnight. Evaporation of the ammonia *in vacuo* gave an orange glass. The glass was dissolved in 25% ethanol, and the residual ammonia was neutralized to pH 5 with acetic acid. The picrate (yellow rosettes), 90 mg, partially melted at 160–165°, 205–210° dec (ammonia evolved), $[\alpha]^{25}D$ +58° (*c*, 1.0, 50% ethanol), precipitated. Paper electrophoresis (acetate buffer, 900 v, 20 ma, 1 hr) of the picrates of 2,2′-anhydro nucleoside **8** and 2-amino-4-imino nucleoside **7** gave cathodic migrations of +8.7 and +7.1 cm, respectively. The picric acid anion in both nucleosides had an anodic migration of +6.7 cm.

Anal. Calcd for $C_{15}H_{17}N_7O_{11}$: C, 38.22; H, 3.64; N, 20.80. Found: C, 38.36; H, 3.84; N, 20.46.

1-(β-D-Arabinofuranosyl)-2-amino-1,4(2H)-4-iminopyrimidine (7) Hydrochloride.—The **8** hydrochloride (53 mg) was left at room temperature in liquid ammonia (10 ml) overnight in a steel bomb. Upon removal of all ammonia, a white sticky, hygroscopic glass, $[\alpha]^{25}D$ +91° (*c*, 1.9, 50% ethanol) was obtained. All attempts to crystallize the **7** hydrochloride failed. Paper electrophoresis (acetate buffer, 900 v, 20 ma, 1.5 hr) gave only one ultraviolet-absorbing spot with a cathodic migration of +11.6 cm. The anhydro nucleoside **8** and arabinosylcytosine **9** gave migrations of +14.1 and 8.1 cm, respectively. Paper chromatography (system 5:1:1) gave the same R_f value (0.26) for the hydrochlorides of **8** and **7**. The ultraviolet absorption properties are reported in Table I.

Even when the above glass (the **7** hydrochloride) was kept in a dessicator (over phosphorus pentoxide) under high vacuum it is slowly converted to salt of **8**. An aqueous solution of the above glass (0.036 *N*) gave an initial pH of 6.6 which changed to 7.2 within 8 min. This result suggests the cyclization of the **7** hydrochloride to the **8** with the concurrent loss of ammonia. Electrophoretic (acetate buffer) results substantiate this conclusion (see Table III). A refrigerated aqueous solution of the **7** hydrochloride showed less cyclization to the **8** than a sample kept at room temperature. The **7** hydrochloride showed less cyclization to the **8** in water than in phosphate buffer (pH 7.3). An aqueous solution of the sulfate of **7** (preparation below) was more stable than that of the hydrochloride. Under the hydrolytic conditions shown in Table III, the **8** hydrochloride was more stable than the **7** hydrochloride.[33]

TABLE III

Hydrolytic conditions	Salt of 7	Time	% products
Water, 25°	Hydrochloride	21 hr	8 (6%), 9 (6%)
Water, 13°	Hydrochloride	19 hr	Trace amount of 8
Water, 25°	Hydrochloride	1 week	8 (22%), 9 (47%)
Water, 13°	Hydrochloride	1 week	8 (13%), 9 (9%)
Phosphate buffer (pH 7.3)	Hydrochloride	22 hr	8 (40%), 9 (40%)
Water, 13°	Sulfate	1 week	No dec of 7

7 Sulfate.—The **7** picrate (0.3 g) was dissolved in 25% ethanol. The solution was passed through a short Dowex 1 (sulfate) column; the effluent containing the picrate was evaporated to dryness *in vacuo*. A white amorphous solid (0.17 g, partially melted at 90–150°, effervesced at 155°) precipitated on the addition of ethanol. Paper electrophoresis (acetate buffer) of the product showed only one ultraviolet-absorbing spot which had the same migration as other salts of **7**. An aqueous solution of the sulfate of **7** (0.04 *N*) gave a pH of 6.8.

1-β-D-Arabinofuranosylisocytosine (14).[14c]—The reaction of 2,2′-anhydro nucleoside **13** (R = H) (1.8 g, 4.8 mmoles) with liquid ammonia (30 ml) in a glass-lined bomb at room temperature for 17 hr gave a colorless glass. The addition of ethanol to the residue gave crystals, 1 g (86%), mp 200–204° with effervescence (lit.[14c] mp 235–236°). *Extreme care must be taken in the recrystallization of nucleosides* **14** *because of their easy conversion to* **2,2′-anhydro nucleosides 13**. Nucleoside **14** (R = H) was purified by dissolving it in 90% methanol with a minimum of heating and filtering. The methanol was evaporated *in vacuo* until white needles, [0.7 g, mp 202–205° with effervescence (ammonia evolved), $[\alpha]^{24}D$ +91° (*c*, 0.34 g, 50% ethanol)] precipitated. Paper chromatography (system 5:1:1) of the isocytosine nucleoside **14** (R = H) showed one ultraviolet absorbing spot with a R_f 0.17 [nucleoside **13** (R = H), R_f 0.44]. The ultraviolet absorption (Table I) and infrared data of **14** (R = H) agreed with that previously reported.[14c]

1-β-D-Arabinofuranosyl-5-methylisocytosine (14).—Nucleoside **13** (R = CH_3) (80 mg, 0.33 mmole) was allowed to react with liquid ammonia (5 ml) for 20 hr at room temperature. A white solid was obtained which was crystallized by dissolving in methanol (20 ml), filtering, and adding ether to incipient cloudiness. White needles, 73 mg (85%), mp 195–199° with effervescence (ammonia evolved), $[\alpha]^{26}D$ +76° (*c* 0.3 g, 50% ethanol), precipitated. Paper chromatography (system 5:1:1) show one spot with a R_f 0.21 [nucleoside **13** (R = CH_3), R_f 0.50]. The ultraviolet absorption properties are found in Table I.

Anal. Calcd for $C_{10}H_{15}N_3O_5$: C, 46.69; H, 5.88; N, 16.34. Found: C, 46.64; H, 5.86; N, 16.29.

1-β-D-Arabinofuranosyl-5-fluoroisocytosine (14).—Nucleoside **13** (R = F) (0.1 g, 0.41 mmole) was allowed to react with liquid ammonia (5 ml) for 2 days at room temperature. A white solid was obtained, 90 mg (84%), mp 195–200° (prior shrinking) with effervescence. Recrystallization from ethanol (5 ml), which contained 1 drop of water, gave colorless plates, mp 198–203° (prior shrinking) with effervescence (ammonia evolved), $[\alpha]^{25}D$ +55° (*c*, 0.1 water). Paper chromatography (system 5:1:1) of the product showed one spot at R_f 0.33 [nucleoside **13** (R = F), R_f 0.64]. The ultraviolet absorption properties are found in Table I.

(38) The **8** acetate was converted to the following salts; **8** HBr, needles from methanol, mp 263–265° dec with effervescence; **8** HNO_3, plates from ethanol, mp 200–203° with effervescence; **8** $HClO_4$, cubes from ethanol, mp 164–165°.

Anal. Calcd for $C_9H_{12}FN_3O_5$: C, 41.38; H, 4.63; F, 7.27; N, 10.09. Found: C, 41.53; H, 4.69; F, 7.24; N, 16.08.

1-β-D-Arabinofuranosyl-4-thiocytosine (16).—Nucleoside **15** (0.3 g, 1.2 mmoles) was allowed to react with liquid ammonia (15 ml) for 2 days at room temperature. After removal of the ammonia, a yellow glass was obtained which on trituration with methanol solidified, 0.22 g, mp 175–180° dec with prior darkening. The product was purified by dissolving in 95% methanol and filtering. The methanol was evaporated *in vacuo* until precipitation occurred. Short yellow needles, mp 180–185° dec with effervescence (ammonia evolved), $[\alpha]^{25}D$ +94° (*c*, 0.27, 50% ethanol), were obtained. Paper chromatography (system 5:1:1) of nucleoside **16** showed one spot at R_f 0.49 (nucleoside **15**, R_f 0.72). The ultraviolet properties are found in Table I.

Anal. Calcd for $C_9H_{13}N_3O_4S$: C, 41.70; H, 5.06; N, 16.20; S, 12.80. Found: C, 41.69; H, 5.10; N, 16.08; S, 12.44.

2',3'-O-Isopropylideneisocytidine (12).[14a]—A few milliliters of liquid ammonia were allowed to react with 2',3'-O-isopropylidene-2,5'-anhydrouridine **10**[39] (8 mg) for 18 hr at room temperature. Cubic crystals, mp 203–205° (lit. mp 206–207°), crystallized from ethanol. The ultraviolet absorption properties were the same as that reported.[14a] Paper chromatography (system 5:1:1) of **12** showed one spot at R_f 0.81 (nucleoside **10**, R_f 0.90]. Paper electrophoresis (acetate buffer, 900 v, 40 ma, 1 hr) of **12** showed one ultraviolet-absorbing spot with a cathodic migration of +3.3 cm (nucleoside **10**, +2.2 cm).

Hyrolysis of 1-(2-Halogeno-2-deoxy-β-D-ribofuranosyl)cytosine and -uracil.—The cytosine derivative **6** (X = F) and uracil derivative **1** (X = F or Cl) were refluxed in water (6 ml at equal concentrations (0.012 *N*) with methyl red and bromothymol blue as internal indicators. The pH was kept between 5 and 6, and the liberated acid was measured by the periodic addition of 0.05 *N* sodium carbonate (theoretical uptake 1.34 ml). The color of the solutions of **1** (X = F or Cl, R = H) and **6** (X = F) were maintained at yellow to pale green.

A plot of ln (per cent 2'-halogeno nucleoside remaining) *vs.* time gave a good linear relationship. No attempt was made to determine the actual order of the reactions. The pseudo-first-order rate constants were 4.65×10^{-4}, 1.04×10^{-3}, and 1.35×10^{-3} min^{-1} for **1** (X = F, R = H), **1** (X = Cl, R = H), and **6** (X = F), respectively. The products from the reaction of the uracil derivative **1** (X = F or Cl) were 2,2'-anhydro nucleoside **13** (R = H) and 1-β-D-arabinosyluracil (**20**, trace amount) as determined by paper chromatography (system 5:1:1). The product from the cytosine derivative **6** (X = F) was found to be 1-β-D-arabinosylcytosine (**9**) by electrophoresis (acetate buffer).

Registry No.—**1** (X = F, R = H), 784-71-4; **2** (X = Cl), 10190-39-3; **2** (X = F), 10212-13-2; **3** (X = Cl), 10190-40-6; **3** (X = F), 10212-14-3; **4** (X = Cl), 10212-15-4; **4** (X = F), 10212-16-5; **5** (X = Cl), 10212-17-6; **5** (X = F), 10212-18-7; **6** (X = Cl), 10212-19-8; **6** (X = F), 10212-20-1; **6** (X = H), 7321-01-9; **7**, 10212-22-3; **7** picrate, 10212-23-4; **7** hydrochloride, 10212-24-5; **8** hydrochloride, 10212-25-6; **8** hydrobromide, 10212-26-7; **8** HNO_3, 10212-27-8; **8** $HClO_4$, 10239-69-7; **8** acetate, 10212-28-9; **8** picrate, 10190-41-7; **12**, 5975-05-3; **14** (R = H), 10212-30-3; **14** (R = CH_3), 10212-31-4; **14** (R = F), 10212-32-5; **15**, 10190-42-8; **16**, 10212-33-6; **17a**, 1073-37-6; **17a'**, 10212-35-8; **18**, 156-81-0; 5-methylisocylosine picrate, 10212-37-0.

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Specifically Deuterated, Acetylated Derivatives of 2-Amino-2-deoxy-D-glucose. Nuclear Magnetic Resonance Studies on Migration of Acetyl Groups[1-5]

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Each of the acetyl group signals in the nmr spectrum of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose (**1**) was identified definitively by synthesis of derivatives **2**, **3**, and **4** that are specifically deuterated in individual acetyl groups. The migration of the *N*-acetyl group during conversion of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranosyl chloride (**7**) into 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose hydrochloride (**9**) was studied by nmr and an effective preparative conversion of **7** into **9** is described. Nmr studies on **9**, its anomer (**6**), and some related derivatives, are described.

In the preceding paper[2] in this series, the signal of the acetamido methyl group in the nmr spectra of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose (**1**) and its β-D anomer (**5**) was assigned definitively by synthesis of analogs that were deuterated specifically in the acetamido methyl group. In both instances, for spectra measured in chloroform-*d*, the methyl group signal at highest field (τ 8.09) was that of the acetamido methyl group. The present report describes further specific deuteration experiments that permit definitive assignment of each of the acetyl group signals in the nmr spectrum of **1** in chloroform-*d* and benzene.

The 60-Mcps nmr spectra of anomers **1** and **5**, measured in chloroform-*d*,[7,8] show discrete signals for three of the five acetyl groups (Figure 1); the signals of two of the acetyl groups are not resolved. The anomers show the same pattern of signals, at essentially the same chemical shifts,[9] except that the α-D anomer (**1**) shows the lowest field, acetyl group signal at τ 7.81, and the corresponding signal in the β-D anomer (**5**) is observed at τ 7.89. Since **1** and **5** differ only by the fact

(1) Part IV in the series "Anomeric Equilibria in Derivatives of Amino Sugars." For part III see ref 2.

(2) Previous paper in this series: D. Horton, J. B. Hughes, J. S. Jewell, Kerstin D. Philips, and W. N. Turner, *J. Org. Chem.*, **32**, 1073 (1967).

(3) A preliminary report of part of this work has been given: D. Horton, 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965, Abstracts, p 5D.

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(6) National Science Foundation Undergraduate Research Participant, 1965–1966.

(7) D. Horton, *J. Org. Chem.*, **29**, 1776 (1964).

(8) F. W. Lichtenthaler and H. P. Albrecht, *Ber.*, **99**, 575 (1966).

(9) The chemical shifts vary to a small extent (~±0.02 ppm) according to the concentration of the sample, for a range of concentrations from 1% to that of a saturated solution, but the relative shifts of the acetyl group signals remain essentially constant. Similar shifts with concentration have been noted with other acetylated sugars: F. W. Lichtenthaler, personal communication.