free base was dissolved in methyl iodide (2 ml) and methanol (1 ml) and refluxed for 0.5 hr. Dilution of the reaction mixture with ethyl ether after cooling induced crystallization. The product was recrystallized from a methanol–ethyl ether mixture to yield compound **20** as needles: yield, 29.12 mg (65%); mp 234–236° dec. Three additional recrystallizations gave a pure product having constant mp 238–238.5° dec; $[\alpha]^{29}D$ +50.7° (*c* 0.523 in methanol).

Anal. Calcd for $C_{10}H_{22}INO_4$: C, 34.59; H, 6.40; N, 4.03. Found: C, 34.86; H, 6.69; N, 4.10.

Methyl 2,3-Di-*O*-acetyl-4,6-dideoxy-4-(*N*,*N*-dimethylamino)-α-D-talopyranoside (21).—Methyl 4,6-dideoxy-4-(*N*,*N*-dimethylamino)-2,3-*O*-isopropylidene-α-D-talopyranoside hydrochloride (**12**, 200 mg) was hydrolyzed as described in the preparation of compound **19** to obtain the free base of compound **19** as a gum, 121 mg (88%). The gum was dissolved in acetic anhydride (1 ml) and pyridine (1 ml) and allowed to stand at room temperature for 3 days. Removal of the solvents *in vacuo* with azeotroping (toluene) yielded 170 mg of crude solid. Recrystallization from hot *n*-hexane gave 110 mg (54% for two steps): mp 84–86°; $[\alpha]^{29}D$ +107° (*c* 6.60 in methanol); pK_a 5.78; nmr δ 1.44 (d, 3, $J_{5,6}$ = 7 Hz, C-6-CH_3), 2.0 [s, 3, equitorial[23] C-2-OC(O)CH_3], 2.13 [s, 3, axial[23] C-3-OC(O)CH_3], 2.2 [s, 6, -N(CH_3)$_2$], 2.44 (q, 1, $J_{3,4}$ = 3 Hz, $J_{4,5}$ = 5.5 Hz, C-4-*H*), 3.44 (s, 3, C-1-OCH_3), 4.35 (octet, 1, $J_{5,6}$ = 7 Hz, $J_{5,4}$ = 5.5 Hz, C-5-*H*), 4.73 (d, 1, $J_{1,2}$ = 0 Hz, $J_{2,3}$ = 1.2 Hz, C-2-*H*), 4.79 (s, 1, $J_{1,2}$ = 0 Hz, C-1-*H*), and 5.66 ppm (unresolved q, 1, C-3-*H*).

Anal. Calcd for $C_{13}H_{23}NO_6$: C, 53.97; H, 8.01; N, 4.84. Found: C, 54.15; H, 8.26; N, 4.73.

The hydrochloride salt of compound **21**, compound **22**, had mp 209–210° dec.

4,6-Dideoxy-4-(*N*,*N*-dimethylamino)-β-D-talopyranose Hydrochloride (23), and 4,6-Dideoxy-4-(*N*,*N*-dimethylamino)-α-D-talopyranose Hydrochloride (24).—Methyl 4,6-dideoxy-4-(*N*,*N*-dimethylamino)-2,3-*O*-isopropylidene-α-D-talopyranoside (compound **12**, 257.1 mg) was heated at 95° in 1.0 *N* hydrochloric acid (3 ml) for 20 hr. The reaction mixture was treated with charcoal, cooled, and lyophilized to yield a foam. The foam was azeotroped twice with an ethanol–toluene mixture. Crystallization of the reaction mixture was accomplished by dissolving it in hot methanol (*ca.* 5 ml), cooling, and adding ethyl ether to incipient turbidity. As crystals deposited over a period of several days, more ethyl ether was added. The yield was 170 mg (96%) of a mixture of anomers **23** and **24**, as evidenced by a mp 140–175° dec. A predominance of the β anomer **23** was obtained by slow recrystallization from a dilute methanol–ethyl ether mixture seeded with the β anomer. After two recrystallizations the yield was 90 mg (51%): mp 154–156° (slight turbidity in melt, cleared at *ca.* 170°) (one more recrystallization lowered the melting point to 152–154°); $[\alpha]^{29}D$ *ca.* 7 → 21° (0.5 hr) (*c* 0.5 in water); pK_a 8.22.

Anal. Calcd for $C_8H_{18}ClNO_4$: C, 42.20; H, 7.97; N, 6.15. Found: C, 42.46; H, 7.98; N, 6.42.

The α anomer **24** was obtained by extracting the crude mixture of α and β anomers, mp 140–175° dec, with several small volumes of hot ethanol. The residue was compound **24**: mp 180–182° dec, with slight softening at 155°; $[\alpha]^{24}D$ 30.8 → 19.0° (0.75 hr) → 19.5° (22 hr); pK_a 7.60.

Anal. Calcd for $C_8H_{18}ClNO_4$: C, 42.20; H, 7.97; N, 6.15. Found: C, 41.97; H, 7.84; N, 6.07.

Registry No.—**2**, 15830-63-4; **4**, 15830-64-5; **5**, 15830-76-9; **6**, 15830-65-6; **7**, 15830-66-7; **8**, 15830-67-8; **9**, 15856-43-6; **11**, 15889-54-0; **12**, 15830-68-9; **14**, 15856-44-7; **15**, 15856-45-8; **16**, 15830-69-0; **17**, 15856-46-9; **18**, 15856-47-0; **19**, 15830-70-3; **20**, 15830-71-4; **21**, 15830-72-5; **22**, 15830-73-6; **23**, 15830-74-7; **24**, 15830-75-8.

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Nucleosides. L. Synthesis of 2,3′-Imino-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine and Related Derivatives[1]

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Reaction of 5′-deoxy-5′-iodo-3′-*O*-mesylthymidine (**3**) with silver acetate in methanol afforded the 2,5′-anhydro derivative of 3′-*O*-mesylthymidine (**4**) in good yield which, by treatment with liquid ammonia, gave 2,3′-imino-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine (**6a**). Compound **6a** was also prepared from the 2-*O*-methyl derivative **8**. Reaction of the 2,5′-anhydro nucleoside **4** with methylamine, hydroxylamine, and hydrazine yielded the corresponding cyclic *N*-methyl, *N*-hydroxy, and *N*-amino derivatives **6b–d**. In the above reactions of **4** or **8** with amines the 2,3′-imino derivatives **6** formed *via* the isocytosine intermediate **5**. The reactions and ultraviolet, pK_a, and pmr data of the 2,3′-imino derivatives **6** are reported and discussed.

Arabinosylcytosine,[3] arabinosyl-5-fluorouracil,[4] and arabinosyl-5-fluorocytosine[5] have demonstrated interesting biochemical and chemotherapeutic activity.[6] In the synthesis of these biologically active compounds, 2,2′-anhydro-1-(β-D-arabinofuranosyl) uracil,[7] and 5-fluorouracil[4] and -cytosine[3,8] (**1a** and **b**, Figure 1) have been important intermediates. In order to obtain pyrimidine nucleosides of modified biological activity, the synthesis of the nitrogen isostere (**6**, Figure 2) of 2,3′-anhydro-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine[9] (**2**, Figure 1) was undertaken. The chemistry of **2** and its derivatives have been studied extensively in this and other laboratories.[9–11] Our recent chemical studies[8] on 2-aminopyrimidine nucleosides suggested that a 2,2′- or 2,3′-imino nucleoside may conceivably act as a chemical precursor for the synthesis of nucleosides containing an amino group in the "up" configuration in the sugar moiety.

(1) (a) 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). (b) A preliminary report of this work has appeared; see J. L. Doerr and J. J. Fox, *J. Amer. Chem. Soc.*, **89**, 1760 (1967).

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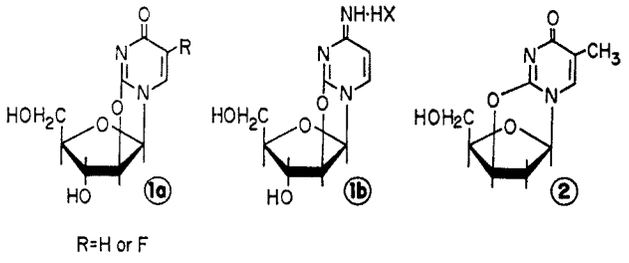

Figure 1.

We have shown[8] that treatment of the known[12] 2′,3′-*O*-isopropylidene-2,5′-anhydrouridine with liquid ammonia at room temperature for 18 hr converted it *directly* into 2′,3′-*O*-isopropylideneisocytidine. It was envisioned that, if a 2,5′-anhydro nucleoside contained a leaving group in the "down" configuration in the sugar moiety, reaction of such a compound with ammonia should lead first to an isocytidine derivative. This derivative should then undergo an intramolecular displacement reaction by the 2-amino group of the aglycon resulting in the formation of a nitrogen bridge analog of an anhydro nucleoside.

As a model compound, the 2,5′-anhydro derivative of 3′-*O*-mesylthymidine (**4**, Figure 2) was prepared in good yield by reaction of the 5′-iodo nucleoside (**3**)[9] with silver acetate in methanol. Proof that **4** is a 2,5′-anhydro nucleoside is shown by the dissimilarity of its melting point, optical rotation, ultraviolet spectral properties, and pmr data (Table I) from the known[9,10] 2,3′-anhydro isomer **7**.[13] Treatment of **4** with liquid ammonia for 5 days at room temperature yielded a crystalline product whose elemental analysis agreed with the 2,3′-imino structure (**6a**). Proof of structure **6a** rests on the following data. The ultraviolet absorption spectral patterns of **6a** under neutral and acidic conditions resemble that of 1-β-D-arabinofuranosyl-5-methylisocytosine (**9**) (Table II). The pmr spectrum of **6a** in DMSO-d_6 (Table I) shows a broad singlet (1 H) at δ 9.62 (>NH) and a broad triplet (1 H) at 5.14 (–OH); both were exchanged by the addition of D_2O. As expected the H_3' signal in **6a** is considerably upfield when compared with the H_3' signal of 2,3′-anhydro-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine (**2**) (Table I).

The methylimino derivative **6b** was prepared in almost quantitative yield by reaction of **4** with methylamine for 5 days at room temperature. The ultraviolet absorption characteristics of **6b** were similar to those for **6a** under acid or neutral conditions. No dissociation of **6b** was observed spectrally in strong alkali. In contrast, the imino derivative **6a**, which has one dissociable proton associated with the pyrimidine, dissociates in strong alkali. As discussed below the pmr data also supports the methylimino bridge in **6b**.

Treatment of the 2,5′-anhydro nucleoside (**4**) with methanolic hydroxylamine or with anhydrous hydrazine gave the *N*-hydroxy and *N*-amino derivatives (**6c** and **6d**, R = OH and NH_2, respectively) in high yields. It is clear that, in the conversion of **4** → **6** by amines, the isocytidine derivatives (**5**) were intermediates.

An alternate route to the synthesis of **6a** was achieved. The 2-methoxy derivative **8** was prepared by treatment of anhydro nucleoside **4** with hot methanol containing triethylamine. Reaction of **8** in liquid ammonia for several days afforded **6a** in high yield.

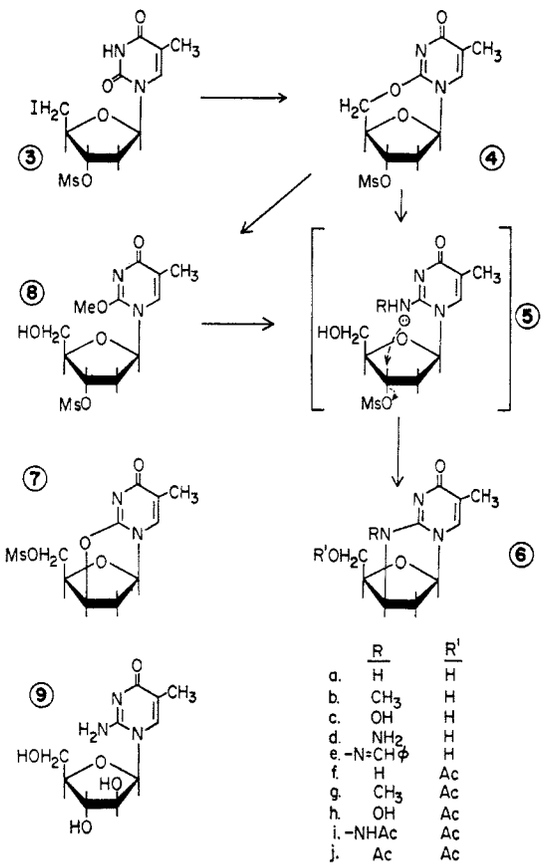

Figure 2.

Treatment of the hydrazino derivative (**6d**) with nitrous acid converted it into **6a**. Reaction of **6d** with benzaldehyde in ethanol containing hydrochloric acid produced the benzalamino derivative **6e** (R = C_6H_5-CH=N—).

Treatment of **6a** with excess acetic anhydride in pyridine at 60–70° for several hours gave an unstable diacetyl derivative **6j** (not isolated)[14] which hydrolyzed slowly to the 5′-*O*-acetate (**6f**). In similar manner, the hydroxylamino derivative **6c** also gave an unstable diacetate which was converted into **6h**. The methyl analog **6b** formed the monoacetate **6g** directly, whereas the hydrazino derivative **6d** gave a stable diacetate **6i**. All the isolated acetate derivatives (**6f–i**) were extremely soluble in water.

A comparison of the acetylation reactions of **6a–d** with the "uncyclized" 1-β-D-arabinofuranosylisocytosine (**10**)[15] (Figure 3) is of interest. Acetylation of **10** afforded a stable crystalline tetraacetate (**11**) which was hydrolyzed in dilute acid (3 hr) at room temperature to the known triacetate (**12**)[16] of 1-β-D-arabinofuranosyluracil. This behavior of **11** is to be contrasted with that of the diacetate of **6a** which, when treated with acid, yields the monoacetate **6f** without cleavage of the

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(13) It should be noted that the conversion of **3** → **4** offers independent confirmation of the structure of **3**. Compound **3** had been prepared[9] by heating 3′,5′-di-*O*-mesylthymidine with sodium iodide in dry acetone.

(14) The actual position of the *N*-acetyl group in structure **6j** (Figure 2) is not known. For convenience the acetyl group is drawn on the bridge nitrogen.

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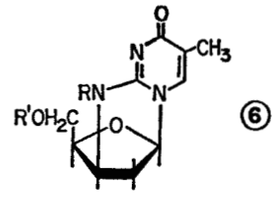
TABLE I

PROTON MAGNETIC RESONANCE DATA



⑥

Compd			Chemical shifts (δ)								
No.	R	R′	H_6	C_5–CH_3	H_1'	$H_2'(H_2')$	H_3'	H_4'	$H_5'(H_5')$	Acetyl	Miscellaneous	Solvent
	H	H	7.43	1.74	5.70	2.34	←~4.13→		3.60	...	(C_5')OH 5.14; >N—H 9.62	DMSO-d_6
6a	H	H	7.55	2.06	5.98	2.73	←~4.66→		4.25	...	...	TFA
	H	H	7.52	1.89	5.88	2.54	←~4.41→		3.90 ($J_{4',5'} \sim 6.0$)	...	...	D_2O
6b	CH_3	H	7.60	2.10	5.98	2.78	←~4.70→		4.25 ($J_{4',5'} \sim 5.0$)	...	>N—CH_3 3.57	TFA
6c	OH	H	7.57	2.09	5.94	2.92	←~4.75→		4.35 ($J_{4',5'} \sim 4.0$)	...	...	TFA
6d	NH_2	H	7.52	1.77	5.77	2.39	←~4.27→		~3.67	...	(C_5')OH 4.92; >N—NH_2 5.01	DMSO-d_6
6f	H	Ac	7.35	1.93	5.81	2.52	←~4.48→			2.08	>N—H 10.48	$CDCl_3$
6g	CH_3	Ac	7.56	1.97	5.95	2.58	←~4.39→			2.09	>N—CH_3 3.33	$CDCl_3$
6h	OH	Ac	7.37	1.95	5.80	2.72	4.89	←~4.54→		2.08	>N—OH 11.98	$CDCl_3$
6i	NHAc	Ac	7.38	1.95	5.76	~2.49 (~2.99)	←~4.47→			2.06	>N—Ac 2.12; >N—H 11.70	$CDCl_3$
1-(2′-Deoxy-2,3′-anhydro-β-D-*threo*-pentofuranosyl)-thymine (2)			7.70	1.80	5.96	~2.56	5.37	4.32	3.59	...	(C_5')OH 5.10	DMSO-d_6
1-(2′-Deoxy-2,5′-anhydro-3′-*O*-methanesulfonyl-β-D-*threo*-pentofuranosyl)thymine (4)			7.78	1.83	6.16	2.72	5.55	4.81	4.68 (4.14)	...	—O—Mes 3.34	DMSO-d_6
1-(2′-Deoxy-2,3′-anhydro-5′-*O*-methanesulfonyl-β-D-*threo*-pentofuranosyl)thymine (7)			7.66	1.79	5.97	2.60	5.41	←~4.44→		...	—O—Mes 3.23	DMSO-d_6
1-β-D-Arabinofuranosylisocytosine (10)			7.57	H_5 = 5.50	5.82	4.18	←~3.80→			...	>N—H 6.78; —OH (three protons), broad peak 4.89–5.98	DMSO-d_6
1-β-D-Arabinofuranosylisocytosine tetraacetate (11)			7.94	H_5 = 6.03	6.61	5.58	5.23	←~4.41→		2.00; 2.13 (nine protons)	>N—H 12.83	DMSO-d_6

TABLE II

SPECTROPHOTOMETRIC[a] AND pK_a[b] DATA

	pH[c]	λ_{max}, mμ	ε	λ_{min}, mμ	ε	pK_a
2,3′-Methylimino- (**6b**)	0	244, 270	9,450, 8,030	220, 260	6,150, 7,760	2.64
	6.9	228, 268–270sh	20,500, 3,360			
2,3′-Imino- (**6a**)	0	240, 266	7,440, 8,100	218, 250	4,850, 7,050	3.33
	7.7 and 12	213, 257–269sh	22,700, 4,000			
	13	267–269sh	3,810			
	7 *N* KOH[d]	237	19,800			
2,3′-Hydroxyimino- (**6c**)	0	235, 264–265sh	8,980, 8,640	225	4,460	3.26[e]
	6.8	228, *265*	20,240, 5,390			
	14	241, 269	17,200, 11,090	269	10,970	
2,3′-Aminoimino- (**6d**)	0	245, 267	8,760, 8,290	221, 257.5	5,560, 8,106	3.60
	6.9	227, *267*	20,340, 4,160			
Arabinofuranosyl-5-methyl-isocytosine (**9**)[f]	0	225, 260	9,130, 8,300	240	6,430	3.80 ± 0.1
	Water	204, 260, *214*, *228*	20,600, 6,740	246	5,910	
	13	262, *225*	7,050	247	5,900	
	5.6 *N* KOH	272–276	4,020	264	3,910	

[a] Italicized numbers refer to inflections; the term sh after the wavelength value refers to a shoulder. [b] The apparent pK_a values in this table are for basic dissociations and were determined spectrophotometrically with an accuracy of ±0.05 pH units unless otherwise indicated. The acidic dissociation observed in the high alkaline region in compounds **6a** and **9** were not determined. [c] The spectrum of the above compounds at pH 0 and 7 are those of pure cationic and neutral species, respectively. The spectrum of **6c** at pH 14 is that of pure anionic species. The spectrum of **6a** in 7 *N* KOH is mainly that of the anionic species. The spectrum of arabinosyl-5-methylisocytosine (**9**) in 5.6 *N* KOH is that of a mixture of neutral and anionic species. On the addition of concentrated hydrochloric acid to the 7 *N* and 5.6 *N* KOH solutions of **6a** and **9**, respectively, the acid spectra of these compounds were reconstituted. [d] The ε value for **6a** in 7 *N* NaOH is 1300 at 290 mμ (no maximum). Compare with the values for **9** (footnote *f*). [e] A second pK_a = 9.34 was also determined. [f] In 7 *N* KOH **9** showed maxima at 291 and 231 mμ (ratio 231:291 mμ of 16.0), minimum at 267 mμ as previously reported.[8]

2,3′-imino bridge.[17] Also, compound **6j** is converted into **6a** in alkali without cleavage of the nitrogen bridge. Treatment of **11** with aqueous 1 *N* alkali at room temperature overnight gave arabinosyluracil (**13**) directly. The conversion of **10** into **13** in 1 *N* alkali had been noted previously.[8] As suggested by the pmr spectrum of the tetraacetate of **11** in $CDCl_3$ (see discussion below), structure **11** (Figure 3) is represented in the acetimido rather than in the acetamido tautomeric form.

A preliminary comparison of the properties of the 2,3′-imino-bridged nucleosides (**6a** and **6b**) with the "oxygen isostere" **2** is also of interest. All of the 2,3′-imino nucleosides were stable under acid and basic conditions which would readily cause the 2,3′-anhydro nucleoside (**2**) to react. For instance, **6a** and **6b** were stable in strong aqueous alkali (7 *N* KOH) for 3 weeks at room temperature. By contrast, anhydro nucleoside **2** is easily cleaved in 0.1 *N* sodium hydroxide (24 hr) to 1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine.[9] These data shows that an "up" 3′-amino-3′-deoxy nucleoside cannot be obtained from the 2,3′-imino nucleosides **6a–d** under alkaline conditions. When the imino-bridged nucleoside (**6a**) was refluxed in 1 *N* hydrochloric acid for 1 hr, only a small amount of degradation occurred, though, after 2 days at reflux temperature, glycosyl cleavage was extensive. This slow degradation of **6a** is to be contrasted with the relatively rapid glycosyl cleavage of **2**, which occurs within 1 hr under similar reaction conditions.[9] When either **6a** or **2** was heated at 65° with liquid ammonia for 4 days, no reaction occurred and starting material was recovered.

The ionization constants and spectral data for the 2,3′-imino derivatives **6a–d** along with the related uncyclized derivative **9** are given in Table II. The ultraviolet spectra of the neutral and cationic species of compounds **6a–d** compared with those of derivative **9** show similarities suggesting the existence of similarly conjugated systems in all of these compounds. As expected, the neutral and cationic spectra of the imino compounds **6a–d** resemble each other more closely than they do the spectra of **9**. The similarity of the neutral and cationic spectra in the imino-bridged compounds **6a–d** suggest that all of these exist predominantly in the *p*-quinonoid form (2-amino-4-oxo) as the neutral species. One may conclude, further, that protonation of compounds **6a–d** occurs on the same site, probably on N_3.

As expected, the monoacetate derivatives of **6f–h** possessed ultraviolet spectral patterns similar with that of the unacetylated compounds (**6a–c**). On the other hand, the diacetate **6i** of the hydrazino derivative exhibited different spectra in water, acid, and base when it was compared with the unacetylated hydrazino derivative **6d**.

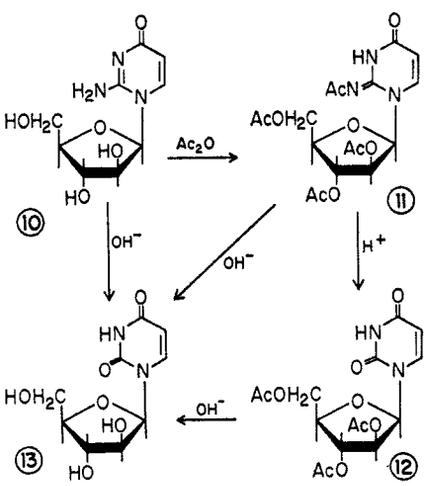


Figure 3.

(17) The behavior of acetate **11** in acid is to be further contrasted to the behavior of unacetylated **10**. Compound **10** (depending on concentration and type of acid) may give either 2,2′-anhydroarabinosyluracil or isocytosine as previously reported.[8]

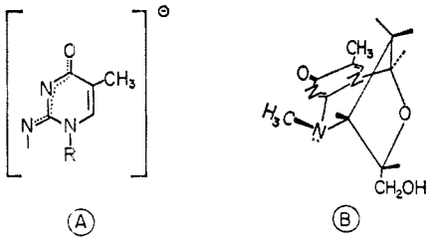


Figure 4.

When compared with the uncyclized 2-amino nucleoside **9**, the introduction of a 2,3′-imino bridge into a nucleoside (**6a–d**) produced an acid-strengthening effect (Table II). As might be expected, the smallest acid-strengthening effect (−0.2 pK units) was exhibited by the amino derivative **6d**. The "unsubstituted" imino derivative **6a** and the hydroxy derivative **6c** showed practically the same drop in pK_a (~0.4 pK units) compared with compound **9** (pK_a = 3.80). As seen in Table II the weakest base in the series is the N-methyl derivative **6b** (pK_a = 2.64) which exhibits a lower pK_a than its unmethylated analog **6a** (pK_a = 3.33). The lower basicity of the N-methyl derivative **6b** *vs.* **6a** may be attributed to a decrease of hydrogen bonding to water in the cation **6b** compared with that in the cation **6a**.

Mention should be made of the second pK_a observed spectrally under alkaline conditions in the 5-methylisocytosine nucleosides **6a**, **6c**, and **9**.[18] The second dissociation in these compounds is attributable to proton removal from the aglycon of the neutral species. The N-hydroxy derivative **6c** has a second pK_a at 9.34. The second pK_a (not determined) of compounds **6a** and **9** is found in the high alkaline range as evidenced by the striking ultraviolet spectral changes observed in alkali. As seen in Table II the ultraviolet spectrum of the 2,3′-imino derivative **6a** and that of the uncyclized 5-methylisocytosine nucleoside **9** in 0.1 N sodium hydroxide (pH 13) are very different from the spectra observed for these compounds in 7 N and 5.6 N potassium hydroxide, respectively. The similarity of the spectral changes in aqueous alkali exhibited by the 2,3′-imino nucleoside **6a** and the 5-methylisocytosine nucleoside **9** strongly suggest a common anion. A representation of this anion is shown in Figure 4A. It is noteworthy that the spectral curve of 1-methylcytosine (a 4-amino derivative) in 6 N sodium hydroxide is identical with that found for pH 7–14.[19] As expected, the spectrum of the N-methyl derivative **6b** (which has no dissociable proton on the aglycon) is the same in 0.1 N sodium hydroxide as in 7 N potassium hydroxide. Like **6b** the aglycon of the N-amino derivative **6d** did not exhibit a dissociation in the ultraviolet in strong alkali.

Proton Magnetic Resonance Data.—The pmr data for the nitrogen bridge analogs of anhydro nucleosides are listed in Table I and are consistent with the structures assigned. Compounds **6b** and **6c** were soluble with difficulty in all solvents but trifluoroacetic acid (TFA). The 5′-*O*-acetates (**6f–i**) were more soluble and their pmr spectra were determined in $CDCl_3$ solution. The nitrogen-bridged compounds gave poorly resolved spectra in all cases. The only signal which could be resolved was that of the C_5-methyl occurring at δ 1.74–2.09 which showed, in most cases, the characteristic J_{CH_3,H_6} ~1.0 Hz. The other signals were either broad singlets (half-band widths of 4.2–6.0 Hz) or broad multiplets. All peaks assigned to –NH or –OH were shown to disappear upon addition of D_2O to the solution.

The N-methyl compound **6b**, although sparingly soluble in DMSO-d_6, did at least show a sharp singlet at δ 3.14 characteristic of an N–CH_3 resonance. That the N-methyl peak in the spectrum of **6b**, and also of **6g**, was unsplit is added proof of the cyclic nature of the compounds **6**.

The pmr data for the nitrogen bridge compounds can be compared with those of 2,3′-anhydro compounds **2** and **7** and 2,5′-anhydro compound **4** (Table I). The $C_{5'}$ protons of **4** form a quartet, an AB subspectrum, δ 4.14 and 4.68 ($J_{4',5'}$ ~ 1.0 Hz, $J_{5',5'}$ ~ 12.5 Hz) which is characteristic of 2,5′-anhydro nucleosides.[20] On the other hand, the 2,3′-anhydro compounds and the nitrogen-bridged compounds (**6**) show the $C_{5'}$ protons, when discernable, as a doublet (pseudo-doublet), which is characteristic of 2,3′- and 2,2′-anhydro nucleosides.[20]

The $H_{3'}$ chemical shifts for the N-bridged compounds (δ ~4.13–4.75 for the average of the $H_{3'}$ and $H_{4'}$ chemical shifts) are found to higher field than the $H_{3'}$ chemical shifts for the anhydro compounds (δ 5.37–5.55) in accordance with the greater electronegativity of oxygen *vs.* nitrogen.

Compounds **6** may be viewed as derivatives of 2,4-diaza-6-oxabicyclo[3.2.1]octane (Figure 4B). In an attempt to determine the configuration of the N substituent in the bicyclo system pmr studies on compound **6g** were carried out in $CDCl_3$ at various temperatures. An extra peak at δ 11.72 was observed which integrated for ~0.6 protons and which disappeared upon addition of D_2O. No other extraneous peaks were found, however, and microanalytical and chromatographic data indicate that the impurity must be present in very small amount. The N-methyl peak at δ 3.33 remained a singlet at temperatures from 43 to −60°. Since the N-methyl peak did not split at lowered temperature, it indicates that the compound exists as a rapidly interconverting mixture of *exo* and *endo* N-methyl conformers owing to rapid nitrogen inversion in the bicyclic system. Very low energy barriers to nitrogen inversion have been found for saturated six-membered heterocycles.[21] A high percentage of conformers should exist at equilibrium with their methyl substituent in the *exo* orientation. The alternate conformer with an *endo* methyl group is disfavored owing to steric hindrance imposed by interaction with the bulky 4′-hydroxymethyl group of the sugar moiety (see Figure 4B).

An indication that the N-methyl substituent occupies predominantly the *exo* orientation is seen in the pmr data for **6i**. In all other compounds (**6**) in the series the bridge methylene protons ($H_{2'}$) have nearly identical chemical shifts. With **6i** the two $C_{2'}$ protons occur at δ 2.49 and 2.99. The proton at δ 2.49 is probably due to the $H_{2'}$ on the same side as the bulky >N–NHAc moiety and its high-field shift may be due to the aniso-

(18) Appreciable changes in the ultraviolet spectrum of 2,3′-*O*-isopropylideneisocytidine, 1-β-D-arabinofuranosylisocytosine, and the 5-methylisocytosine derivative **9** under various alkaline conditions were reported and dissociation of the isocytosine moiety was suggested.[8] The present study confirms this hypothesis.

(19) T. Ueda and J. J. Fox, *J. Amer. Chem. Soc.*, **85**, 4024 (1963).

(20) R. J. Cushley, unpublished results.

(21) A. T. Bottini and J. D. Roberts, *J. Amer. Chem. Soc.*, **80**, 5203 (1958).

tropic effect of the acetyl group. Diamagnetic effects due to neighboring acetyl groups have been reported recently.[22] The nonequivalence of the two $H_{2'}$ signals is not found in the other cases since the anisotropy is known to decrease rapidly with distance.[23]

The pmr data for 1-β-D-arabinofuranosylsocytosine (**10**) and its tetraacetate (**11**) are also given in Table I. Since the C_5-methyl group is no longer present, column 3 contains the chemical shift of H_5. An interesting effect is seen in the spectrum of **11** in dry DMSO-d_6. The H_5 signal at δ 6.03 has a half-band width of 3.2 Hz while the H_6 signal has a half-band width of 2.0 Hz. Thus there is a long-range coupling of H_5 to H_3 of about 0.2–0.4 Hz which vanishes when D_2O is added to the solution. Cushley, *et al.*, have reported a long-range coupling between H_5 and H_3 (J = 1–2 Hz) for pyrimidine nucleosides and 1-methyluracil when dry DMSO-d_6 is used as solvent.[24] That such a long-range coupling is observed in the spectrum of compound **11**, and the magnitude is smaller than observed previously,[24] shows that at least part of the compound exists in the imino form as depicted in Figure 3.

Experimental Section

General Procedure.—Pmr spectra were determined with a Varian A-60 spectrometer fitted with a V-6057 variable-temperature accessory. Chemical shifts (δ) are given in parts per million (ppm) from internal TMS (DSS for the D_2O solutions). Ultraviolet absorption data were determined with a Cary recording spectrophotometer, Model 15. The apparent pK_a values were determined spectrophotometrically using buffers and techniques previously employed.[25] Infrared data were obtained using a Perkin-Elmer Model 221 spectrophotometer. Paper chromatograms were determined on Schleicher and Schuell paper No. 597 (ascending technique) using system A, acetone–chloroform–water (5:1:1), or system B, butanol–water–ethanol (40:19:11). Melting points of all compounds except **6b** were taken on a Thomas–Hoover capillary melting point apparatus. The melting point of **6b** was taken on a Mel-Temp apparatus. All melting points are corrected. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. Pmr and ultraviolet absorption data are reported in Tables I and II, respectively.

2,5′-Anhydro-1-(2-deoxy-3-*O*-mesyl-β-D-*erythro*-pentofuranosyl)thymine (4).—The 5′-iodo nucleoside **3**[9] (25 g, 0.058 mol) was dissolved in 3 l. of methanol. Silver acetate (75 g) was added and the reaction mixture refluxed with stirring for 30 min. The hot reaction mixture was filtered using a filter aid of diatomaceous earth. Hydrogen sulfide gas was passed through the solution until all silver ions were removed. Norit was added to the silver sulfide suspension and the mixture was filtered using a Filter-aid pad. The colorless filtrate was aerated with nitrogen in order to drive off excess hydrogen sulfide. Precipitation of the anhydro nucleoside occurred. Filtration afforded 4.5 g, mp 180–183° dec.

Triethylamine (0.058 mol) was added to the filtrate which was then concentrated. An additional 7.3 g, mp 180–183° dec (total yield 67%), of **4** was obtained. A sample of **4** was recrystallized from 95% methanol. White, hair-like crystals appeared, mp 182–183° dec, $[\alpha]^{26}D$ +52° (c 0.4, DMF). The ultraviolet absorption properties in water were bands appearing at λ_{max} 248 mμ and λ_{min} 218 mμ.

Anal. Calcd for $C_{11}H_{14}N_2O_6S$: C, 43.70; H, 4.65; N, 9.27; S, 10.62. Found: C, 43.76; H, 4.70; N, 9.16; S, 10.70.

2-*O*-Methyl-3′-*O*-mesylthymidine (8).—To a suspension of 2,5′-anhydro nucleoside **4** (4 g, 0.013 mol) in methanol (1400 ml) was added triethylamine (80 ml). The mixture was refluxed for 3 hr during which time solution occurred. The reaction mixture was concentrated *in vacuo* to an amorphous white powder. The powder was crystallized from ethanol (~150 ml) to give white needles (3.9 g), mp 130–135°. A sample, on recrystallization, melted at 135–138°, $[\alpha]^{24}D$ +19° (c 0.6, DMF). The ultraviolet absorption properties in water were bands appearing at λ_{max} 254–255 and 227 mμ and λ_{min} 217 and 233 mμ. It was noted that the spectrum of **8** bears a striking resemblance to that of 2,2′-anhydro-1-β-D-arabinofuranosylthymine.[26]

Pmr spectrum in pyridine-d_5 consisted of H_6 (δ 8.10, doublet, J_{CH_3,H_6} = 1.1 Hz), OH(C-5′) (δ 7.13, broad peak), $H_{1'}$ (δ 6.48, triplet, $J_{1',2'}$ = 7 Hz), $H_{3'}$ (δ 5.40, mulitplet), $H_{4'}$ (δ 4.65, multiplet), $H_{5'},H_{5'}$ (δ 4.19, broad singlet), $-OCH_3$ (δ 3.19, singlet), $-OSO_2CH_3$ (δ 3.45, singlet), $H_{2'},H_{2'}$ (δ 2.82, quartet, $J_{2',3'}$ = 4 Hz), $C-CH_3$ (δ 1.93, doublet). Upon addition of D_2O the peak at δ 7.13 disappeared and the peak at 4.19 became a pseudodoublet, $J_{4',5'}$ = 3.1 Hz).

Anal. Calcd for $C_{12}H_{18}N_2O_7S$: C, 43.11; H, 5.43; N, 8.38; S, 9.59. Found: C, 43.08; H, 5.63; N, 8.26; S, 9.66.

2,3′-Imino-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine (6a). Method A.—The 2,5′-anhydro nucleoside **4** (0.8 g, 2.6 mmol) was allowed to react with liquid ammonia (40 ml) for 5 days at room temperature in a glass-lined steel bomb. Evaporation of the ammoniacal solution gave a white powder which was shown to contain only **6a** by chromatographic analysis. The product was dissolved in water and purified by absorption on a column of Dowex 50 (H^+, 100–200 mesh). The column was washed with water until the effluent was acid free, then eluted with 2 N NH_4OH. The ammonia eluates containing ultraviolet absorbing material were evaporated to a crystalline residue which was recrystallized from 90% ethanol. Compound **6a** crystallized as white prisms (0.5 g, 84%), mp 245° (sintering) and 265° dec (with effervescence), $[\alpha]^{26}D$ +23° (c 0.7, 0.1 N HCl).

Anal. Calcd for $C_{10}H_{13}N_3O_3$: C, 53.80; H, 5.87; N, 18.82. Found: C, 53.54; H, 5.87; N, 19.05.

Method B.—The 2-*O*-methyl nucleoside (**8**) (0.6 g, 1.8 mmol) was treated with liquid ammonia (40 ml) for 4 days at room temperature. Compound **6a** was isolated as described in method A. White prisms (0.29 g, 72%) were obtained, mp 250° (sintering) and 268° dec (with effervescence). The ultraviolet absorption spectra in acid and water, ir spectrum, and chromatographic properties of the product were identical with those of the compound obtained by method A.

2,3′-Methylimino-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine (6b).—The 2,5′-anhydro nucleoside **4** (1.2 g, 4 mmol) was allowed to react with liquid monomethylamine (40 ml) for 5 days at room temperature. The monomethylamine was evaporated and a crystalline residue was obtained which by chromatographic analysis (systems A and B) showed only one blue fluorescent spot. Upon addition of water (20 ml) to the residue crystallization occurred: 0.9 g (98%) of **6b**, mp 265° (sintering) and 295° dec (with effervescence). The product on recrystallization from water (80 ml) afforded white needles (0.6 g), mp 334° (sintering) and 345° dec (with effervescence), $[\alpha]^{26}D$ ~0° (c 0.7, 0.1 N HCl). On evaporation the mother liquor yielded an additional 0.16 g, mp 270° (sintering) and 278° dec (with effervescence). The ir spectra of the low and high melting recrystallized products were identical as were their chromatographic properties.

Anal. Calcd for $C_{11}H_{15}N_3O_3$: C, 55.68; H, 6.37; N, 17.71. Found: C, 55.60; H, 6.32; N, 17.82.

2,2′-Hydroxyimino-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine (6c).—A methanolic hydroxylamine solution was prepared by dissolving hydroxylamine hydrochloride (2.2 g, 32 mmol) in methanol (50 ml) containing phenolphthalein as indicator. Enough methanolic 1 N KOH (~40 ml) was added to the solution to produce a red color. To the precipitated KCl suspension a solution of hydroxylamine hydrochloride was added until a pH of about 7.4 was reached. The potassium chloride was removed by filtration.

The above filtrate was immediately added to a suspension of the 2,5′-anhydro nucleoside **4** (2 g, 6.6 mmol) in methanol (75 ml). Solution of **4** occurred upon reflux. After 2.5 hr of reflux, prisms of **6c** (1.1 g, 69%) crystallized from the hot solution and were removed by filtration. During the melting point determination, the compound darkened at 200° and decomposed with effervescence at 240°. Paper chromatography (system A)

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showed that the mother liquor contained **6c** (R_f 0.36) in addition to some starting material (**4**, R_f 0.90). Concentration of the mother liquor afforded additional **6c** (0.4 g), mp 190° (sintering) and 240° dec (with effervescence). Recrystallization of **6c** from water yielded prisms, mp 262° (sintering) and 274° dec (with effervescence), $[\alpha]^{25}D$ −17° (*c* 0.7, 0.1 *N* HCl).

6c gave an intense dark blue color with ferric chloride solution. Aqueous solutions of **6c** were slightly blue in color.

Anal. Calcd for $C_{10}H_{13}N_3O_4$: C, 50.20; H, 5.48; N, 17.57. Found: C, 50.28; N, 5.42; N, 17.70.

2,3′-Aminimino-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine (6d).—The 2,5′-anhydro nucleoside **4** (2 g, 6.6 mmol) was allowed to react with anhydrous hydrazine (~15 ml) for 1 hr at room temperature. During this time solution of **4** occurred. The reaction mixture was taken to dryness *in vacuo* and dissolved in water. This mixture was evaporated *in vacuo* to dryness. The residue, which by chromatographic analysis (systems A and B) contained only one ultraviolet absorbing spot (**6d**), was dissolved in water and the solution neutralized with 2 *N* acetic acid. The product was purified by a batchwise treatment with Dowex 50 (H^+, 100–200 mesh). The resin was washed free of acid and treated with 2 *N* NH_4OH. The resin was removed by filtration and the ammonium hydroxide filtrate (~200 ml) was evaporated to dryness. On trituration with ethanol a white solid (1.3 g, 77%) was obtained, mp 230° (sintering) and 260° dec (with effervescence). Crystallization from methanol (80 ml) afforded rodlike crystals (0.8 g), mp 273–275° dec (with effervescence), $[\alpha]^{25}D$ +7° (*c* 0.8, 0.1 *N* HCl). On further concentration of the mother liquor an additional 0.26 g, mp 273–276° dec, was obtained.

Anal. Calcd for $C_{10}H_{14}O_3N_4$: C, 50.41; H, 5.92; N, 23.52. Found: C, 50.46; H, 5.89; N, 23.36.

2,3′-Imino-1-(5-*O*-acetyl-2-deoxy-β-D-*threo*-pentofuranosyl)thymine (6f).—To the 2,3′-imino compound **6a** (0.2 g, 0.9 mmol) suspended in pyridine (15 ml) was added acetic anhydride (5.3 mmol). The reaction mixture was allowed to stand overnight at room temperature. Ethanol was then added to the solution and the pyridine was removed *in vacuo* by repeated distillation with water and then ethanol. A glass (containing residual acetic acid) was obtained. Paper chromatography (system A) showed the presence of two ultraviolet absorbing products: the diacetate **6j** (R_f 0.92)[27] and the 5′-*O*-acetate **6f** (R_f 0.73). The glass was dissolved in ethyl acetate and 170 mg of white crystals of **6f** (mp 240° dec with prior shrinking) slowly (~1 day) appeared. (The diacetate **6j** remained in the mother liquor and was slowly converted into the monoacetate **6f**.) Recrystallization of **6f** from ethyl acetate gave rodlike crystals (90 mg), mp 260–265°, $[\alpha]^{25}D$ +35° (*c* 0.4, water). This product exhibited essentially the same ultraviolet spectral data in water, acid, and base as **6a** (Table II). Paper chromatography of **6f** in systems A and B showed one ultraviolet absorbing spot with R_f 0.80 and 0.64, respectively (nucleoside **6a**, R_f 0.54 and 0.47).

Anal. Calcd for $C_{12}H_{15}N_3O_4$: C, 54.33; H, 5.70; N, 15.84. Found: C, 54.38; H, 5.66; N, 15.96.

2,3′-Methylimino-1-(5-*O*-acetyl-2-deoxy-β-D-*threo*-pentofuranosyl)thymine (6g).—To 2,3′-methylimino **6b** (0.15 g, 0.63 mmol) suspended in pyridine (35 ml) was added acetic anhydride (1.6 mmol). The mixture was heated at 75° for 5 hr during which time solution of **6b** slowly occurred. The reaction mixture was allowed to stand overnight at room temperature. The same procedure as that described for the isolation of **6f** was used. A white glass was obtained which was dissolved in ethyl acetate. A white amorphous solid, 0.1 g, mp 170–175°, precipitated slowly. The product was purified by dissolving in hot ethyl acetate. A white amorphous solid (60 mg), mp 175–176°, $[\alpha]^{26}D$ + 10.5° (*c* 0.3, water), precipitated. The product exhibited essentially the same ultraviolet spectral data in water, acid, and base as **6b**. Paper chromatography in systems A and B showed one fluorescent spot with R_f 0.85 and 0.70, respectively (nucleoside **6b**, R_f 0.59 and 0.62].

Deacetylation of **6g** occurred readily in alkali. On the addition of 1 *N* sodium hydroxide to the *O*-acetate **6g**, the nucleoside **6b** precipitated immediately. This product had the same melting point and ultraviolet and infrared data as an authentic sample of **6b**. Chromatographic analysis of the filtrate showed that **6b** was the only product.

Anal. Calcd for $C_{13}H_{17}N_3O_4$: C, 55.90; H, 6.14; N, 15.05. Found: C, 55.95; H, 6.06; N, 15.14.

2,3′-Acetylaminoimino-1-(5-*O*-acetyl-2-deoxy-β-D-*threo*-pentofuranosyl)thymine (6i).—To 2,3′-aminoimino **6d** (0.1 g, 0.42 mmol) suspended in pyridine (15 ml) was added acetic anhydride (1.1 mmol). The reaction mixture was stirred at room temperature for 18 hr. The same procedure used in the isolation of the monoacetate **6f** was followed. A white glass was obtained which was dissolved in ethyl acetate. White prisms (76 mg), mp 253–257° (prior darkening), crystallized. Recrystallization of **6i** gave 54 mg, mp 257–262° (prior darkening), $[\alpha]^{25}D$ −84° (*c* 0.4, water). The ultraviolet absorption spectrum in water showed a maximum at 223 mμ (ε 17,350) and an inflection at 260 mμ (ε 4700). The 3-ml aqueous aliquot was acidified with 1 *N* hydrochloric acid, and then made basic with 1 *N* sodium hydroxide. The ultraviolet spectrum in acid showed a maximum at 224 mμ and a broad shoulder centered at 257 mμ. The spectrum in alkali showed a maximum at 240 mμ and a shoulder centered at 268 mμ. Paper chromatography of **6i** in systems A and B showed one ultraviolet absorbing spot with R_f 0.82 and 0.69, respectively (nucleoside **6d**, R_f 0.45 and 0.44).

Anal. Calcd for $C_{14}H_{18}O_5N_4$: C, 52.17; H, 5.63; N, 17.89. Found: C, 52.22; H, 5.54; N, 16.79.

2,3′-Hyroxyimino-1-(5-*O*-acetyl-2-deoxy-β-D-*threo*-pentofuranosyl)thymine (6h).—To 2,3′-hydroxyimino **6c** (0.1 g, 0.42 mmol) suspended in pyridine (15 ml) was added acetic anhydride (1.1 mmol). The reaction mixture was heated at 75° for a few hours until solution occurred and then allowed to stand overnight at room temperature. The same procedure as that described for the isolation of **6f** was used. A white glass (containing residual acetic acid) was obtained. The ultraviolet spectrum of the glass in water had a maximum at 224 mμ and an inflection at 255 mμ. In acid the spectrum had a maximum at 224 mμ and a broad inflection at 245–255 mμ. This pattern changed within 1.5 hr to one similar to **6c** at pH 0 (Table II). The data suggest that the glass contained mainly an unstable diacetate of **6c** with similar chemical properties to those observed for diacetate **6j**. The diacetate of **6c** was converted into the monoacetate **6h** under the following conditions. The glass was dissolved in ethanol and the solution was allowed to stand overnight at room temperature. Short white needles (72 mg), mp 214–219° dec (prior darkening), crystallized. Recrystallization from ethanol yielded 40 mg, mp 222–227° dec (with effervescence, prior darkening), $[\alpha]^{25}D$ +9° (*c* 0.5, water). Compound **6h** exhibited essentially the same ultraviolet spectral data in water, acid, and base as **6c** (Table II). Paper chromatography of **6h** in systems A and B showed one ultraviolet absorbing spot with R_f 0.76 and 0.59, respectively (nucleoside **6c**, R_f 0.42 and 0.46).

Anal. Calcd for $C_{12}H_{15}O_5N_3$: C, 51.24; H, 5.38; N, 14.94. Found: C, 51.27; H, 5.56; N, 14.76.

2-*N*-Acetyl-1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)isocytosine (11).—To isocytosine nucleoside **10** (0.4 g, 1.6 mmol) suspended in pyridine (20 ml) was added acetic anhydride (8.2 mmol). The reaction mixture was heated at 55° for 1 hr and then allowed to stand at room temperature overnight. Almost complete solution had occurred. Some starting material (~10 mg) was removed by filtration. Ethanol (0.2 ml) was added and the pyridine was evaporated *in vacuo*. A syrup was obtained. Upon dissolving the syrup in ethanol, white crystals (0.5 g, mp 106–110°) appeared. A small sample, on recrystallization from water, afforded prisms, mp 108–110°, $[\alpha]^{25}D$ +89° (*c* 0.4, water). Ultraviolet absorption properties in water were maxima at 256 mμ (ε 17,200) and 217 mμ (ε 10,800), and a minimum at 234 mμ (ε 7200). Compound **11** was unstable in acid and alkali and was converted into 1-β-D-arabinofuranosyluracil or an acetyl derivative thereof (see below).

Anal. Calcd for $C_{17}H_{21}N_3O_9$: C, 49.64; H, 5.15; N, 10.21. Found: C, 48.96; H, 5.19; N, 10.28.

Hydrolysis of the Tetraacetate 11. A. In Acid.—The tetraacetate **11** (0.1 g) dissolved in 0.08 *N* sulfuric acid (25 ml) was allowed to react for 3 hr at room temperature. During this time the ultraviolet spectra of the reaction mixture changed to that of arabinofuranosyluracil (**13**). The acid solution was neutralized with barium carbonate, and the resulting filtrate was evaporated to dryness. On the addition of ethanol to the residue, triacetate **12** precipitated. The yield of **12** was 36%, mp 127–128° (lit.[7] mp 129–130°). The ir, pmr, and analytical data of the triacetate were identical with those of an authentic sample of **12**.

(27) The fast-moving diacetate of **6j** spot was eluted with water. The ultraviolet spectrum of the solution had a maximum at 230 mμ, and a shoulder at 255 mμ. On the addition of acid to the solution, the spectrum changed (2–3 hr) to the acid spectrum of **6a** (Table II). Upon the addition of base to the aqueous solution of **6j** the spectrum immediately changed to that of **6a**.

B. In Alkali.—The tetraacetate **11** (25 mg) dissolved in 1 *N* sodium hydroxide (3 ml) was allowed to stand overnight at room temperature. During this time the ultraviolet spectra of the reaction mixture changed to that of arabinosyluracil (**13**). The basic solution was treated with Dowex 50 resin (H^+ form) and the resulting filtrate was subjected to chromatographic analysis using systems A and B. Only one ultraviolet absorbing spot corresponding to **13** was detected.

Reactions of 2,3′-Aminoimino Nucleoside 6d. A. Reaction with Nitrous Acid.—The 2,3′-aminoimino nucleoside **6d** (50 mg, 0.21 mmol) was dissolved in 70% acetic acid (3 ml). The solution was cooled, sodium nitrite (19 mg) in water (1 ml) was slowly added, and the reaction mixture was allowed to stand for 1 hr. Th imino nucleoside **6a** was purified by absorption on a column of Dowex 50 (H^+, 100–200 mesh). The nucleoside was eluted with 2 *N* NH_4OH, and the ultraviolet absorbing eluate was evaporated *in vacuo* to dryness. The product was crystallized from ethanol. Compound **6a** (16 mg), mp 250° (sintering) and 268° (dec with effervescence), was obtained.

B. Reaction with Benzaldehyde.—A mixture of the 2,3′-aminoimino derivative **6d** (0.2 g, 0.84 mmol), benzaldehyde (0.2 ml), ethanol (10 ml), and three drops of concentrated hydrochloric acid was refluxed for 15 min. The solution was cooled, and concentrated ammonium hydroxide was added until a pH of about 8 was reached. Evaporation of the ethanol afforded a yellowish residue. The residue was first triturated with ether and then water was added. A white solid (**6e**) precipitated and was filtered. The solid was crystallized from ethanol. The benzal compound **6e** was obtained as white rodlike crystals (0.12 g, 44%), mp 258–292° dec (with effervescence). Ultraviolet absorption properties in ethanol were maxima at 308 and 228 mμ, inflections at 314 and 246 mμ, and a minimum at 269 mμ.

Anal. Calcd for $C_{17}H_{18}N_4O_3$: C, 62.57; H, 5.56; N, 17.17. Found: C, 62.43; H, 5.53; N, 17.23.

Stability of the Imino Bridge. In Alkali.—The 2,3′-imino compound (**6a**, 50 mg) was dissolved in 15 ml of 1 *N* NaOH. After standing 1 day at room temperature the ultraviolet absorption spectrum remained unchanged. The solution was placed on a Dowex 50 resin (H^+) and elution with 0.1 *N* NH_4OH followed by evaporation afforded 20 mg of crystals, mp 258–261° dec. The ir and chromatographc properties were identical with those obtained from **6a**.

Solutions of **6a** or **6b** in 7 *N* KOH for 3 weeks did not alter the uv spectra. (The uv spectrum of **6b** in 7 *N* KOH was identical with the uv spectrum in pH 6.92 solution except that the maximum at 228 mμ was masked by buffer absorption.)

In Acid.—The 2,3′-imino derivatives **6a–d** were more stable in aqueous acid than the "oxygen isostere" **2** or the uncyclized 2-amino nucleoside **9**.[8]

A paper chromatogram of the derivatives **6a–d**, **2**, and 2-deoxyribose was developed using a butanol–water system (84:14), and then spraying with acid cysteine reagent.[28] Only the 2,3′-anhydro nucleoside **2** (R_f 0.37) and 2-deoxyribose (R_f 0.32) gave the characteristic pink color with the cysteine reagent. The nucleosides **6a–d** were visualized using uv determinations and gave R_f 0.35, 0.48, 0.29, and 0.28 respectively.

The imino derivative **6a** (8.85 mg) was refluxed in 0.1 *N* hydrochloric acid (10 ml). An aliquot (0.05 ml) was removed and added to 5 ml of buffer (pH 7, pH 12, and pH 0) the ultraviolet spectrum was taken. After 1.1 hr, the spectra at these pHs were almost the same as those reported in Table II. [A small increase (6%) in absorption in the 260-mμ region was observed at pH 12]. After 22 hr, the spectra had changed appreciably: at pH 7, maxima at 214–218 mμ (ε 22,000), 270 (4000), and 302 (4300), minima of 257 mμ (ε 3800) and 282 (3700); at pH 12, maxima at 220 mμ (ε 21,400) and 275 (5000) and a shoulder at 300–310 (3000), minimum at 256 mμ (ε 3900); at pH 0, maxima at 227 mμ (ε 13,600) and 267 (9300), minimum at 249 mμ (ε 7300). After refluxing for 2 days, the uv spectra at pH 0 and 7 were essentially the same as the spectra after 1 day. At pH 12 there was a broad maximum between 275 and 302 mμ in addition to the maximum at 220 mμ. The above spectral changes indicate that some glycosyl cleavage had occurred. The 22-hr spectral patterns bore some resemblance to the spectral patterns of 5-methylisocytosine. 5-Methylisocytosine has been prepared previously[8] from 1-β-D-arabinofuranosyl-5-methylisocytosine (**9**). 5-Methylisocytosine had ultraviolet absorptions at λ_{max}^{water} 205 mμ (ε 7700) and 263 (2900), shoulders at 215 and 286; λ_{min} 246 (2300); $\lambda_{max}^{pH\ 12}$ 228 (4350) and 279 (3800); λ_{min} 220 (4130) and 246 (2400); $\lambda_{max}^{1\ N\ HCl}$ 221 (5500) and 261 (4000); λ_{min} 242 (3100).

It is probable that the sugar moiety or a derivative thereof remains attached to the 2-amino group thus accounting for the different spectral characteristics of the acid hydrolysate of **6a** and 5-methylisocytosine.

Registry No.—**2**, 15981-92-7; **4**, 15981-78-9; **6a**, 15981-79-0; **6b**, 15981-80-3; **6c**, 15981-81-4; **6d**, 15981-82-5; **6e**, 15981-83-6; **6f**, 16031-78-0; **6g**, 15981-84-7; **6h**, 15981-85-8; **6i**, 16065-64-8; **7**, 15981-86-9; **8**, 15981-87-0; **9**, 10212-31-4; **10**, 10212-30-3; **11**, 15981-93-8; **12**, 14057-18-2; 5-methylisocytosine, 15981-91-6.

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(28) J. G. Buchanan, *Nature*, **168**, 1091 (1951).