2,2′-Anhydropyrimidine Nucleosides. Novel Syntheses and Reactions

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A novel synthesis of 2,2′-anhydropyrimidine nucleosides is described. D-Arabinose and D-ribose react with cyanamide to yield the aminooxazoline derivatives **2** and **14**, respectively. The reactions of **2** with propiolonitrile, methyl propiolate, and dimethyl acetylenedicarboxylate yield the β-anhydronucleosides **4**, **8**, and **11**, respectively. Similarly, the reactions of **14** with the same acetylene derivatives yield the novel α-anhydronucleosides **16**, **18**, and **20**. The ring-opening reactions of certain of these anhydronucleosides with water and with other nucleophiles are described.

As a result of a series of investigations into the origins of nucleosides under primitive earth conditions, a new synthesis of certain pyrimidine anhydronucleosides was discovered. The preliminary results of these studies were described in the context of prebiological chemical evolution.[1]

We have continued with these studies and have developed suitable procedures for the preparation of both the α and β anomers of 2,2′-anhydrocytidine, 2,2′-anhydrouridine, and 2,2′-anhydroorotidine methyl ester. In the past some of these anhydronucleosides have only been available through tedious procedures that afford inadequate yields.[2,3] Recently, improved procedures have been described for the synthesis of the β anomers of 2,2′-anhydrocytidine[4] and 2,2′-anhydrouridine.[5]

We wish to describe simple, general procedures for the synthesis of anhydronucleosides and to emphasize the versatility of these compounds as intermediates in the synthesis of 2′-substituted 2′-deoxycytidines, α-ribosides, β-arabinosides, and other nucleoside analogs.

Experimental Section

General.—Melting points were taken in open capillaries in a Mel-Temp heating block and are uncorrected. Elemental microanalyses were performed by Midwest Microlab. Ultraviolet spectra were measured on a Unicam SP 800 recording spectrophotometer. Paper chromatography was performed by descending elution with the following solvent systems: A, *n*-butyl alcohol saturated with water; B, *n*-butyl alcohol–5 *N* acetic acid (2:1); and C, 95% ethanol–1 *M* ammonium acetate (pH 5.0) (7:3, with 0.001 *M* EDTA). Paper electrophoresis was carried out in Varsol-cooled tanks at 4000 V with the following buffer systems: D, 0.05 *N* formic acid buffered to pH 2.6 with ammonia; E, 0.05 *N* boric acid buffered to pH 8.5 with NaOH; and F, 0.03 *N* H_3PO_4 buffered to pH 7.1 with KOH. Whatman No. 3MM paper was used in every case.

2-Amino-β-D-arabinofurano[1′,2′:4,5]-2-oxazoline (2).—The synthesis and characterization of this compound was previously described.[1] Improved yields are obtained by the following modification. Concentrated ammonia solution (5.0 ml) and crystalline cyanamide (8.4 g, 0.20 mol) were added to a stirred slurry of D-arabinose (**1**, 15.0 g, 0.10 mol) in 50 ml of methanol. The mixture was stirred for 4 hr at 40–45° and then chilled in an ice bath. After filtering, washing with cold methanol, and air drying, the white powder weighed 14.1 g (81%) and melted at 175–176°. The pK_a in water was determined titrimetrically to be 6.52.

2-Amino-α-D-ribofurano[1′,2′:4,5]-2-oxazoline (14).—The synthesis and characterization of this compound has also been described.[1] The following method is more convenient for the synthesis of pure material, although the yield is lower. A slurry of D-ribose (**13**, 45.0 g, 0.30 mol) and cyanamide (25.0 g, 0.60 mol) in 50 ml of 1 *N* NH_4OH was swirled in a warm water bath until solution was essentially complete and the temperature was about 30°. After about 30 min at room temperature an exothermic crystallization commenced and was allowed to proceed for several minutes. The slurry of solids was heated in a 60° water bath for 30 min, then 100 ml of CH_3OH were added and the mixture was refrigerated overnight. The solids were filtered off, then washed with methanol and ether and finally dried under vacuum. The yield of free-flowing white powder was 34.2 g (65.5%), mp 197° dec. The pK_a value in water was determined titrimetrically to be 6.54.

Reaction of Cyanamide with Other Sugars.—The sugars (0.10 *M* each) were heated at 60° in aqueous solution containing NH_3 (0.10 *M*) and cyanamide (0.20 *M*). Aliquots were withdrawn periodically for analysis by chromatography in system A. Sugars were detected by development with aniline phthalate at 100°, and the oxazoline products were detected by the appearance of a characteristic blue color after spraying with dibromoquinone-*N*-chloroimide in ethanol at room temperature. The sugars used, and their half-lives in these reactions, were glycolaldehyde, glyceraldehyde, erythrose (1–2 min), ribose, arabinose (10–40 min), glucose, fructose, and lactose (3 hr). All of the products (presumably oxazoline derivatives) travelled with R_f values in the range 0.17–0.29 except for that from lactose, for which the R_f was 0.02 (system A).

2,2′-Anhydro-1-β-D-arabinofuranosylcytosine Salts (4a–c).—A suspension of **2** (6.96 g, 0.040 mol) in 20 ml of *N*,*N*-dimethylacetamide was stirred in a water bath at *ca.* 15° and propiolonitrile (2.50 ml, 0.040 mol) was added. After about 30 min the reaction was complete, giving a darkly colored but clear solution in which the major component is thought to be the cyanovinyl adduct **3**.[1] Solutions of **3** prepared in this way were found to survive unchanged after storage at room temperature for several weeks or heating at 60° for several hours. No attempts were made to isolate adduct **3**. In aqueous ammonia it is rapidly converted to β-arabinosylcytosine; in water the conversion is slower and 2,2′-anhydrocytidine is detectable as an intermediate.[1]

The acetate salt **4** was obtained by adding 4.6 ml of glacial acetic acid and 30 ml of water to the reaction mixture containing **3**. After 30 min at room temperature the solution was evaporated under high vacuum to a syrupy residue. The residue was dissolved in 100 ml of boiling methanol and diluted with 200 ml of warm ethyl acetate, whereupon crystallization soon occurred. The chilled mixture was filtered and the crystals were washed with ethyl acetate. In several preparations the yield of **4a** varied between 10.3 and 10.8 g (85–89%), and the product melted at 175–176°. Recrystallization from methanol–ethyl acetate yielded 9.4–10.0 g (78–83%): mp 178–179° (lit.[6] mp 190–192° dec, with sintering at 165–185°); uv (H_2O) λ_{max} 231.5, 262.5 nm (ε 9780, 10,800) [lit.[2] for the HCl salt (pH 1–7) λ_{max} 231, 262 nm (ε 9400, 10,600)].

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(2) E. R. Walwick, W. K. Roberts, and C. A. Dekker, *Proc. Chem. Soc.*, 84 (1959); W. K. Roberts and C. A. Dekker, *J. Org. Chem.*, **32**, 816 (1967).

(3) D. M. Brown, A. Todd, and S. Varadarajan, *J. Chem. Soc.*, 2388 (1956); J. J. Fox and I. Wempen, *Advan. Carbohyd. Chem.*, **14**, 282 (1959), and references cited therein.

(4) K. Kikugawa and M. Ichino, *Tetrahedron Lett.*, 867 (1970); *J. Org. Chem.*, **37**, 284 (1972).

(5) A. Hampton and A. W. Nichol, *Biochemistry*, **5**, 2076 (1966); J. P. H. Verheyden, D. Wagner, and J. G. Moffatt, *J. Org. Chem.*, **36**, 250 (1971).

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

Anal. Calcd for $C_{11}H_{15}N_3O_6 \cdot 0.5CH_3OH$: C, 45.84; H, 5.68; N, 13.94. Found: C, 45.70; H, 6.04; N, 14.17.

The hydrofluoride salt **4** was obtained by adding 2.8 ml of 48% HF solution and 30 ml of H_2O to the reaction mixture containing **3**. After 2 hr at room temperature 300 ml of cold dioxane was added to yield 7.85 g (80%) of crystalline **4b**: mp 186–188°; uv (H_2O) λ_{max} 232, 262 nm (ϵ 9760, 10,600); λ_{min} 244 nm (ϵ 7080).

Anal. Calcd for $C_9H_{12}N_3O_4F \cdot 0.5H_2O$: C, 42.55; H, 5.15; N, 16.51; F, 7.48. Found: C, 41.92; H, 5.24; N, 15.99; F, 8.79. The analytical values indicate the presence of additional fluorine and correspond most closely to the average composition $C_9H_{12}N_3O_4F \cdot 0.5H_2O \cdot 0.2HF$.

The phosphate salt **4c** was obtained by the addition of 1 molar equiv of H_3PO_4 to a solution of **4a** in water. After removal of water and acetic acid under high vacuum, a hygroscopic gum was obtained which was further dried by the repeated addition and evaporation of anhydrous ethanol, uv (H_2O) λ_{max} 232, 263 nm (ϵ 9600, 10,500), λ_{min} 244 nm (ϵ 7000).

Both **4b** and **4c** were chromatographically homogeneous (systems A and B) and travelled with the same R_f values as those of **4a**. Further purifications were not attempted.

2,2′-Anhydro-1-β-D-arabinofuranosyluracil (8) and 2-Imino-1-carbomethoxyvinyl-β-D-arabinofurano[1′,2′:4,5]oxazolidine (9).—A suspension of **2** (6.96 g, 0.040 mol) in 100 ml of absolute ethanol and methyl propiolate (10.1 ml, 0.12 mol) was heated under reflux with magnetic stirring for 1 hr. The suspension was chilled in an ice bath and filtered. The crystals of **8** were washed with cold ethanol and air dried. The product melts at 242–243° (lit.[5] mp 238–240°) and the yields in several preparations varied between 6.0 and 6.4 g (66–71%): uv (H_2O) λ_{max} 223, 250 nm (ϵ 8400, 8200); λ_{min} 235 nm (ϵ 6600) (lit.[3] λ_{max} 222, 250 nm; λ_{min} 239 nm).

The filtrate was evaporated to dryness and the residual syrup was dissolved in boiling acetonitrile. A small amount of **8** was filtered and the filtrate was refrigerated. The crystalline precipitate of the open-chain adduct **9** was filtered, washed with cold acetonitrile, and air dried. A yield of 1.85 g (18%) was obtained: mp 167–168°; uv (H_2O) pH 1, λ_{max} 248 nm (ϵ 17,500), λ_{min} 213 nm (ϵ 5000); pH 12, λ_{max} 264 nm (ϵ 18,000), λ_{min} 227 nm (ϵ 4300); nmr (DMSO-d_6, TMS internal standard) δ 7.64 (doublet, 1, α-vinyl H), 5.43 (doublet, 1, β-vinyl H, $J_{\alpha,\beta}$ = 3.6 Hz), 6.66 (singlet, 1, vinyl NH), 3.63 (singlet, 3, $-OCH_3$).

Isomerization of 9.—A 0.10 *M* solution of **9** in ethanol was heated at 100° for 1 hr in a tightly stoppered tube. The uv spectrum of a diluted aliquot showed that no change had taken place. Heating in the presence of charcoal, palladium black, or iodine also failed to produce any change in the spectrum, although with iodine there was a decrease in absorbance and a discharging of the violet color.

A 1.0×10^{-4} *M* solution of **9** in water was placed in a 10-mm quartz cell and irradiated with 2537-Å light (Rayonet photochemical reactor). The uv spectrum of the solution was scanned periodically. The spectrum changed rapidly to that of the anhydronucleoside **8**, and was followed by a slower decrease in absorbance due to photodestruction. The same photodestruction process was observed when a 1.0×10^{-4} *M* solution of **8** was irradiated under the same conditions.

2,2′-Anhydro-1-β-D-arabinofuranosylorotic Acid Methyl Ester (11).—A mixture of **2** (3.48 g, 0.020 mol) and dimethyl acetylenedicarboxylate (5.68 g 0.040 mol) in 50 ml of absolute ethanol was heated under reflux for 1 hr. The clear amber solution was chilled in an ice bath and the precipitate of cream-colored needles was filtered off, washed with ethanol, and air dried. The yield of solid was 3.95 g (69%), mp 228–230°. Recrystallization from hot water yielded 3.10 g (55%) of fine white needles: mp 233–233.3°; uv (H_2O) pH 1.5, λ_{max} 276 nm (ϵ 7100), λ_{min} 245 nm (ϵ 3400); pH 13, λ_{max} 267 nm (ϵ 7100), λ_{min} 246 nm (ϵ 5600).

Anal. Calcd for $C_{11}H_{12}N_2O_7$: C, 46.50; H, 4.26; N, 9.85. Found: C, 46.54; H, 4.30; N, 9.93.

Compound **11** was hydrolyzed in 0.1 *N* HCl at 100° for 3 days. Paper chromatography in several chromatographic systems including A–C confirmed the presence of orotic acid and arabinose as major products.

2,2′-Anhydro-1-α-D-ribofuranosylcytosine Hydrochloride (16).—A suspension of **14** (20.88 g, 0.12 mol) in 200 ml of *N*,*N*-dimethylacetamide was stirred magnetically in an ice bath, and propiolonitrile (7.50 ml, 0.12 mol) was added. After about 1 hr the ice bath was removed and the solution was stirred for 2 days at room temperature. The dark solution (still containing some undissolved solids) was stirred in an ice bath while 30 ml of glacial acetic acid and 100 ml of water were added. The solution was stirred at *ca.* 4° for 4 days and then evaporated to a thick syrup under high vacuum. The syrup was taken up in water and applied to a column of 400 g of Dowex 50 (H^+) ion exchange resin. The column was eluted with hydrochloric acid in a step-gradient (1 *M*, 2 *M*, 3 *M*) and the band of **16** was located in the 3 *M* HCl eluates by paper chromatography. The combined fractions were evaporated to dryness and the solid was recrystallized from ethanol–water, yielding 16.9 g (51%) of **16** as off-white crystals, mp 235° dec, uv (H_2O) λ_{max} 232 nm (ϵ 9700), 261 (10,800).

Anal. Calcd for $C_9H_{12}N_3O_4Cl \cdot {}^1/_2H_2O$: C, 39.93; H, 4.84; N, 15.52. Found: C, 39.82; H, 5.17; N, 14.97.

2,2′-Anhydro-1-α-D-ribofuranosyluracil (18).—The synthesis and characterization of this compound was described earlier.[1] The following is an improved procedure: **14** (61.7 g, 0.354 mol) and methyl propiolate (59.4 g, 0.708 mol) in 800 ml of water were heated under reflux for 30 min. The clear solution was evaporated to dryness and the residue was extracted with 400 ml of boiling methanol. The clear filtrate was refrigerated for 2 days and yielded 21.4 g (27%) of **18** as pale yellow crystals: mp 223–225°; uv (H_2O) λ_{max} 225, 250 nm (ϵ 8600, 8400); λ_{min} 235 nm (ϵ 7100).

2,2′-Anhydro-1-α-D-ribofuranosylorotic Acid Methyl Ester (20).—A suspension of **14** (25.0 g, 0.143 mol) in dimethyl acetylenedicarboxylate (40.6 g, 0.286 mol) and 300 ml of methanol was heated under reflux for 1 hr. Undissolved solids were removed by filtration and the clear, darkly colored filtrate was refrigerated. The crystals of **20** were filtered off and dried under high vacuum, yielding 21.6 g (52%), mp 230–232°, uv (H_2O) λ_{max} 267 nm (ϵ 6900), λ_{min} 245 nm (ϵ 3200).

Anal. Calcd for $C_{11}H_{12}N_2O_7$: C, 46.48; H, 4.25; N, 9.85. Found: C, 46.26; H, 4.16; N, 9.76.

Compound **20** was hydrolyzed in acid and analyzed in the same way as described for **11**. The presence of orotic acid and ribose as major products was confirmed.

1-β-D-Arabinofuranosylcytosine (5).—The synthesis of the cyanovinyl adduct **3** was carried out as described above for the synthesis of **4a**, but using 21.6 g of **2**, 9.8 ml of propiolonitrile, and 100 ml of *N*,*N*-dimethylacetamide. Water (50 ml) was added to the clear, dark solution, which was then heated at *ca.* 50° for 1 hr. The uv spectrum of a diluted aliquot indicated that the conversion to **5** was complete. The solution was evaporated under vacuum to a thick red oil, which was then taken up in 200 ml of hot 95% ethanol and refrigerated overnight. The crystalline product was filtered off, washed with ethanol and ether, and then air dried. The yield of **5** was 29.7 g (81%). This product was identical with authentic 1-β-D-arabinofuranosylcytosine (Upjohn Co.) in all respects (melting point, uv, ir, ORD, chromatography).

The synthesis of the HCl salt of **5** in a similar fashion was previously reported.[1] Yields of 82–84% were obtained. **5** is also obtained in high yield by the hydrolysis of **4** in warm aqueous ammonia, followed by evaporation of the solution to dryness and recrystallization of the residue from small volumes of hot water.

1-β-D-Arabinofuranosyluracil (10).—A solution of **8** (5.0 g, 0.022 mol) in 10 ml of 1 *N* HCl was heated at 100° for 40 min and then evaporated to dryness. The residue was taken up in water, adjusted to pH 9 with aqueous NH_3, and reevaporated to dryness. The residue was crystallized from a minimum volume of boiling water, and yielded 4.15 g (77%) of **10**, mp 215–216° (lit.[3] mp 220–221°). A second crop of 0.47 g was obtained by concentration of the filtrates (overall yield 86%): uv (H_2O) pH 2, λ_{max} 263 nm (ϵ 10500), λ_{min} 231 nm (ϵ 2300); pH 11, λ_{max} 262.5 nm (ϵ 8000), λ_{min} 242 nm (ϵ 5200) [lit.[3] (H_2O) λ_{max} 262.5–263.5 nm (ϵ 10,500), λ_{min} 230–231 nm (ϵ 2000)].

This material is chromatographically homogeneous in several systems, and travels with the same R_f as that of authentic 1-β-D-arabinofuranosyluracil.

1-α-D-Ribofuranosylcytosine (17).—The cyanovinyl adduct **15** was synthesized as previously described for the preparation of anhydronucleoside **16**, but using 15.1 g (0.086 mol) of **14** and 5.4 ml (0.086 mol) of propiolonitrile in 100 ml of *N*,*N*-dimethylacetamide. The dark solution (which contains undissolved solids) is presumed to contain the adduct **15** as a major component, in analogy to the synthesis of the β analog **3**. Water (100 ml) was added to the mixture and stirring was continued for 3 days at room temperature. Undissolved solids (2.85 g) were removed by filtration and the filtrate was evaporated to a dark syrup under high vacuum. The syrup was dissolved in 50 ml of

hot ethanol and then refrigerated. The dark-colored crystals which formed (12.3 g) were dissolved in 20 ml of hot water, and then treated with Norit A and filtered. The filtrate was diluted with 80 ml of hot isopropyl alcohol, and the clear supernatant was decanted from some dark gums and refrigerated. The crystals which formed were again recrystallized from water–isopropyl alcohol and yielded, after air drying, 7.50 g (35%) of **17** as granular crystals. The methods used in the identification of this compounds were previously discussed.[1]

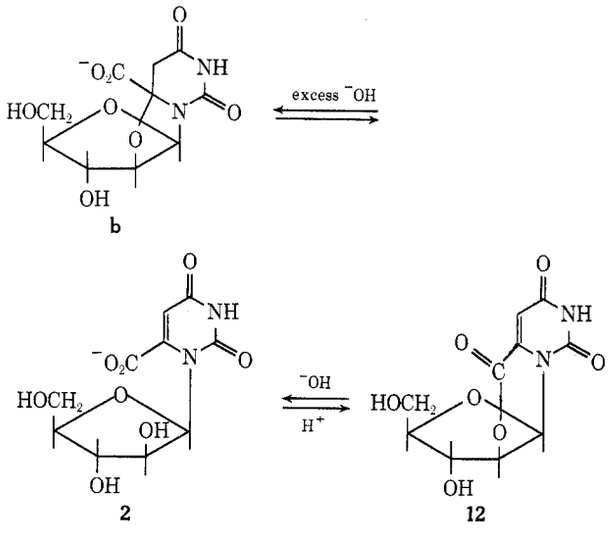
Compound **17** could also be prepared in high yield by the hydrolysis of **16** in aqueous ammonia, in the same way as described for the conversion of **4** to **5**.

1-α-D-Ribofuranosyluracil (19).—A solution of **18** (10.0 g, 0.044 mol) in 22 ml of 0.2 *N* HCl was heated under reflux for 2 hr, and the pale yellow solution was then evaporated to dryness under vacuum. The viscous residue was taken up in water and adjusted to a pH of 6 by the addition of Dowex 1X8 (OH^-) resin. The mixture was filtered and the resin was rinsed with water. The combined filtrates were freeze dried and yielded **19** as a hygroscopic white powder in essentially quantitative yield. The identification of this compound was previously described.[1]

1-β-D-Arabinofuranosylorotic Acid 2′,6-Lactone (12).—A suspension of 3.0 g of **11** in 50 ml of water was heated under reflux for 1 hr. Refrigeration of the clear solution at 4° produced a stiff gel which slowly crystallized. After 2 days the white precipitate of **12** was filtered off, washed with a little water, and dried under vacuum. The yield was 2.3 g (81%). An analytical sample was obtained by crystallization from boiling water: mp 248–250°; ir (KBr disc) 1735 cm^{-1} (lactone C=O); uv (H_2O) λ_{max} 290 nm (ϵ 7700), λ_{min} 245 nm (ϵ 2000).

Anal. Calcd for $C_{10}H_{10}N_2O_7$: C, 44.45; H, 3.73; N, 10.36. Found: C, 44.50; H, 3.92; N, 10.19.

On standing in water the uv spectrum of **12** slowly shifts to λ_{max} 273 nm (ϵ 8200), λ_{min} 236 nm (ϵ 2700), presumably the result of hydrolysis to 1-β-D-arabinofuranosylorotate. Upon acidification with HCl lactonization occurs and the original spectrum is regenerated. In strong alkali an additional reaction occurs which results in a partial loss of absorbance. We believe that this is due to the establishment of an equilibrium between 1-β-D-arabinofuranosylorotate and 2′,6-anhydro-1-β-D-arabinofuranosyl-5,6-dihydroorotate. Similar additions have been reported previously.[7]



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1-α-D-Ribofuranosylorotic Acid 2′,6-Lactone (21).—This compound was prepared by the hydrolysis of ester **20**, in the same manner as described for the synthesis of the β analog **12** from **11**. White platelets, mp 268–269°, were obtained, uv (H_2O) λ_{max} 291 nm (ϵ 7600), λ_{min} 247 nm (ϵ 2200).

Anal. Calcd for $C_{10}H_{10}N_2O_7$: C, 44.45; H, 3.73; N, 10.36. Found: C, 43.13; H, 3.33; N, 9.95.

The aqueous solution chemistry of this compound is essentially the same as that described above for the β analog **12**.

2′(3′)-O-Acetylcytidine (6a).—A suspension of **4a** (1.0 g, 0.035 mol) in 35 ml of anhydrous *N*,*N*-dimethylformamide was heated in an oil bath at 100° for 2.5 hr. An aliquot of the clear brown solution was chromatographed in system A. The chromatogram showed a major spot of **6a** (*ca.* 60% yield, estimated by visual comparison of the spot intensity with those of standards of known concentration; R_f 0.29), smaller amounts of starting material **4a** (R_f 0.09), cytidine (R_f 0.14), 1-β-D-arabinofuranosylcytosine (**17**, R_f 0.18), and traces of other unidentified products. The solution was evaporated under vacuum to an oil, which was then applied to a 4 × 35 cm column of silica gel. The column was eluted with $CHCl_3$–CH_3OH (95:5) and the eluates were monitored by chromatography in system A. The fractions containing **6a** (contaminated by cytidine) were pooled and evaporated, yielding a yellow oil which could not be made to crystallize. An aliquot was further purified by tlc on silica gel plates with $CHCl_3$–CH_3OH (88:15), on which **6a** travelled with an R_f of 0.38. The band was eluted with methanol, and **6a** was crystallized after concentration: mp 118–122°; uv (H_2O) pH 2, λ_{max} 278.5 nm (ϵ 11,400), λ_{min} 252.5 nm (ϵ 1900); pH 10, λ_{max} 270 nm (ϵ 7700), λ_{min} 252.5 nm (ϵ 6300); nmr (D_2O, TMS internal standard) δ 2.19 (s, 3, acetyl CH_3). The uv spectrum, as well as the remaining features of the nmr spectrum, were consistent with the proposed structure.

Anal. Calcd for $C_{11}H_{15}N_3O_6$: C, 45.37; H, 5.42; N, 15.59. Found: C, 45.55; H, 5.38; N, 15.90.

The compound did not migrate in system E, which establishes that the acetyl group is on the 2′ and/or 3′ oxygen. Alkaline hydrolysis (1 *N* NH_4OH, 100°, 15 min) yielded cytidine quantitatively which was identified by the coincidence of its R_f with that of authentic cytidine in the chromatographic systems A and E.

2′-Fluoro-2′-deoxy-β-D-ribofuranosylcytosine (6b).—A suspension of **4b** (21.3 g, 0.086 mol) in 860 ml of anhydrous *N*,*N*-dimethylformamide was heated at 100° with magnetic stirring for 24 hr. An aliquot of the dark solution was chromatogrammed in system A, in which a major spot of R_f 0.18 corresponded to 1-β-D-arabinofuranosylcytosine (**5**) and two others corresponded to starting material **4b** (R_f 0.09) and cytidine (R_f 0.14). Product **6b** travelled with an R_f of 0.27 and its yield was estimated at 30% by the uv spectrum of the eluted spot. The reaction mixture was concentrated under high vacuum at 45° to a dark syrup which was then applied to a column of Dowex 1X8 (OH^-) ion exchange resin and eluted with methanol–water (25:75). The first major uv-absorbing band contained **6b**, and the combined fractions were concentrated under vacuum at room temperature until crystallization began. A yield of 4.26 g (16%, mp 161–163°) of **6b** was obtained after filtration and air drying. The elemental composition of this material was found to correspond approximately to that of a trihydrate.

Material dried at 60° under high vacuum for 2 days had the following properties: uv (H_2O) pH 1, λ_{max} 278, 212 nm (ϵ 12,900, 9900), λ_{min} 241 nm (ϵ 2300); pH 12, λ_{max} 272, 230 nm (ϵ 9100, 8300), λ_{min} 251 nm (ϵ 6400); mp 173° (with much prior shrinking; measured in a preheated block) [lit.[6] uv (H_2O) pH 1, λ_{max} 277, 211 nm (ϵ 12,910, 9550), λ_{min} 239 nm (ϵ 1840); pH 14, λ_{max} 271 nm (ϵ 9110), λ_{min} 248 nm (ϵ 5730); mp 171–173° with prior shrinking]; mass spectrum (70 eV), *m/e* 20 (HF^+). The mass spectrum of deoxycytidine does not show significant ion intensity at *m/e* 20.

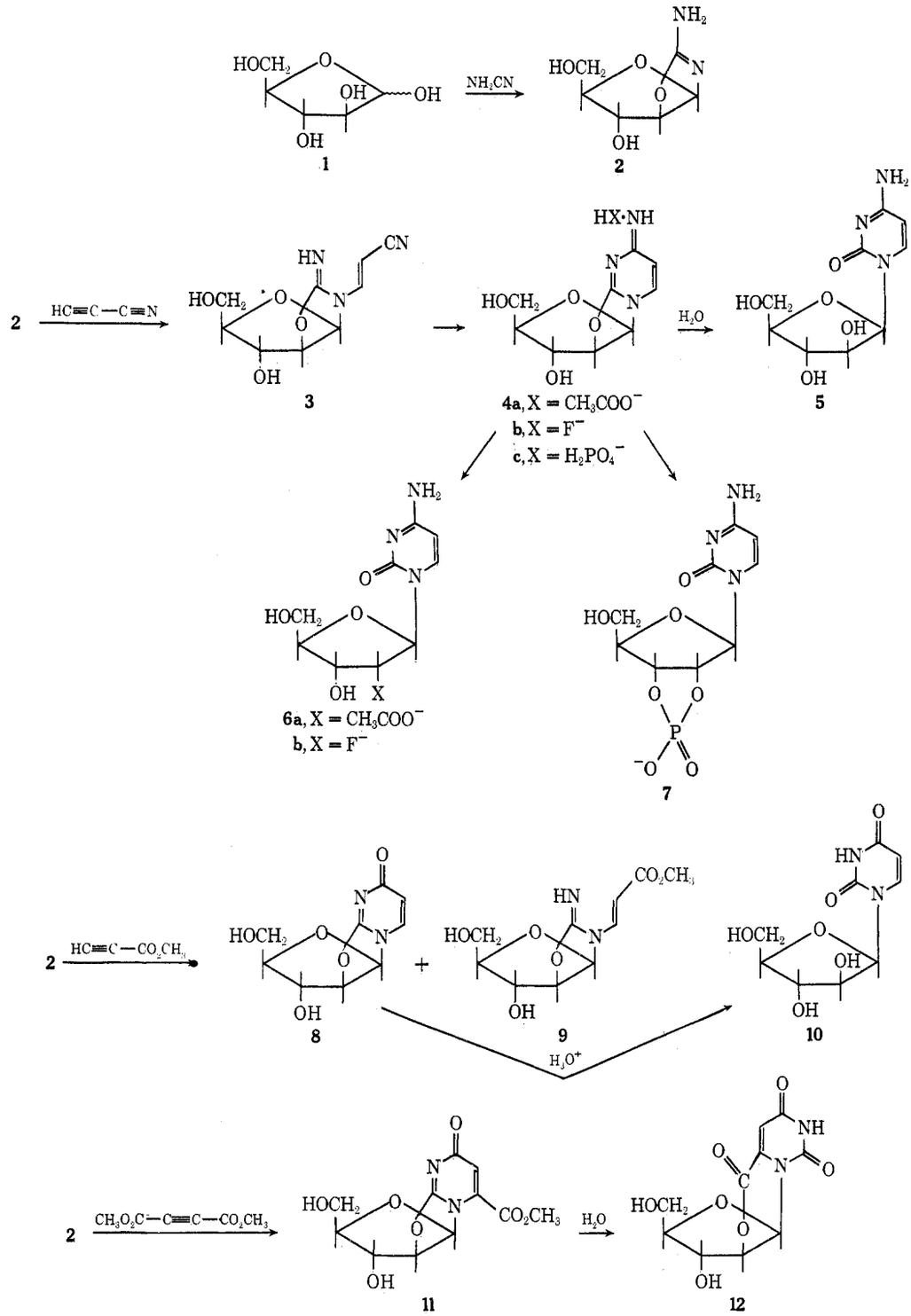
The synthesis of **6b** by this procedure generally ceases before all of **4b** is consumed, and further heating does not increase the yield. Anhydrous conditions appear to be necessary for optimum yields.

Preliminary attempts to synthesize **6b** by heating **4a** with HF in dioxane at 110° resulted only in recovery of **4a**.

Cytidine 2′,3′-Cyclic Phosphate (7).—A suspension of **4c** (14.6 mg, 0.045 mmol) in 0.45 ml of anhydrous hexamethylphosphoric triamide (HMPT) was heated at 100° for 21 hr. Aliquots of the resulting light brown solution were chromatogrammed in several systems (B–F) alongside authentic standards, and yields were estimated from the uv spectra of eluted spots. The major products present were cytidine 2′,3′-cyclic phosphate (**7**, 26%), unreacted starting material **4c** (44%), and 1-β-D-arabinofuranosylcytosine (**5**, 30%).

The following control reactions were carried out and analyzed in the same way. Cytidine and 1 molar equiv of H_3PO_4 produced a mixture of 5′- and 2′,3′-cyclic monophosphates in a combined yield of 5% or less. Cytidine 5′-monophosphoric acid was recovered unchanged and did not yield any 2′(3′)-monophosphate or 2′,3′-cyclic phosphate. Cytidine 2′(3′)-monophosphoric acid was converted in high yield (>50%) to the 2′,3′-cyclic phosphate.

(7) W. J. Wechter and R. C. Kelly, *Collect. Czech. Chem. Commun.*, **35**, 1991 (1970); B. A. Otter and J. J. Fox, *J. Amer. Chem. Soc.*, **89**, 3663 (1967).



Results

The condensations of D-arabinose (**1**) and of D-ribose (**13**) with cyanamide yield the novel aminooxazoline derivatives **2** and **14**, respectively. The compounds are obtained in the furanose ring forms, and the configuration of the heterocyclic ring in each case is uniquely specified by the orientation of the 2′-hydroxyl group, *i.e.*, β in **2** and α in **14**.

Preliminary results suggest that analogous aminooxazolines might be formed from a variety of other sugars. We have not yet confirmed this, however, by isolating and characterizing the products.

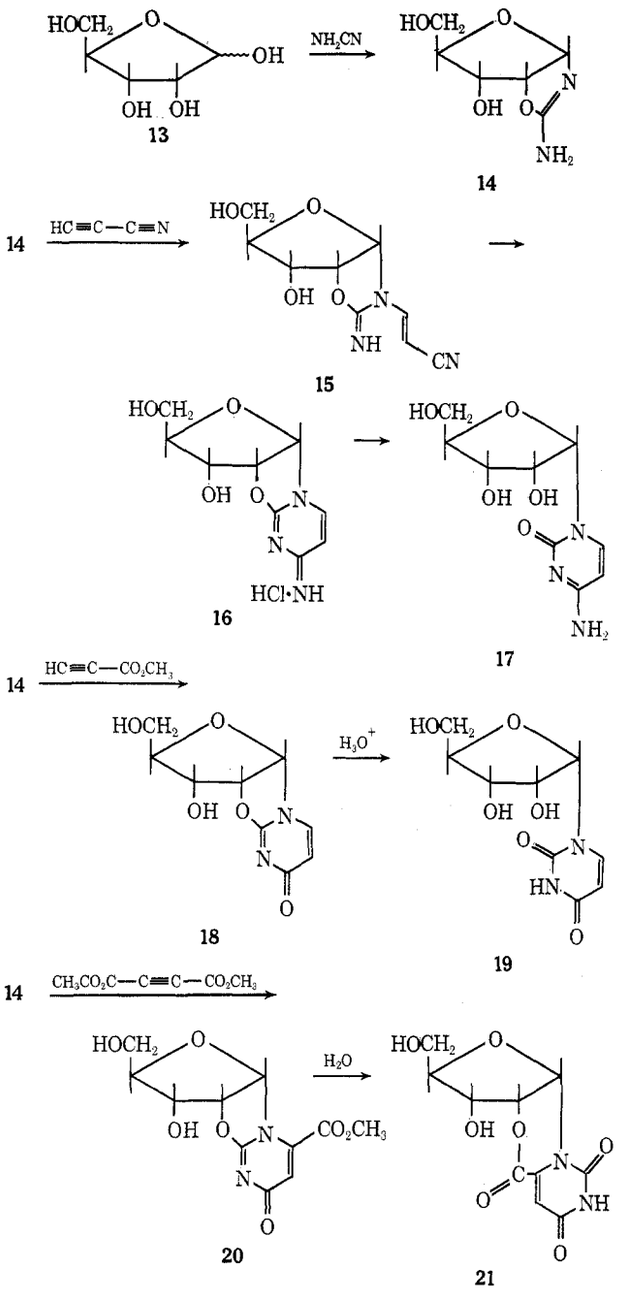
The aminooxazolines were found to react readily with a variety of electrophiles. Our results with propiolonitrile (cyanoacetylene), methyl propiolate, and dimethyl acetylenedicarboxylate are discussed here.

The arabinooxazoline **2** reacts rapidly with 1 molar equiv of propiolonitrile in *N*,*N*-dimethylacetamide at room temperature to yield a dark solution in which an open-chain cyanovinyl adduct **3** is thought to be the major component. This adduct is unchanged in the presence of anhydrous acids or by mild heating. However, in the presence of water or of aqueous acids it undergoes cyclization to 2,2′-anhydro-1-β-D-arabinofuranosylcytosine (**4**). We infer from this behavior

that **3** is possibly formed in a stable trans configuration, and that the function of water is to mediate an isomerization to the cis configuration, which then cyclizes spontaneously.

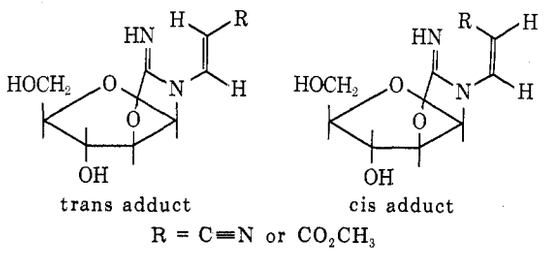
The pH of an aqueous solution of the free base **4** is sufficiently high to result in autohydrolysis, from which **5** is formed in quantitative yields.[2] In the presence of acids, however, **4** is stable. Both **4a** (acetate salt) and **5** may be isolated in yields of 80–90% based on **2**.

The reaction of the ribooxazoline **14** with propiolonitrile is presumed to occur predominantly *via* an analogous open-chain adduct **15**. However, the yield is



lower and tarry side products are also formed. 2,2′-Anhydro-1-α-D-ribofuranosylcytosine (hydrochloride salt, **16**) and 1-α-D-ribofuranosylcytosine (**17**) were isolated in yields of 51 and 35%, respectively.

The reaction of **2** with methyl propiolate in refluxing ethanol led to the formation of the *trans*-carbomethyoxyvinyl adduct **9** (18% yield) and of 2,2′-anhydro-1-β-D-arabinofuranosyluracil (**8**, 66–71% yield), the latter presumably resulting from the spontaneous cyclization of a *cis*-carbomethoxyvinyl adduct. The trans adduct **9** was thermally stable but could be photoisomerized to **8**, undoubtedly *via* the *cis*-carbomethoxyvinyl isomer.



The corresponding reaction of **14** with methyl propiolate in refluxing water gave 2,2′-anhydro-1-α-D-ribofuranosyluracil (**18**) in 27% yield. No attempt was made to isolate the corresponding *trans*-carbomethoxyvinyl adduct.

The aqueous hydrolyses of the β-anhydronucleosides **4** and **8** lead exclusively to the β-arabinosides **5** and **10**, respectively, as is already well known.[2,3] We find that the hydrolyses of the α-anhydronucleosides **16** and **18** also occur in high yield with retention of the 2′-oxygen configuration, yielding exclusively the α-ribosides **17** and **19**, respectively.

The reactions of **2** and **14** with dimethyl acetylenedicarboxylate in refluxing ethanol result in the formation of the methyl esters of the anhydroorotidine derivatives **11** (69% yield) and **20** (52% yield), respectively. In both cases conversion (to the corresponding lactones **12** and **21** could be achieved in boiling water.

Nucleophilic ring opening reactions (other than hydrolysis) of 2,2′-anhydro-1-β-D-arabinofuranosyluracil (**8**) and related anhydronucleosides have been described in the literature for a variety of nucleophiles. Anions such as I^-, Br^-, Cl^-, F^-,[8] N_3^-,[5] and thioacetate[9] have been reported to yield 2′-substituted 2′-deoxynucleosides in which the 2′ substituent was in the α configuration.

We decided to attempt the synthesis of certain 2′-substituted 2′-deoxycytidines by the application of related methods to the now easily available anhydrocytidine derivative **4**. In the past the preparation of such derivatives have generally involved a thiation–ammonation sequence applied to the corresponding uridine derivatives.[5,6]

When heated in dimethylformamide at 100°, the acetate salt **4** was converted to 2′(3′)-*O*-acetylcytidine (**6**) in *ca.* 60% yield. In a similar fashion the hydrofluoride salt **4b** was converted to the fluoronucleoside **6b** in 30% yield. Limiting factors in the synthesis of **6b** (and perhaps also of **6a**) appear to include a very facile hydrolysis of the starting anhydronucleoside as well as of the 2′-substituted product by small amounts of water, and the establishment of (or approach to) an equilibrium **4** ⇌ **6**. The conversion of **6b** to **4b** in dioxane at 100° has already been reported.[6]

When heated at 100° in HMPT, the phosphate salt **4c** was converted to a single phosphate ester, cytidine 2′,3′-cyclic monophosphoric acid (**7**), in 26% yield. Compound **7** was not an appreciable product from the

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reaction of cytidine 5′-monophosphoric acid, or of cytidine and orthophosphoric acid under the same conditions. On the other hand, cytidine 2′(3′)-monophosphoric acid was extensively cyclized to **7**. Therefore, we conclude that the predominant pathway in the conversion **6** → **7** proceeds *via* a direct ring opening of the anhydro link by orthophosphate to yield cytidine 2′-monophosphoric acid (**6c**) which then undergoes cyclization to **7**.

Discussion

The synthetic methods described in this paper provide easy routes to a variety of pyrimidine anhydronucleosides, many of which have not been previously described[10] or have been accessible only with difficulty. Because our method utilizes free sugars as starting materials, it is equally applicable, for example, to the synthesis of L nucleosides from the readily available L-arabinose.[11,12] A great variety of nucleosides could be generated by varying the sugars and electrophiles used in the standard synthesis.

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Registry No.—**2**, 36994-58-8; **4a**, 10212-28-9; **4b**, 36963-54-9; **6a**, 36963-55-0; **6b**, 10212-20-1; **9**, 36963-57-2; **11**, 36963-58-3; **12**, 33886-19-0; **14**, 27963-97-9; **16**, 36963-61-8; **18**, 27964-04-1; **20**, 36959-85-0; **21**, 36959-86-1.

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Elimination Reactions on the Di- and Trimesylated Derivatives of N^3-Benzyluridine

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To investigate the base-catalyzed elimination reactions on multiply mesylated pyrimidine ribonucleosides, N^3-protected uridine derivatives, 3-benzyl-2′,3′-di-*O*-mesyluridine (**7**) and 3-benzyl-2′,3′,5′-tri-*O*-mesyluridine (**8**), were synthesized as model compounds, which would be less likely to undergo cyclonucleoside formation. Sodium benzoate catalyzed elimination reaction on **7** and **8** gave the 2′-uridinenes, **9** and **17**, with a mesyloxy group at $C_{2'}$, which were converted to the crystalline 2′-uridinenes, **10** and **11**. Treatment of **10** with potassium carbonate gave a new class of compound, *endo*-3-(3-benzyluracil-1-yl)-2-oxabicyclo[3.1.0]-4-oxocyclohexane (**13**). **8** with sodium acetate and sodium iodide gave 5′-substituted compounds, **18** and **19**, respectively. **8** with potassium carbonate gave 2′,3′-epoxy nucleoside (**20**) and **13**. This suggests the intervention of two synchronous reaction paths.

Although didehydronucleosides are potentially useful intermediates for the transformations of the sugar moieties of nucleosides, examples of their use in synthesis are limited.[1,2] This reflects the fact that this class of compounds are less accessible than the cyclonucleosides. In the pyrimidine series, 2′,3′-[3-6] and 3′,4′-unsaturated[7] nucleosides have been obtained by base-catalyzed elimination reactions. An elegant synthesis of 4′,5′-unsaturated uridine was also reported.[8] However, similar investigations on the introduction of 2′,3′-unsaturated bonds into the ribonucleosides are quite few.[4,9] This spurred us to examine the direction of base-catalyzed elimination reactions on N^3-protected uridine derivatives, where cyclonucleoside formation was considered less probable.

This paper deals with a simple revised method for selective N^3-benzylation of uridine and its derivatives, and the results of some elimination studies on their di- and tri-O-mesylated derivatives.

Preparation of the Starting Materials for the Elimination Reactions.—Benzylation of uridine and its derivatives was studied previously for the purpose of working out selective 2′-*O*-benzylation required for ribooligonucleotide synthesis.[10-12] In these cases, concomitant N^3-benzylation was also noted. For the present purpose, it was necessary to find better reaction conditions for the selective N^3-benzylation. Combination of benzyl chloride and potassium carbonate in a mixture of acetone and *N*,*N*-dimethylformamide (DMF) as a medium eventually proved to be satisfactory. Thus, 5′-*O*-trityluridine (**1**) (Scheme I) with a slight excess of benzyl chloride and potassium carbonate gave exclusively 3-benzyl-5′-*O*-trityluridine (**2**) after 3-hr reflux in a mixture containing equal amounts of acetone and DMF. The use of benzyl bromide revealed at least one more product in a lesser amount. Detritylation of **2** gave 3-benzyluridine (**3**)[11,12] in good yield. To establish structure **3**, 2′,3′-*O*-isopropylideneuridine (**4**) was benzylated to give 3-benzyl-2′,3′-*O*-isopropylideneuridine (**5**) as a homogeneous foam whose nmr spec-

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