The Convenient Synthesis of Anhydronucleosides *via* the 2′,3′-*O*-Sulfinate of Pyrimidine Nucleosides as the Active Intermediates

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The synthesis of 2,2′-*O*-anhydro(1-β-D-arabinofuranosyl) cytosine (**3a**) and 2,2′-*O*-anhydro(1-β-D-arabinofuranosyl) uracil (**3b**) is described. The active intermediates, 2′,3′-*O*-sulfinyl cytidine (**2a**) and 2′,3′-*O*-sulfinyl uridine (**2b**), were prepared from the corresponding nucleosides (**1a** or **1b**) by successive treatment with thionyl chloride in acetonitrile and with water. The 2,2′-*O*-anhydropyrimidine nucleosides were formed quantitatively by heating the intermediates in an acidic aqueous solution or in *N*,*N*-dimethylformamide in the presence of sodium acetate. The treatment of the anhydronucleosides with alkali gave the corresponding arabinonucleosides.

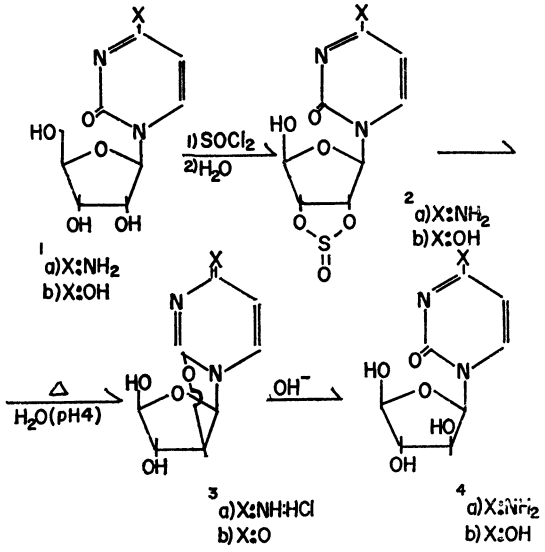
Anhydronucleosides are important modifications of natural nucleosides and have been used as intermediates in the chemical synthesis of arabinonucleosides. 2,2′-*O*-Anhydro-1-β-D-arabinofuranosyl cytosine has been shown to be an useful intermediate[1–3] for the synthesis of a carsinostatic nucleoside, 1-β-D-arabinofuranosyl cytosine,[4] and by itself to be a potent carsinostatic agent.[5] Anhydronucleosides have been synthesized by several procedures from natural nucleosides *via* the corresponding activated intermediates, such as tosyl,[6,7] mesyl,[8,9] carbonyl[10,11] and thiocarbonyl[12] derivatives, but most of those procedure involve tedious steps. Recently, 2,2′-*O*-anhydro(1-β-D-arabinofuranosyl)cytosine was successfully synthesized directly from cytidine with a Vilsmeier-Haack reagent.[13] We wish to describe another simple method for the synthesis of anhydronucleosides.

Results and Discussion

The synthesis of anhydronucleosides is outlined in Scheme 1. The essential step in the scheme is the introduction of a good leaving group at the 2′ and 3′ positions in the ribose moiety of natural nucleosides. In order to establish a convenient method usable on a large scale, a cyclic sulfinyl group was used as the leaving group; it was considered to be more active than either the cyclic carbonyl group[10,11] or the cyclic thiocarbonyl group.[12]

Thionyl chloride, which is readily obtainable on a commercial scale, was reacted with cytidine (**1a**) in dry acetonitrile at room temperature for 2 hr. Paper chromatography showed a single spot with an R_f-value of 0.7, different from that of **1a** (R_f 0.33). The reaction product was isolated in an 80% yield by adding a small amount of water to the reaction mixture. The structure of the acylation product was identified as 2′,3′-*O*-sulfinyl cytidine hydrochloride (**2a**), which was negative to a periodate-benzidine test[14] and which gave an ultraviolet spectrum similar to that of **1a**. The alkali hydrolysis of **2a** eliminated the cyclic sulfinyl group and so yielded **1a**. The **2a** product had three fairly sharp bands due to S=O stretching at 1010, 1021, and 1053 cm^{-1}, and three sharp bands associated with the five-membered cyclic sulfinyl group at 1201, 1205, and 1210 cm^{-1} in the infrared spectrum.[15] The elemental analysis of **2a** agreed with the proposed structure. The structure of **2a** was further confirmed by a subsequent reaction in which it was converted into 1-β-D-arabinofuranosyl cytosine (**4a**) through 2,2′-*O*-anhydro(1-β-D-arabinofuranosyl) cytosine hydrochloride (**3a**).

In the acidic solution, **2a** was stable at room temperature but was transformed into **3a** at an elevated tem-



Scheme 1.

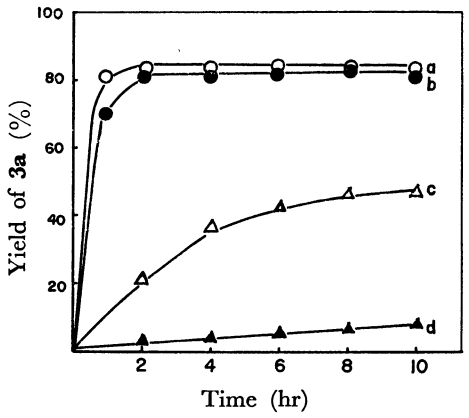


Fig. 1. Effect of reaction temperature on the preparation of 2′,3′-*O*-sulfinyl cytidine (**3a**).
Experimental conditions:
CH_3CN 10 ml, $SOCl_2$ 15 mmol, cytidine 3 mmol at pH 4.0, at the temperature of a) 100 °C, b) 80 °C, c) 60 °C, d) 40 °C.

Table 1. Properties of nucleoside derivatives

Compd.	Mp (dec.)	R_f, P. C.		$[\alpha]_D^{20}$	UV spectrum[a]	
		solv. A	solv. B		λ_{max}	λ_{min}
1a	220—230 °C	0.33	0.53	29.9	280	241
2a	253—254 °C	0.70	—	−50.4	278	240
3a	262 °C		0.81	−22.0	232, 263	243
4a	215 °C	0.33	0.65	158.0	280	241
1b	165 °C	0.51	0.54	4.0	263	230
2b	104—105 °C	0.69	—	—	260	229
3b	234—236 °C	0.48	0.67	11.0	223, 252	236
4b	215—216 °C	0.48	0.58	116.8	263	230

a) at pH 2.0

perature (Fig. 1). When **2a** was refluxed in the acidic aqueous solution at pH 4.0, the reaction went essentially to completion after 1 hr to form a 2,2′-*O*-anhydro-linkage and it could be heated at 80 °C for 2 hr without any deterioration in the reaction yield. By anion-exchange column chromatography, **3a** was isolated in a 74.7% yield and was identified as 2,2′-*O*-anhydro(1-β-D-arabinofuranosyl)cytosine hydrochloride by a comparison of its physical properties with those of an authentic sample.[13] The overall yield of **3a** from **1a** could be increased to 72.8% by omitting the isolation of the intermediate, **2a**.

Uridine (**1b**) was reacted with thionyl chloride under the same conditions as **1a** to give 2′,3′-*O*-sulfinyl uridine (**2b**) as a single product, as was shown by paper chromatography. Its chromatographic behavior and ultraviolet absorption properties are shown in Table 1. By heating **2b** in *N*,*N*-dimethylformamide (DMF) at 80 °C in the presence of sodium acetate, 2,2′-*O*-anhydro-(1-β-D-arabinofuranosyl)uracil (**3b**) was obtained from **1b** in an overall yield of 46.7%. The physical properties of **3b** were in good agreement with those of an authentic material.[11] Compounds **3a** and **3b** were easily converted to 1-β-D-arabinofuranosyl cytosine (**4a**) and 1-β-D-arabinofuranosyl uracil (**4b**) respectively by treating them with alkali. The physical properties of **4a** and **4b** are listed in Table 1; they are identical with those of authentic samples.

The method of synthesizing anhydronucleosides described above is superior to that of Kikugawa[13] or Furukawa[11] in respect to the yield and the simplicity of the procedure.

Experimental

The ultraviolet spectra were recorded on a Hitachi DPS-3T spectrophotometer, and the infrared spectra, on a Hitachi EPI-G2 apparatus. The paper chromatography of the reaction products was carried out by the ascending technique on Toyo-Roshi No. 51 paper (40×40 cm) using the following solvent systems: Solvent A; *n*-butanol-acetic acid-water (4 : 1 : 5); Solvent B; isopropanol-saturated ammonium sulfate-1M-sodium acetate (2 : 79 : 19).

2′,3′-O-Sulfinyl Cytidine Hydrochloride Obtained by the Reaction of Cytidine with Thionyl Chloride. Freshly distilled thionyl chloride (6 g, 50 mmol) was placed in 30 ml of acetonitrile, and the mixture was set aside at room temperature. To the solution was then added cytidine (2.6 g, 10 mmol), and the mixture was stirred at room temperature for 2 hr. A 15-ml portion (0.8 mol) of water was then added to the reaction mixture to destroy the reactant under vigorous stirring. Paper chromatography showed a single spot having an R_f value of 0.7, an aqueous extract of which showed the absorption maximum at 278 nm (pH 1.0). The resulting reaction mixture was kept in the refrigerator overnight, and then the white precipitate thus obtained was collected by filtration, washed with water and then twice with methanol, and dried to yield 2.63 g (81.0%) of 2′,3′-*O*-sulfinyl cytidine hydrochloride; mp 253—254 °C (dec.), $[\alpha]_D^{20}$-50.4° (*c*, 0.5, H_2O). The UV and chromatographic properties are shown in Table 1.

Found C; 33.30, H; 3.70, N; 11.30, S; 9.30, Cl; 11.53%. Calcd for $C_9H_{12}N_3O_6SCl$, C; 33.18, H; 3.69, N; 11.90, S; 9.83; Cl; 10.91%.

*2,2′-O-Anhydro(1-β-D-arabinofuranosyl) Cytosine Hydrochloride (**3a**).* *From 2′-3′-O-Sulfinyl Cytidine Hydrochloride (**2a**):* 2′,3′-*O*-Sulfinyl cytidine hydrochloride (32.5 g, 100 mmol) was dissolved into 1 l of water, and then the solution was adjusted to pH 4.0 with 6M-sodium hydroxide. After refluxing the resulting solution for 4 hr, the reaction mixture was cooled and was then applied to a Diaion W.A.-30 (acetate form) column (2.5×40 cm). The column was washed with 3 l of water, the washings were evaporated *in vacuo* to about 200 ml after the pH of the solution had been adjusted to 4.0 with 6M-hydrochloric acid. A 300-ml portion of ethanol was added to the concentrated solution, and the solid thus precipitated was collected by filtration, washed twice with ethanol, and dried to give 19.5 g (78.4% yield) of 2,2′-*O*-anhydro(1-β-D-arabinofuranosyl) cytosine hydrochloride. Mp 262 °C (dec.), $[\alpha]_D^{22}$ −22° (*c*, 0.5, H_2O). The UV and chromatographic properties are shown in Table 1. Found, C; 41.30, H, 4.65, N; 15.85, Cl; 13.60%. Calcd for $C_9H_{11}O_4N_3$ HCl, C; 41.32, H; 4.63; N; 16.07, Cl; 13.61%.

*From Cytidine (**1a**):* Cytidine (2.6 g, 10 mmol) and thionyl chloride (6 g, 50 mmol) were mixed in acetonitrile (30 ml) at room temperature for 2 hr, and then the reaction mixture was poured into ice water (1 l). The aqueous solution was adjusted to pH 4.0 with 6M-sodium hydroxide and then refluxed for 2 hr. The resulting solution was cooled to room temperature and treated essentially by the above method. 1.9 g of 2,2′-*O*-anhydro(1-β-D-arabinofuranosyl) cytosine hydrochloride was obtained as white needles in the yield of 72.8% from material cytidine. mp 262—263 °C (dec.), $[\alpha]_D^{20}$ −22° (*c*, 0.5, H_2O). Found, C; 41.28, H; 4.70, N; 16.01, Cl; 13.63%.

*1-β-D-Arabinofuranosyl Cytosine (**4a**).* 2,2′-*O*-Anhydro-(1-β-D-arabinofuranosyl) cytosine hydrochloride (52 g, 200 mmol) was dissolved in 3 l of water, after which the solution was adjusted to pH 10.0 with 2M-sodium hydroxide. The solution was allowed to stand at room temperature for

1 hr and was then applied to an Amberlite 200 (H^+ form) column (5×200 cm). The column was washed with water and eluted with 2 l of 1M-ammonium hydroxide. Fractions having an UV absorption at 260 nm (as checked on an UV spectrophotometer) were collected and concentrated to dryness. The residual crystalline product was recrystallized from ethanol to give 43.7 g of 1-β-D-arabinofuranosyl cytosine; mp 215 °C (dec.), $[\alpha]_D^{20}+158°$ (*c*, 0.5, H_2O). Found, C; 44.49, H; 5.41, N; 17.21%. Calcd for $C_9H_{12}O_5N_3$, C; 44.44, H; 5.39, N; 17.28%.

*2′,3′-O-Sulfinyl Uridine (**2b**).* Into a mixed solution of thionyl chloride (20 g, 160 mmol) and acetonitrile (100 ml) was vigorously stirred uridine (10 g, 40 mmol), and then the reaction mixture was maintained for 4 hr at 5 °C. After water (4.3 g, 240 mmol) had been added to the resulting mixture, the white precipitates were collected, washed with methanol, and then dried to yield 11.1 g (85%) of 2′,3′-*O*-sulfinyl uridine dihydrate. mp 104—105 °C (dec.), Found; C; 33.04, H; 2.98, N; 8.49, S; 9.78%. Calcd for $C_9H_{10}O_2N_7S\cdot 2H_2O$ C; 33.13, H; 3.06, N; 8.58, S; 9.81%.

*2′,3′-O-Anhydro(1-β-D-arabinofuranosyl) Uracil (**3b**)* 2′,3′-*O*-Sulfinyl uridine dihydrate (6.96 g, 21 mmol) and sodium acetate (7.9 g, 96 mmol) were heated in *N,N*-dimethylformamide (400 ml) at 80 °C for 4 hr and then cooled. The solid thus precipitated was filtered off and washed with 50 ml of DMF. The filtrate and washings were combined and concentrated to dryness. The resulting syrup was crystallized from ethanol. The crystalline product (4.7 g) was recrystallized from water to give 2.7 g of 2,2′-*O*-anhydro-(1-β-D-arabinofuranosyl) uracil; mp 234—236 °C (dec.), $[\alpha]_D^{20}-11.0°$ (*c*, 0.5, H_2O). Found, C; 48.15, H; 4.41, N; 12.40%. Calcd for $C_9H_{10}N_2O_5$, C; 48.23, H; 4.45, N; 12.41%.

*1-β-D-Arabinofuranosyl Uracil (**4b**).* 2,2′-*O*-Anhydro(1-β-D-arabinofuranosyl) uracil (1.1 g, 5 mmol) was dissolved in 100 ml of water, and then the solution was adjusted to pH 10.0 with 2M-ammonium hydroxide. The mixture was allowed to stand at room temperature for 1 hr and was then applied to a column containing 20 g of active carbon, granule type. The column was subsequently washed with water and eluted with 50 ml of 0.5 M-ammonium hydroxide. The effluent was evaporated *in vacuo*. The crystallization of the residue from ethanol afforded 1.03 g of 1-β-D-arabinofuranosyl uracil, mp 215—216 °C (dec.), $[\alpha]_D^{20}+114.3°$ (*c*, 0.5, H_2O). Found, C; 44.29, H; 5.01, N; 11.40%. Calcd for $C_9H_{12}N_2O_6$, C; 44.26, H; 4.92, N; 11.48%.

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