TABLE IV
PHYSICAL PROPERTIES OF 2,4-DINITROPHENYLHYDRAZONE DERIVATIVES ISOLATED FROM THE PYROLYSIS PRODUCTS

	Found				Literature			
			Uv ($CHCl_3$)			Uv ($CHCl_3$)		
	R_f[b]	Mp, °C	λ_{max}, mμ[a]	$\epsilon \times 10^{-4}$	Mp, °C	λ_{max}, mμ	$\epsilon \times 10^{-4}$	Ref
2-Furaldehyde	0.32	222–224	388*	2.90	225	386	2.65	40
2,3-Butanedione (bis)	0.16	312–314	394*, 442	2.92	314–315			41
Pyruvaldehyde (bis)	0.11	298–301	394*, 444	3.81	299–300			41
					304–305			42
Acetaldehyde	0.30	164–165	354*	2.22	167	354	2.22	40
Glyoxal (bis)	0.07	330–333	390, 445*	2.42	326–328			41
					336–338			42

[a] Starred wavelengths denote major maxima. [b] In benzene.

tives produced a strong quenching effect on scintillation that was measured by using ^{14}C-labeled toluene as an internal standard. The counting efficiency varied within the range of 10–30% according to the sample and concentration.

Esr Spectroscopy.—Samples of the anhydro sugar (1 part) were mixed with ground glass (9 parts) and ground together thoroughly to ensure uniform mixing. The ground samples (4–7 mg) were accurately weighed into a 2-mm capillary tube. The tube was placed into the cavity of a Varian E-3 esr spectrometer heated with a specially designed variable-temperature accessory.

Registry No.—1,6-Anhydro-β-D-glucopyranose, 498-07-7.

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Studies on the Vilsmeier–Haack Reaction. IV.[1] Convenient Synthesis of 2,2′-Anhydro-1-β-D-arabinofuranosylcytosine (2,2′-Cyclocytidine) and Its Derivatives[2]

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A carcinostatic nucleoside, 2,2′-anhydro-1-β-D-arabinofuranosylcytosine (2,2′-cyclocytidine) (**5**), was prepared in a yield of 55% by treatment of cytidine (**4**) with Vilsmeier–Haack reagent **1** or **2**. 5′-Chloro-5′-deoxy-2,2′-anhydro-1-β-D-arabinofuranosylcytosine (**6**) and 2′,5′-dichloro-2′,5′-dideoxycytidine (**7**) were also prepared by prolonged treatment of **4** with **1**. Treatment of **5** and **6** with mild alkali gave 1-β-D-arabinofuranosylcytosine (**9**) and 5′-chloro-5′-deoxy-1-β-D-arabinofuranosylcytosine (**10**), respectively, whereas treatment of either of **6** and **7** with strong alkali gave 2′,5′-anhydro-1-β-D-arabinofuranosylcytosine (**11**).

2,2′-Anhydro-1-β-D-arabinofuranosylcytosine (2,2′-cyclocytidine) (**5**) has been shown to be an intermediate[3–5] for the synthesis of a carcinostatic nucleoside, 1-β-D-arabinofuranosylcytosine (**9**),[6] and by itself a potent carcinostatic agent.[7] 1-β-D-Arabinofuranosylcytosine (**9**) has been synthesized by several procedures, such as (a) from cytidine *via* 2,2′-anhydro intermediates,[3,8,9] (b) from 1-β-D-arabinofuranosyluracil,[10] or (c) from the appropriate sugars,[11–13] but most of these involve tedious steps. Recently, **5** and **9** were successfully synthesized[14] directly from **4** by use of a partially hydrolyzed phosphorus oxychloride.[15] We wish to report an improved method to prepare **5**, **9**, and their derivatives.

N,*N*′-Dimethylformamide (DMF) combines with inorganic acid halides to form active reagents (Vilsmeier–Haack reagents),[16–18] which are useful as formylating, halogenating, and dehydroxylating agents.[19] Thus, phosphorus oxychloride and thionyl chloride react with DMF to form the complex **1**[18] and the complex **2**,[17] respectively (Scheme I). The latter may be converted into the crystalline complex **3** by removal of sulfur dioxide,[17] and **3** re-forms **2** on addition of sulfur dioxide.[20] The reaction of nucleosides with the com-

(1) Part III: K. Kikugawa, M. Ichino, and T. Kawashima, *Chem. Pharm. Bull.*, **19**, 2466 (1971).

(2) (a) A preliminary account of part of this work has appeared in K. Kikugawa and M. Ichino, *Tetrahedron Lett.*, 867 (1970). (b) This paper was presented at the 91th Annual Meeting of the Pharmaceutical Society of Japan, April 8, 1971, Fukuoka, Japan. (c) The commonly applied term "2,2′-cyclocytidine" has been retained in the title for the convenience of "key word index" users.

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

(4) I. L. Doerr and J. J. Fox, *J. Org. Chem.*, **32**, 1465 (1967).

(5) W. V. Ruyle and T. Y. Shen, *J. Med. Chem.*, **10**, 331 (1967).

(6) S. S. Cohen, "Progress in Nucleic Acid Research and Molecular Biology," Vol. V, J. N. Davidson and W. E. Cohn, Ed., Academic Press, New York, N. Y., 1966, p 1.

(7) A. Hoshi, F. Kanzawa, K. Kuretani, M. Saneyoshi, and Y. Arai, *Gann*, **62**, 145 (1971).

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

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(12) B. Shimizu and F. Shimizu, *Chem. Pharm. Bull*, **18**, 1060 (1970).

(13) L. E. Orgel and R. A. Sanchez (*Chem. Eng. News*, Oct 6, 1969) synthesized **9** with a plausible prebiosynthetic route.

(14) T. Kanai, T. Kojima, O. Maruyama, and M. Ichino, *Chem. Pharm. Bull.*, **18**, 2569 (1970).

(15) Y. Furukawa and M. Honjo, *ibid.*, **16**, 2286 (1968).

(16) A. Vilsmeier and A. Haack, *Chem. Ber.*, **60**, 119 (1927).

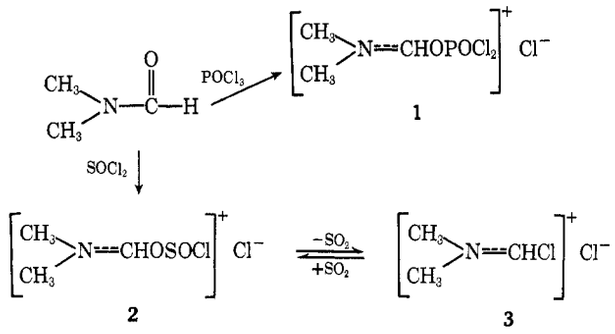
(17) H. H. Bosshard, R. Mory, M. Schmid, and H. Zollinger, *Helv. Chim. Acta*, **42**, 1653 (1959).

(18) H. H. Bosshard and H. Zollinger, *ibid.*, **42**, 1659 (1959).

(19) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," Wiley, New York, N. Y., 1967, p 284.

(20) K. Kikugawa and T. Kawashima, *Chem. Pharm. Bull.*, **19**, 2627 (1971).

SCHEME I



plex **3** have been studied by several researchers, affording nucleosides chlorinated at the base[21] or the sugar moiety.[22] This time, we studied the reaction of **1** or **2** with cytidine (**4**) and obtained the anhydro nucleoside **5** and its derivatives, **6** and **7**.

Cytidine (**4**) was treated with the complex **1** in DMF at room temperature for 3 hr and then the mixture was treated with water. Paper chromatography showed a major spot corresponding to the anhydro nucleoside **5**. It was isolated as a formate **5a** and then converted to a hydrochloride **5b** (yield 55%) by use of ion exchange column chromatographies. The product **5b** was identified as 2,2′-anhydro-1-β-D-arabinofuranosylcytosine hydrochloride by comparison of the physicochemical properties with those of the authentic sample.[3-5]

When **5b** was hydrolyzed with ammonia, **9** was obtained quantitatively. The yield of **9** from **4** can be increased to 60% by omitting the isolation of the intermediate **5**. Thus, this procedure constitutes a simple method to synthesize 1-β-D-arabinofuranosylcytosine (**9**) in contrast to other complicated methods.

Treatment of cytidine (**4**) with **1** was performed at room temperature for 24 hr (Scheme II). Paper chromatography showed another spot having an R_f value larger than that of **5**. The new product (**6**) was isolated from the aqueous reaction mixture in a yield of 65% by use of successive cation and anion exchange columns. The product (**6**) was identified as the 5′-chloro-5′-deoxy derivative of the anhydro nucleoside **5**. It is known that the 5′-hydroxyl function of nucleosides can be readily replaced by halogen atoms.[22,23] Treatment of **6** with mild alkali gave a monochlorinated 1-β-D-arabinofuranosylcytosine (**10**), which could be further converted into the compound **11** by treatment with strong alkali. Elemental analysis and ultraviolet absorption spectrum suggested that **11** was 2′,5′-anhydropentofuranosylcytosine. The compound **11** could be converted into the known 2′,5′-anhydro-1-β-D-arabinofuranosyluracil (**12**)[24] by treatment with nitrous acid. Hence the structure of **11** was firmly established to be 2′,5′-anhydro-1-β-D-arabinofuranosylcytosine. Thus, the structures of the reaction products, **6** and **10**, were elucidated to be 5′-chloro-5′-deoxy-2,2′-anhydro-1-β-D-arabinofuranosylcytosine hydrochloride and 5′-chloro-5′-deoxy-1-β-D-arabinofuranosylcytosine, respectively.

(21) (a) M. Ikehara, H. Uno, and F. Ishikawa, *Chem. Pharm. Bull.*, **12**, 267 (1964); (b) M. Ikehara and H. Uno, *ibid.*, **13**, 221 (1965); (c) J. Žemlička and F. Šorm, *Collect. Czech. Chem. Commun.*, **30**, 1880 (1965); (d) J. Žemlička, J. Smrt, and F. Šorm, *Tetrahedron Lett.*, 379 (1962).

(22) (a) R. F. Dods and J. S. Roth, *ibid.*, 165 (1969); (b) R. F. Dods and J. S. Roth, *J. Org. Chem.*, **34**, 1627 (1969).

(23) K. Kikugawa and M. Ichino, *Tetrahedron Lett.*, 87 (1971).

(24) I. L. Doerr, J. F. Codington, and J. J. Fox, *J. Org. Chem.*, **30**, 467 (1965).

Treatment of cytidine (**4**) with **1** for 240 hr gave a new product (**7**) containing two chlorine atoms in a yield of 70%. When **7** was heated at 80° for 1 hr in water, it was quantitatively converted into the anhydro compound **6**. Since 2′-chloro-2′-deoxycytidine can be easily converted into the anhydro nucleoside **5**,[4] the structure of **7** must be 2′,5′-dichloro-2′,5′-dideoxycytidine. Physicochemical properties of **7** supported the above structure. Treatment of **7** with ammonia afforded **10**, probably *via* the anhydro intermediate **6**, and with strong alkali afforded 2′,5′-anhydro compound **11** quantitatively.

Thus, cytidine (**4**) undergoes the transformation with **1** in the following sequence: (1) anhydro bond formation between the 2 and 2′ positions; (2) chlorination at the 5′ position; (3) cleavage of the 2,2′-anhydro bond with chlorine.

Treatment of cytidine (**4**) with the complex **2** also afforded **5**–**7**. In this case, however, 5′-chloro-5′-deoxycytidine (**8**), whose structure was established by comparison with the authentic sample,[23] was also produced. Thus, the yield of the anhydro compound **5** obtained was lower than that obtained by the complex **1**. Reaction of cytidine (**4**) with the crystalline complex **3** was also attempted, but no reaction was observed.

In order to prepare 5′-substituted derivatives of 1-β-D-arabinofuranosylcytosine, replacement of the 5′ chlorine atom of **10** with nucleophiles was attempted but was unsuccessful because it was readily attacked by the 2′-hydroxyl function affording **11**. Treatment of the anhydro compound **11** with acid gave cytosine (**13**) instead of **9**. This observation was not unexpected in view of the known lability of the glycosidic linkage of **12** toward acid affording uracil (**14**).[24] Cleavage of the anhydro ring of **11** by halide, azide, or benzylthio ion failed, although it is known that the anhydro ring in the 3′,5′-anhydroxylofuranosyl nucleosides can be attacked by these nucleophiles.[25]

Experimental Section[26]

2,2′-Anhydro-1-β-D-arabinofuranosylcytosine (5) by the Reaction of Cytidine (4) with 1.[29]—Phosphorus oxychloride (6.0 g, 39 mmol) was placed in 20 ml of DMF and the mixture was set aside at room temperature for 30 min. To the solution was added 1.0 g

(25) (a) J. P. Horwitz, J. Chua, J. A. Urbanski, and M. Noel, *ibid.*, **28**, 942 (1963); (b) Y. Mizuno, T. Ueda, K. Ikeda, and Y. Miura, *Chem. Pharm. Bull.*, **16**, 262 (1968); (c) K. Kikugawa, M. Ichino, and T. Ukita, *ibid.*, **17**, 785 (1969); (d) K. Kikugawa, M. Ichino, T. Kusama, and T. Ukita, *ibid.*, **17**, 798 (1969).

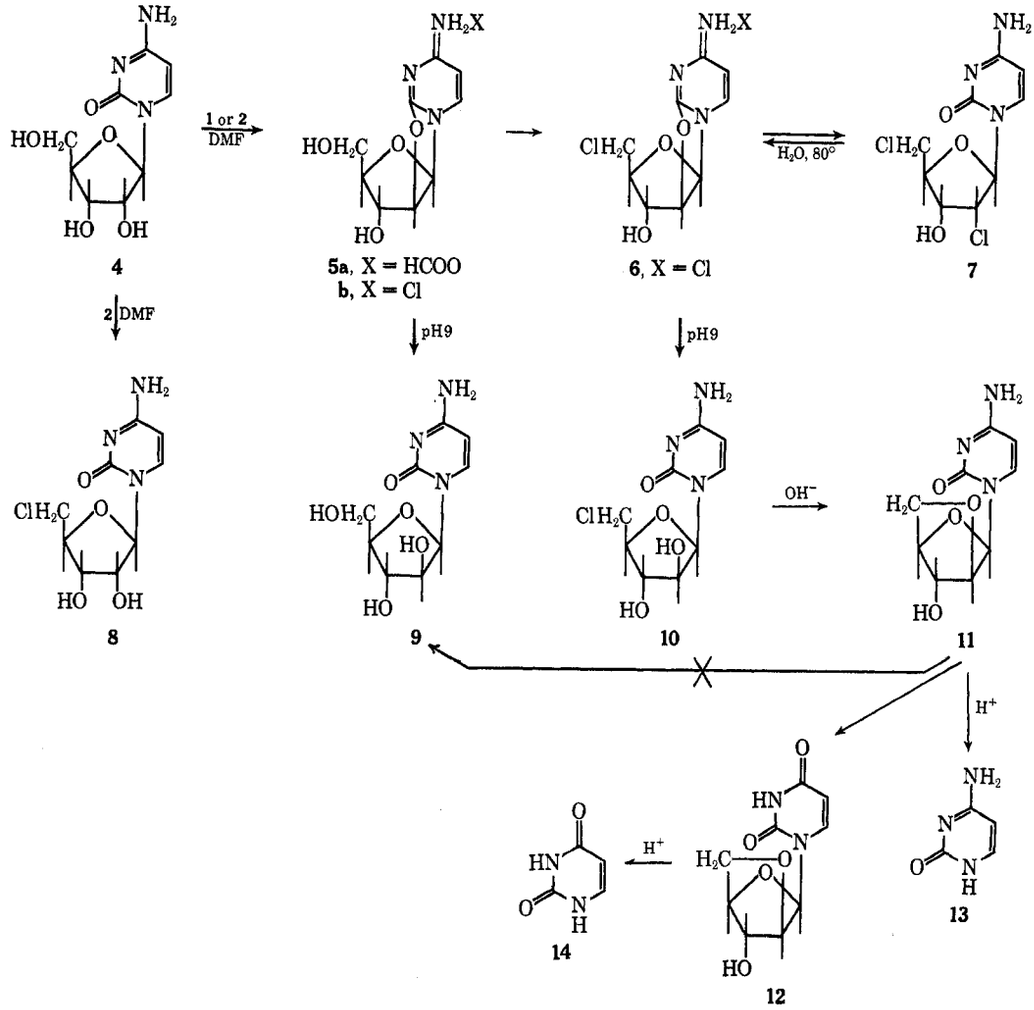
(26) Melting points were determined on a Büchi Schmerzpunktbestimungsapparat nach Dr. Tottli and not corrected. Ultraviolet absorption was measured with a Hitachi recording spectrophotometer, ESP-3T. Optical rotations were obtained with a JASCO automatic polarimeter, Model DIP-SL. Paper chromatograms were run by the ascending technique on Tōyō Roshi No. 51A paper, using the following solvent systems: (1) *i*-PrOH–1 *M* NH_4OAc (pH 4.0) (7:3); (2) *n*-BuOH–H_2O (84:16); (3) 5 *M* NH_4OAc–0.5 *M* EDTA·Na_4–saturated $Na_2B_4O_7$–EtOH (12:0.3:48:132);[27] (4) *i*-PrOH–NH_4OH–H_2O (7:1:2). The spots were detected under uv light and was represented by the symbol R_f with suffix corresponding to the number of the solvents. Paper electrophoresis was carried out in the borate buffer system (pH 6.0),[28] and the mobility was represented by the relative value to that of cytidine (**4**). TOD is total optical density.

(27) A. W. Schrecker and I. A. R. Mead, *Biochem. Pharmacol.*, **15**, 1443 (1966).

(28) J. F. Codington, R. Fecher, and J. J. Fox, *J. Amer. Chem. Soc.*, **82**, 2794 (1960).

(29) The complex **1** could be isolated as a gummy solid when $POCl_3$ was treated with an equimolar amount of DMF in anhydrous ether.[18] When **4** was treated with this gummy solid in DMF the same results as described here were obtained.

SCHEME II



(4.1 mmol) of cytidine (4) and the mixture was stirred at room temperature for 3 hr, then poured into 100 ml of water to destroy the reagent. The ultraviolet absorption spectrum of the aqueous reaction mixture showed the maxima at 260 and 320 nm, and the latter maximum completely disappeared after 3 hr standing at room temperature.[30] Paper chromatography showed one main spot having R_{f_1} 0.58 and R_{f_3} 0.73, an aqueous extract of which showed the absorption maxima at 232 and 262 nm (pH 1–6). The aqueous reaction mixture [$TOD_{280\ nm}$ (pH 1) 50,500] was applied to a Dowex 50 × 4 (pyridinium form) column (2.5 × 40 cm). The column was eluted with 0.1 *M* pyridinium formate (pH 4.8) to give **4** at the 1700–2300-ml fraction, and subsequently with 0.4 *M* pyridinium formate (pH 4.8) to give the product **5** at the 500–1300-ml fraction. The fraction containing the product **5** [$TOD_{280\ nm}$ (pH 1) 13,000] was evaporated to dryness after the pH of the solution was adjusted to 4.0 with formic acid in order to avoid the degradation of the product. Repeated evaporation of the residue with EtOH gave a gum. Crystallization from EtOH gave **5a** as granules which melted at 173–174° dec and weighed 735 mg, uv max (pH 1–6) 232 and 263 nm. It was redissolved in 20 ml of water and passed through a Dowex 1 × 4 (Cl^-) column (2 × 3 cm). The column was washed with 100 ml of water. The combined effluent and washings were evaporated to dryness to give a crystalline material. Recrystallization from aqueous EtOH gave **5b** as white needles which melted at 262–264° dec and weighed 615 mg (55%): uv max (pH 1–6) 231 nm (ε 9600), 263 (10,900), min (pH 1–6) 218 (7000), 243 (6600), shoulder (pH 1–6) 282 (3200); $[\alpha]^{20}D$ −21.0° (*c* 2, H_2O) [lit.[3] mp 248–250°; uv max (pH 1–7) 231 nm (ε 9400), 262 (10,600), min (pH 1–7) 243 (6500); $[\alpha]^{23}D$ −21.8° (*c* 2, H_2O)]; R_{f_1} 0.58, R_{f_2} 0.05, R_{f_3} 0.73.

(30) The substance having the characteristic absorption maxima at 260 and 320 nm could not be isolated because of its unstability. Thus, it seemed that the initial reaction of **4** with **1** afforded the unstable substitution at the cytosine moiety.

Anal. Calcd for $C_9H_{11}O_4N_3 \cdot HCl$: C, 41.32; H, 4.63; N, 16.07. Found: C, 41.44; H, 4.45; N, 16.30.

1-β-D-Arabinofuranosylcytosine (9). A. From 2,2′-Anhydro-1-β-D-arabinofuranosylcytosine (5b).—The compound **5b** (100 mg) was dissolved in 2 ml of water and the solution was adjusted to pH 9 with ammonia. The mixture was allowed to stand at room temperature for 15 min, acidified with HCl, and applied to a column (1 × 1.5 cm) of Dowex 50 × 4 (H^+). The column, which was washed well with water, was eluted with 50 ml of 1 *N* NH_4OH. The effluent was evaporated *in vacuo*. Crystallization of the residue from EtOH afforded 78 mg (90%) of the pure material of **9**: mp 210–212° dec; uv max (pH 1) 282 nm (ε 13,400), min (pH 1) 241 (1600), max (pH 7) 271 (9700), min (pH 7) 251 (6500); $[\alpha]^{20}D$ +158° (*c* 0.5, H_2O) [lit.[3] mp 212–213° dec; uv max (pH 1) 280 nm (ε 13,400), max (pH 13) 273.5 (10,000); $[\alpha]^{25}D$ +151° (*c* 0.5, H_2O)]; R_{f_2} 0.18, R_{f_3} 0.71; paper electrophoretic mobility +0.45.

Anal. Calcd for $C_9H_{13}O_5N_3$: C, 44.44; H, 5.39; N, 17.28. Found: C, 44.65; H, 5.21; N, 17.08.

B. By the Reaction of Cytidine (4) with 1.—The reaction mixture, containing 6.0 g of $POCl_3$, 1.0 g of **4**, and 20 ml of DMF, was stirred at room temperature for 3 hr. It was poured into 100 ml of water and the aqueous mixture was treated with ammonia at pH 9 and room temperature for 15 min. The mixture was reacidified with HCl and was applied to a column (2.5 × 40 cm) of Dowex 50 × 4 (H^+). The column, which was washed well with water, was eluted with 1.0 l. of 1 *N* NH_4OH. The eluate was evaporated *in vacuo* to dryness, giving a gummy residue. It was crystallized from EtOH to afford 604 mg (60%) of **9**, mp 208–211° dec.

5′-Chloro-5′-deoxy-2,2′-anhydro-1-β-D-arabinofuranosylcytosine (6). A. By the Reaction of Cytidine (4) with 1.—The reaction mixture, containing 6.0 g of $POCl_3$, 1.0 g of **4**, and 20 ml of DMF, was stirred at room temperature for 24 hr. Paper chromatography showed two spots having R_{f_1} 0.58 and 0.67, corre-

sponding to **5** and **6**, respectively. An aqueous extract of both of these spots showed identical absorption maxima at 232 and 264 nm (pH 1–6). To the reaction mixture was added 100 ml of water. The solution was then applied to a column of Dowex 50 × 4 (pyridinium form). The column was eluted with 0.1 *M* pyridinium formate (pH 4.8) to give two peaks at the 2500–4500-ml fraction (**5**) and at the 6000–8000-ml fraction (**6**). The fraction containing **6** was evaporated to dryness, and the residue was dissolved in 5 ml of water. The solution was passed through a column (2 × 3 cm) of Dowex 1 × 4 (Cl^-). The column was eluted with 50 ml of water. Effluent and washings were combined and evaporated to dryness. The residue was crystallized from EtOH to afford 750 mg (65%) of fine needles of **6**. Recrystallization from aqueous EtOH gave a pure sample of **6**: mp 263–265° dec; uv max (pH 1–7) 233 nm (ε 9900), 263 (11,300) min (pH 1–7) 244 (7800); $[\alpha]^{20}D$ −25.3° (*c* 0.5, H_2O); R_{f_1} 0.67, R_{f_2} 0.11, R_{f_3} 0.78.

B. From 2′,5′-Dichloro-2′,5′-dideoxycytidine (7).—2′,5′-Dichloro-2′,5′-dideoxycytidine (**7**) (100 mg) was dissolved in 2 ml of water and heated at 80° for 1 hr. The mixture was evaporated to dryness *in vacuo*, and a crystalline residue was obtained. Recrystallization from aqueous EtOH gave 80 mg (80%) of fine needles of **6**: mp 263–265° dec; uv max (pH 1–7) 231, 263 nm; R_{f_1} 0.67, R_{f_2} 0.11, R_{f_3} 0.78.

Anal. Calcd for $C_9H_{10}O_3N_3Cl \cdot HCl$: C, 38.60; H, 3.96; N, 15.01; Cl, 25.33. Found: C, 38.82; H, 4.00; N, 15.23; Cl, 24.97.

2′,5′-Dichloro-2′,5′-dideoxycytidine (7).—The reaction mixture, containing 12.0 g of $POCl_3$, 2.0 g of **4**, and 40 ml of DMF, was stored at room temperature for 240 hr. Paper chromatography showed a major spot having R_{f_1} 0.84, an aqueous extract of which showed an absorption maximum at 280 nm (pH 1). The reaction mixture was mixed with 2000 ml of water and was applied to a column of Dowex 50 × 4 (pyridinium form). The column was eluted with 0.1 *M* pyridinium formate (pH 4.0) to give a major peak at the 5000–8000-ml fraction. The fraction was evaporated to dryness *in vacuo* at below 40°. Repeated evaporation with EtOH gave a gummy residue which was crystallized from aqueous EtOH to give 1.61 g (70%) of **7**. Recrystallization from aqueous EtOH gave fine needles of **7**: mp 242–245° dec; uv max (pH 1) 282 nm (ε 13,500), max (pH 7) 272 (9600); $[\alpha]^{26}D$ +29° (*c* 0.25, H_2O); R_{f_1} 0.85, R_{f_2} 0.66, R_{f_3} 0.82; paper electrophoretic mobility 0.0.

Anal. Calcd for $C_9H_{11}O_3N_3Cl_2 \cdot {}^1/_2H_2O$: C, 37.40; H, 4.19; N, 14.54; Cl, 24.54. Found: C, 37.54; H, 4.18; N, 14.59; Cl, 24.18.

The compound is negative to HIO_4–benzidine reagent.[31]

5′-Chloro-5′-deoxy-1-β-D-arabinofuranosylcytosine (10). A. By the Reaction of Cytidine (4) with 1.—The reaction mixture, containing 6.0 g of $POCl_3$, 1.0 g of **4**, and 20 ml of DMF, was kept at room temperature for 240 hr. The mixture was treated with water and then with ammonia, and desalted as in method B of the preparation of **9**, affording a residual gum. Paper chromatography of the residue showed a major spot having R_{f_2} 0.52 corresponding to **10** and two minor spots having R_{f_2} 0.66 and 0.26, corresponding to **7** and **11**, respectively. Compound **10** was isolated from the residue in a yield of 55% (590 mg) by use of a cellulose column (1.8 × 57 cm) with the elution solvent, *n*-BuOH–H_2O (84:16). Recrystallization from aqueous EtOH gave white needles of **10**: mp 202–204.5° dec; uv max (pH 1) 281 nm (ε 13,550), min (pH 1) 241 (1580), max (pH 7) 272 (9650), min (pH 7) 251 (6270); $[\alpha]^{20}D$ +163.8° (*c* 0.5, H_2O); R_{f_1} 0.72, R_{f_2} 0.52, R_{f_3} 0.78.

Anal. Calcd for $C_9H_{12}O_4N_3Cl$: C, 41.30; H, 4.62; N, 16.06; Cl, 13.55. Found: C, 41.05; H, 4.59; N, 16.16; Cl, 13.16.

B. From 5′-Chloro-5′-deoxy-2,2′-anhydro-1-β-D-arabinofuranosylcytosine (6).—5′-Chloro-5′-deoxy-2,2′-anhydro-1-β-D-arabinofuranosylcytosine (**6**) (100 mg) was dissolved in 5 ml of water and the mixture was adjusted to pH 9 with ammonia. After standing at room temperature for 15 min it was evaporated to dryness. Crystallization from aqueous EtOH afforded 65 mg of **10**, mp 202–204° dec.

2′,5′-Anhydro-1-β-D-arabinofuranosylcytosine (11). A. By the Reaction of Cytidine (4) with 1.—The desalted reaction mixture obtained as in method A of the preparation of **10** was applied to a column (2.5 × 40 cm) of Dowex 1 × 4 (OH^-),[32] which was eluted with 30% MeOH. From the 2000–3000-ml fraction 740 mg (80.0%) of **11** was obtained. Recrystallization from aqueous EtOH gave pure needles of **11**: mp 257–258° dec; uv max (pH 1) 282.5 nm (ε 13,400), min (pH 1) 242 (1300), max (pH 7) 273 (9300), min (pH 7) 250 (5000); $[\alpha]^{20}D$ +232.3° (*c* 0.5, H_2O); R_{f_1} 0.57, R_{f_2} 0.26, R_{f_3} 0.72, R_{f_4} 0.63; paper electrophoretic mobility 0.0.

Anal. Calcd for $C_9H_{11}O_4N_3$: C, 47.99; H, 4.93; N, 18.66. Found: C, 48.17; H, 5.17; N, 18.27.

The compound is negative to HIO_4–benzidine reagent.[31]

B. From 5′-Chloro-5′-deoxy-1-β-D-arabinofuranosylcytosine (10).—5′-Chloro-5′-deoxy-1-β-D-arabinofuranosylcytosine (**10**) (100 mg) was dissolved in 2 ml of 2 *N* NaOH and heated at 80° for 1 hr. The mixture was acidified with HCl and absorbed to a Dowex 50 × 4 (H^+) column (2 × 3 cm). The column, which was washed well with water, was eluted with 20 ml of 1 *N* NH_4OH, and the effluent was evaporated to dryness. Crystallization from EtOH gave 70 mg of white needles of **11**, mp 257–258° dec.

2′,5′-Anhydro-1-β-D-arabinofuranosyluracil (12).—2′,5′-Anhydro-1-β-D-arabinofuranosylcytosine (**11**) (300 mg) was treated with 1.5 g of $NaNO_2$, 2.2 ml of AcOH, and 5 ml of water at room temperature for 3 hr. After the mixture was diluted with 10 ml of water, it was passed successively through columns of Dowex 50 × 4 (20 ml) and Dowex 1 × 4 (HCO_3^-) (5 ml). Combined eluate and washings (about 100 ml) were evaporated to dryness, affording 254 mg (85%) of the crystalline product **12**. Recrystallization from aqueous EtOH gave white needles of **12**: mp 258–259.5° dec; uv max (pH 7) 265 nm (ε 10,500), min (pH 7) 233 (2000), max (pH 13) 265 (8500), min (pH 13) 242 (4600); $[\alpha]^{20}D$ +206.3° (*c* 0.3, H_2O) [lit.[24] mp 260–262° eff dec; uv max (pH 6.9) 264 nm (ε 10,700), min (pH 7) 231 (1900), max (1 *N* NaOH) 264 (8400), min (1 *N* NaOH) 240 (4270); $[\alpha]^{23}D$ +193° (*c* 0.3, H_2O)]; R_{f_2} 0.34.

Anal. Calcd for $C_9H_{10}O_5N_2$: C, 47.79; H, 4.42; N, 12.39. Found: C, 47.91; H, 4.31; N, 12.40.

A mixture of **12** and an authentic compound,[24] mp 257–259° dec, melted at 257–258.5° dec.

Reaction of Cytidine (4) with 2.[33]—Thionyl chloride (3.0 ml) was dissolved in 20 ml of DMF and the mixture was set aside at room temperature for 30 min. To the solution was added 2.0 g of **4** and the mixture was stirred at room temperature for 3 hr. It was then poured into about 50 ml of water and the aqueous solution was stirred for 1 hr to remove sulfur dioxide that evolved by the decomposition of the reagent. The product (**5a**) was isolated in a yield of 30% by a procedure similar to that described in the preparation of **5a** using **1**. **5a** was converted into **5b**, which melted at 262–264° dec: uv max (pH 1–6) 231 nm (ε 9600), 262.5 (10,800), min (pH 1–6) 218 (7100), 243 (6700), shoulder (pH 1–6) 282 (3300); $[\alpha]^{20}D$ −22° (*c* 2, H_2O); R_{f_1} 0.58, R_{f_2} 0.05.

The reaction mixture, containing 3 ml of $SOCl_2$, 2 g of **4**, and 20 ml of DMF, was allowed to stand at room temperature for 240 hr. After addition of water, the mixture was absorbed to Dowex 50 × 4 (H^+) (2.5 × 40 cm). The column was eluted with 1 *N* NH_4OH, and the effluent was evaporated to dryness *in vacuo*. Paper chromatography of the residue showed four spots having R_{f_2} 0.26, 0.34, 0.52, and 0.66 corresponding to **11**, **8**, **10**, and **7**, respectively. From the gummy residue 5′-chloro-5′-deoxycytidine (**8**), which melted at 167–170° dec, was isolated in a yield of 20%. Paper chromatographic comparison of **8** (R_{f_2} 0.34) with the authentic sample,[23] and the mixed fusion test confirmed the structure of **8**. From the mother liquor, 2′,5′-anhydro compound **11**, which melted at 257–258° dec, was isolated in a yield of 35% by use of a Dowex 1 × 4 (OH^-)[32] column.

Attempted Cleavage of the Anhydro Ring of 11.—2′,5′-Anhydro-1-β-D-arabinofuranosylcytosine (**11**) (10 mg) was treated with 0.15 ml of 0.4 *N* H_2SO_4 at 100° for 2.5 hr. Paper chromatography revealed a spot having R_{f_4} 0.53, identical with that of cytosine **13**, an aqueous extract of which showed the absorption maxima at 268 (pH 7) and 282 nm (pH 13). Several attempts to open the anhydro ring of **11** by nucleophiles such as NaI–AcOH, complex **3**–$CHCl_3$, LiN_3–DMF, and $NaSCH_2Ph$–MeOH under heated conditions were made but they were unsuccessful.

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(33) The complex **2** could also be formed by the addition of sulfur dioxide to the complex **3**.[20] When **4** was treated with this fuming liquid in DMF, the same results were obtained.

Registry No.—**1**, 28528-49-6; **2**, 25575-32-0; **4**, 65-46-3; **5a**, 26790-12-5; **5b**, 10212-25-6; **6**, 32659-29-3; **7**, 32659-30-6; **9**, 147-94-4; **10**, 32659-31-7; **11**, 32830-01-6; **12**, 3257-75-8.

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Nucleoside Peptides. III. The Synthesis of *N*-[1-(9-Adenyl)-β-D-ribofuranuronosyl] Derivatives of Certain Amino Acids and Peptides

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Benzyl esters of several amino acids and peptides have now been successfully coupled with 1-(9-adenyl)-2,3-*O*-isopropylidene-β-D-ribofuranuronic acid (**1**) by the DCC method to afford *N*-[1-(9-adenyl)-2,3-*O*-isopropylidene-β-D-ribofuranuronosyl]amino acid and peptide benzyl esters. Concomitant acylurea side-product formation was inhibited by the addition of *N*-hydroxysuccinimide. The title compounds were produced in excellent yields when the isopropylidene and benzyl blocking groups were removed by acid hydrolysis and catalytic hydrogenolysis, respectively. These procedures provide a general method for the attachment of the amino terminus of an amino acid or peptide to a carboxylic acid moiety of a nucleoside.

Recently there has been a great deal of interest in the isolation and synthesis of nucleoside amino acids and peptides.[1-3] Reasons for the preparation and study of this class of compounds has been outlined in an earlier publication submitted from these laboratories.[4] Most syntheses of nucleoside peptides have involved either the coupling of an amino[1,2,4] or hydroxyl[5] group of a nucleoside to the carboxyl group of a blocked amino acid or displacement of a leaving group on a nucleoside by the amino group of an amino acid.[6] In one report[7] purine and pyrimidine ribofuranuronic acids have been coupled to unblocked high molecular weight polypeptides in yields ranging from 2 to 10%. The work described in this article has provided a general method for the coupling of the amino terminus of an amino acid or peptide to a free carboxylic acid moiety of a nucleoside in good yield.

1-(9-Adenyl)-2,3-*O*-isopropylidene-β-D-ribofuranuronic acid (**1**) was selected as the nucleoside reagent because of its solubility properties and ease of preparation.[8a,b] *N*,*N*′-Dicyclohexylcarbodiimide (DCC) was chosen to effect coupling, since it has been known to provide peptide linkages in high yield with little or no racemization.[9] When **1** was coupled to various amino acid benzyl esters by the action of DCC, yields ranging from 40 to 50% of the desired products (**2**) were obtained (Scheme I). Purification was complicated by the presence of a second product (**3**), 15–30% yields, from which **2** could not be readily separated. Consideration of the mechanism of action of DCC proposed by Khorana and coworkers[10,11] led to the assumption that this by-product could be the acylurea adduct[12,13] of **1** and DCC. This assumption was substantiated by elemental analysis. Examination of its infrared spectrum, which exhibited a strong band at 1640 cm^{-1} (-NHCONH, ~1660 cm^{-1}),[14] suggested that this by-product was actually *N*-acylurea (**3**) rather than the *O*-acylisourea.[10]

Attempts to suppress the formation of acylurea by-product by changing the solvent medium to methylene chloride[12] were without success. Addition of *N*-hydroxysuccinimide (NHS) with DCC has been shown to improve the yields in peptide syntheses[15] without increasing racemization;[16] therefore **1** was coupled to glycine benzyl ester in the presence of DCC and NHS and gave a 91% yield of *N*-[1-(9-adenyl)-2,3-*O*-isopropylidene-β-D-ribofuranuronosyl]glycine benzyl ester (**2a**). Under these conditions only a trace of the side product was detected in the reaction mixture. Similarly, compounds **2b**, **2c**, and **2d** benzyl ester were prepared in high yield by treating **1** with the benzyl esters of L-alanine, L-phenylalanine, and L-glutamic acid (Scheme I).

Hydrolysis of the isopropylidene blocking groups with 88% formic acid was very slow at room temperature. When the temperature was raised to 60–65° the reaction was complete in 2–4 hr. *N*-[1-(9-Adenyl)-β-D-ribofuranuronosyl]glycine benzyl ester (**4a**), -L-alanine benzyl ester (**4b**), -L-phenylalanine benzyl ester (**4c**), and -L-glutamic acid dibenzyl ester (**4d**) were produced in good yields by this procedure. Facile hydrogenolysis of the benzyl blocking groups of **4a–d** was accomplished utilizing palladium on charcoal as catalyst. The title compounds *N*-[1-(9-adenyl)-β-D-ribofuran-

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