Notes

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A Convenient Synthesis of Cytosine Nucleosides

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It has been reported that 1-(β-D-arabinofuranosyl)cytosine has antileukemic activities as well as other biological activities.[2] The synthesis of 1-(β-D-arabinofuranosyl)cytosine was reported by several investigators.[3a–h] This investigation was undertaken to see if a practical method could be worked out for converting the readily available 1-(β-D-arabinofuranosyl)uracil (Ara-U) to 1-(β-D-arabinofuranosyl)cytosine (Ara-C).

We found that the reaction of acylated uracil nucleosides with phosphorous oxychloride using various amines as catalysts gave 4-chloro-acylated uracil nucleosides in good yields. In this paper, we wish to report a convenient synthesis for Ara-C starting from uracil derivatives *via* 4-chlorouracil derivatives.

In 1962, Žemlička, *et al.* reported the introduction of a chloro substituent at the 4-position of uridine and 6-azauridine derivatives[4a,b] using dimethylchloromethyleneammonium chloride in chloroform. We investigated the use of phosphorous oxychloride instead of dimethylchloromethyleneammonium chloride as a chlorinating reagent for acylated uracil nucleoside derivatives: 2′,3′,5′-tri-O-benzoyluridine[5] was first treated with phosphorous oxychloride in the presence of diethylaniline hydrochloride as a catalyst.

Chlorinated 2′,3′,5′-tri-O-benzoyluridine (IIa) was obtained as crystals in about 50% yield by heating 2′,3′,5′-tri-O-benzoyluridine (Ia) with phosphorous oxychloride and diethylaniline hydrochloride in refluxing ethyl acetate for 7 hours. The chloride (IIa) was identical with 4-chloro-2′,3′,5′-tri-O-benzoyluridine reported by Žemlička, *et al.*[4b] in its physical properties. Excess liquid ammonia was added to the chloroform solution of IIa and the solution was allowed to stand for 15 hours at room temperature. The reaction mixture was purified through Dowex 1×4 (OH^-) resin column chromatography to give cytidine (III) in 70% yield, which was identical with commercially available 1-(β-D-ribofuranosyl)cytosine in its physical properties.

As it became clear that phosphorous oxychloride was effective on chlorinating at the 4-position of one uracil nucleoside, this chlorination procedure was applied to other uracil nucleosides in order to convert them to cytosine nucleosides.

2′,3′,5′-Tri-O-acetyluridine[6] (Ib), 2′,3′,5′-tri-O-benzoyl-1-(β-D-arabinofuranosyl)uracil

1) Location: 2-58, 1-*chome*, *Hiromachi*, *Shinagawa-ku*, *Tokyo*.
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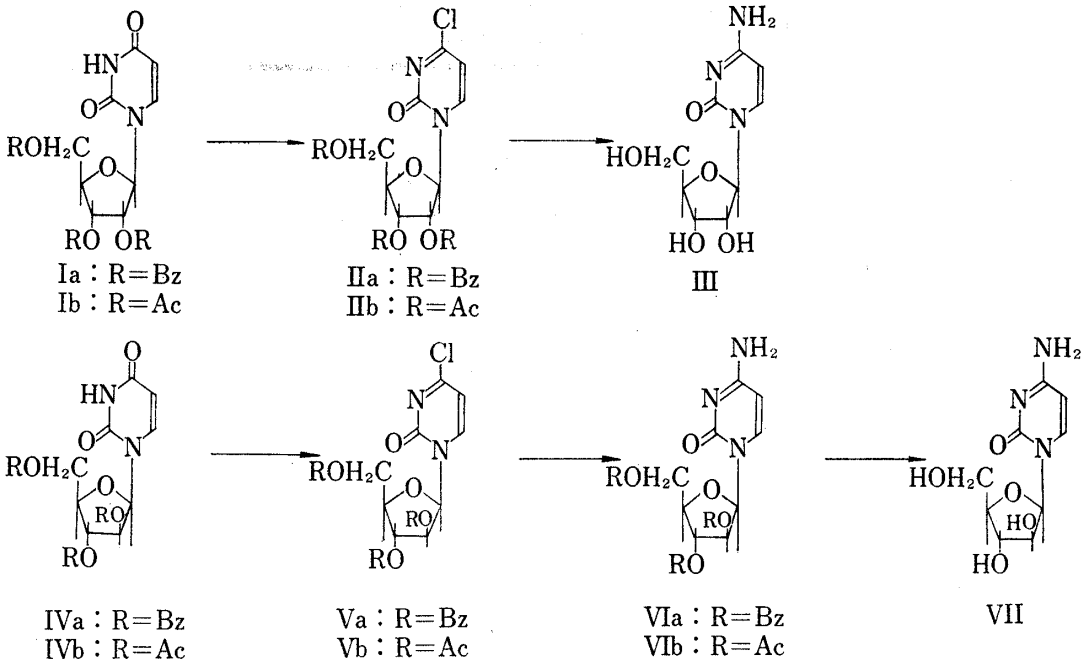

Bz=benzoyl

Chart 1

Table I. Ultraviolet Spectra and Thin-Layer Chromatographic Behavior of 4-Chlorouracil Derivatives

	UV $\lambda_{max}^{CHCl_3}$ (mμ)	$Rf^{a)}$
Tri-O-benzoyl-U (IIa)	277, 284, 307	0.59
Tri-O-benzoyl-Ara-U (Va)	277, 284, 307	0.61
Tri-O-acetyl-U (IIb)	307	0.25
Tri-O-acetyl-Ara-U (Vb)	307	0.28

a) Silica gel F_{254}; benzene–acetone (22:3)

(IVa) and 2′,3′,5′-tri-O-acetyl-1-(β-D-arabinofuranosyl)uracil (IVb)[6] were reacted with phosphorous oxychloride under the same conditions as Ia to give the corresponding 4-chloro derivatives as a single product as shown by thin-layer chromatography (TLC). The chromatographic behavior and ultraviolet absorption data of the 4-chloro derivatives are shwon in Table I. In the practical procedure starting from Ib, IVa, and IVb, amination was carried out without purification of 4-chloro derivatives (IIb), (Va), and (Vb), because of the difficulty of crystallization and of their instability toward moisture. Thus the resulting syrupy 4-chloro derivatives were dissolved in dry chloroform and the solutions were saturated with ammonia at 0° with stirring. Inorganic salts, appearing during the saturation of ammonia, were filtered off while moisture was excluded. The filtrate was again saturated with ammonia at 0° and was allowed to stand for 12 hours at room temperature. Crude acylated arabinosylcytosines (VIa) and (VIb) were obtained after removal of the solvent. Deacylation of crude (VIa) and (VIb) with sodium methoxide or methanolic ammonia followed by purification through Dowex 1×4 (OH^-) column afforded Ara-C (VII) in about 70% yield, which was identical with an authentic sample of 1-(β-D-arabinofuranosyl)cytosine.[3a)]

In these chlorinating reactions, it is possible to use other amines or their salts such as pyridine, pycoline, and lutidine *etc.* as catalysts, but the reaction solution turns dark brown and the yield is reduced in these cases (see Table II). Coloration of the solution also occurred in chlorinations in several solvents except ethyl acetate.

Table II. Isolation Yield of Cytosine Nucleosides from acylated Uracil Nucleosides using Diethylaniline Hydrochloride or Pyridine as a Catalyst[a]

Starting material	Catalyst diethylaniline HCl (%)	Pyridine (%)
Tri-O-benzoyl-U (IIa)	32	40
Tri-O-acetyl-U (IIb)	69	43
Tri-O-benzoyl-Ara-U (Va)	69	37
Tri-O-acetyl-Ara-U (Vb)	74	39

a) Reaction conditions and isolation techniques were the same as the case of (III)-(a).

Thus, the best condition of this chlorination reaction is to use phosphorous oxychloride in the presence of diethylaniline hydrochloride and ethyl acetate.

The synthetic method of cytosine nucleosides, described above, is superior to that of Žemlička, *et al.* in respect of the high yield and the simplicity of experimental procedure which makes the isolation of intermediate 4-chloronucleosides unnecessary.

Experimental

4-Chloro-2′,3′,5′-tri-O-benzoyluridine (IIa)——Diethylaniline hydrochloride (1.1 g) dried for five min. at 150° under 1 mmHg was dissolved in 10 ml of dry ethyl acetate and 10 ml of phosphorous oxychloride with heating on an oil bath. Then 1.1 g of 2′,3′,5′-tri-O-benzoyluridine was added to the solution and the reaction solution was refluxed for 7 hr with protection from moisture. After the solution was concentrated under diminished pressure, 10 ml of dry ethyl acetate was added and the solution was concentrated *in vacuo*. This operation was repeated twice. Resulting gummy product was dissolved in 10 ml of dry benzene and allowed to stand for several hr giving 520 mg (45%) of 4-chloro-2′,3′,5′-tri-O-benzoyluridine: mp 200—202°. UV $\lambda_{max}^{CHCl_3}$ mμ: 278 (sh), 284, 308. IR: no band corresponding to C_4 carbonyl. *Anal.* Calcd. for $C_{30}H_{23}O_8N_2Cl$: C, 62.66; H, 4.03; N, 4.87; Cl, 6.17. Found: C, 62.88; H, 4.08; N, 4.56; Cl, 6.24.

1-(β-D-Ribofuranosyl)cytosine (III)——(a) III from IIa: To a 10 ml of dry chloroform solution of IIa (575 mg) was added 10 ml of liquid ammonia under dry ice-acetone cooling. After the reaction solution was allowed to stand at room temperature for 16 hr, the inorganic salt was filtered off and washed with 10 ml of dry chloroform. The filtrate and the washings were combined and concentrated to dryness. The resulting pale yellow syrup was dissolved in 10 ml of 0.1N sodium methoxide solution and the solution was refluxed for 10 min and then neutralized with 1N HCl. The solvent was removed under reduced pressure and the residue was dissolved in 20 ml of water. The solution was extracted twice with 10 ml portions of chloroform for removal of the benzoate. The aqueous layer was concentrated to about 5 ml. This solution was applied to the top of Dowex 1×4 (OH^-) column (2.0 cm×7 cm) and the column was washed with 300 ml of water and eluted with 50% methanol. Fractions having a UV λ_{max} at 272 mμ (checked on an ultraviolet (UV) spectrophotometer) were collected and concentrated to dryness. The residual crystalline product was recrystallized from 90% methanol to give 175 mg of 1-(β-D-ribofuranosyl) cytosine, mp 220—230° (decomp.); $[\alpha]_D^{25}$ +31° (c=0.7 H_2O). *Anal.* Calcd. for $C_9H_{13}O_5N_3$: C, 44.44; H, 5.39; N, 17.27. Found: C, 44.48; H, 5.39; N, 17.45. UV $\lambda_{max}^{0.1N\ HCl}$ mμ (ε): 279.4 (9870); $\lambda_{max}^{H_2O}$ mμ (ε): 270.5 (8890); $\lambda_{max}^{0.1N\ NaOH}$ mμ (ε) 271.5 (8870).

(b) III from Ib: Anhydrous diethylaniline hydrochloride (550 mg) was dissolved in 5 ml of ethyl acetate and 5 ml of phosphorous oxychloride with heating and 370 mg of Ib was added to the solution. This mixture was allowed to react for 7 hr under reflux. The solvent was removed under reduced pressure. The resulting syrup was aminated and deacylated by the same process as for tri-O-acetyl-Ara-U described below. Purification of the deacylated product through Dowex 1×4 (OH^-) column gave 158 mg of the cytidine (III) after recrystallization from 95% methanol.

1-(β-D-Arabinofuranosyl)cytosine (VII)——(a) VII from IVa: Dry diethylaniline hydrochloride was added to the solution of ethyl acetate (5 ml) and phosphorous oxychloride (5 ml) and dissolved at refluxing temperature. 2′,3′,5′-Tri-O-benzoyl-Ara-U (IVa) (556 mg) was added to the solution and refluxed using a drying tube. After 7 hr, solvent was removed *in vacuo* at 70°. Addition of 10 ml of ethyl acetate and evaporation was repeated twice and reduced pressure was maintained for additional one hour. The resulting yellow caramel was dissolved in 10 ml of chloroform and cooled in a dry ice-acetone bath. Dry ammonia gas was bubbled into the solution with stirring until the volume of the solution increased to about 20 ml. The reaction solution was set aside for 16 hr at room temperature. The resulting inorganic salts were filtered off, and chloroform layer was evaporated to dryness. Crude (VIa) was obtained (946 mg) as a yellow cara-

mel. This gummy product was refluxed in 10 ml of 0.1N sodium methoxide for 10 min and the solution was neutralized with 1N hydrochloric acid. After evaporation of the solvent, the product was dissolved in 20 ml of water, and extracted twice with 10 ml of chloroform. The aqueous layer was concentrated to about 5 ml and was applied to the Dowex 1×4 (OH^-) resin column (2 cm × 7 cm). The column was washed with 300 ml of water and eluted with 50% methanol. Fractions which showed a UV λ_{max} at 272 mμ were collected. The combined solutions were concentrated to dryness *in vacuo* giving 184 mg of a crystalline residue which was recrystallized from 95% methanol, giving 157 mg of 1-(β-D-arabinofuranosyl)cytosine (VII). mp 212—213° (uncorr.). $[\alpha]_D^{20}$ +146° (c=1.04, H_2O). UV $\lambda_{max}^{0.1N\ HCl}$ mμ (ε): 280.5 (9910); $\lambda_{max}^{H_2O}$ mμ (ε): 271.3 (9290); $\lambda_{max}^{0.1N\ NaOH}$ mμ (ε): 273.9. (9920). NMR (in DMSO-d_6 TMS internal 60 Mc) (δ): 7.63 (1H, doublet, J=7.5 cps, C_5-H), 7.03 (2H, singlet, NH_2), 6.07 (1H, doublet, J=4.0 cps, C_1-H), 5.72 (1H, doublet, J=7.5 cps, C_6-H). IR no absorption band corresponding to the C_6 carbonyl group. *Anal.* Calcd. for $C_9H_{13}O_5N_3$: C, 44.44; H, 5.39; N, 17.27. Found: C, 44.87; H, 5.53; N, 17.00.

(b) VII From Tri-O-acetyl-AraU (IVb): Anhydrous diethylaniline hydrochloride (14.8 g) was added to a solution containing 74 ml of ethyl acetate and 74 ml of phosphorous oxychloride and the mixture was refluxed for 10 min. Ten grams of IVb was added to the solution. After refluxing the reaction mixture protected by a drying tube for 7 hr, the solvent was removed under reduced pressure in an oil bath adjusted to 70—80°. Further evaporation was continued for about 30 min. The residue was dissolved in 270 ml of chloroform and saturated with dry ammonia gas at 0° with vigorous stirring. The resulting inorganic salts were filtered off under dry conditions. The resulting solution was saturated again with dry ammonia gas at 0° and allowed to stand at room temperature. After 13 hr, the resulting inorganic salts were removed by filtration and the filtrate was evaporated to dryness *in vacuo*. The residual viscous solution was dissolved in 100 ml of methanolic ammonia (saturated at −10°) and allowed to stand at room temperature for 18 hr. The solution was evaporated to dryness *in vacuo*. The residue was extracted twice with 200 ml of hot benzene.

The powdery product was dissolved in small amount of water and applied to a Dowex 1×4 (OH^-) resin (200 ml) column. The column was washed with one liter of water and eluted with 5 liter of 50% methanol. Ara-C fractions were collected and concentrated to dryness. Crude Ara-C was recrystallized from 95% methanol using active charcoal to afford 4.6 g of Ara-C. All physical properties of this compound were identical with those described above.

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Mechanism of the Intestinal Absorption of Drugs from Oil in Water Emulsions. III.[1] Absorption and Biotransformation of Methyl Orange

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The sulfonated water-soluble azo dyes are widely used as colorings for foods and pharmaceutical preparations. As many azo dyes are potent carcinogens, and some azo dyes are known to be toxic, much attention have recently been paid to their biological dispositions. Scheline and Longberg[3] reported on the absorption, metabolism, and excretion of a sulfonated

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