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Pyrimidine Nucleosides. I. The Convenient Synthesis of 1-β-D-Lyxofuranosylcytosine from Cytidine

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Fox, *et al.*[2] have prepared 1-β-D-lyxofuranosylcytosine (I) *via* 2,2′-anhydroarabinofuranosyluracil, starting from uridine. However, the synthesis of I from cytidine has not been reported, yet, mainly because of the difficulty encountered in the specific protection of N^4-amino group and because of instability of 2,2′- or 2,3′-anhydro-linkage of N^4-acyl protected anhydro-derivatives of cytosine series.[3]

Now we successfully synthesized N^4-unprotected 2,2′-anhydro-3′-O-mesylarabinofuranosylcytosine and isolated it as the mesylate salt (V). Its conversion to I was effected by treatment with weak alkali (*viz.*, aqueous pyridine).

Thus, cytidine (II) was converted to 5′-O-tritylcytidine in a yield of 88%, which in turn was mesylated with three equivalents of methanesulfonyl chloride at −20° to 5′-O-trityl-2′,3′-di-O-mesylcytidine (IV). Compound (IV), obtained in the amorphous form was found to be quite pure on the criteria of thin—layer chromatographic and ultraviolet absorption spectral properties. Therefore, it was used for the subsequent step without further purification.

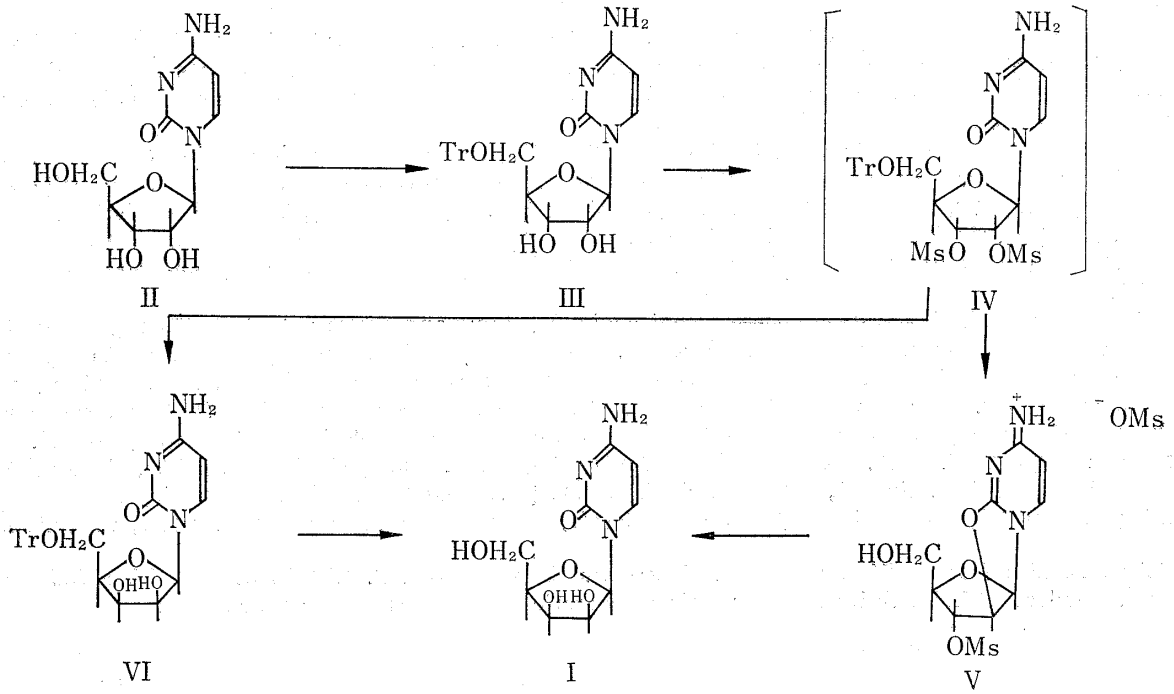

Chart 1

1) Location: *Higashihama-11277, Saiki city, Oita.*
2) N.C. Yung and J.J. Fox, *J. Org. Chem.*, **27**, 1477 (1962).
3) H.P.M. Fromagot and C.B. Reese, *Tetrahedron Letters*, **1966**, 3499.

It is to be noted that whereas treatment of III with *p*-toluenesulfonyl chloride afforded a large proportion of N^4-sulfonylated compound, treatment of III with mesyl chloride afforded only N^4-unsubstituted mesylate.

Compound (IV) was dissolved in 20% aq. methanol and the solution was heated at 80° for 1.5 hours and then kept at the refluxing temperature for 20 minutes to afford 3′-O-mesyl-2,2′-anhydroarabinofuranosylcytosine (V) in 70% yield. 1-β-D-Lyxofuranosylcytosine was obtained on treatment of V with refluxing aq. pyridine (pyridine–water 2:1 v/v) in a good yield. The structural assignment rests upon mp of the hydrochloride (174°), the positive periodate spray test, the mobility in the paper electrophoresis (18.3 cm,[4] borate buffer pH 6.0, 700 volt, 30 mA, 3 hr[5]), spectral properties (see experimental) and combustion values.

Compound (I) was also prepared by alternate route (IV→VI→I); 5′-O-trityl-2′,3′-di-O-mesylcytidine (IV) was dissolved in 65% aq. pyridine and the solution was heated at 70° for 5 minutes. The product was assigned the 5-O-trityl-1-β-D-lyxofuranosylcytosine structure (VI) on the basis of the thin-layer chromatographic mobility, the positive periodate spray test and the ultraviolet spectral properties.

The trityl compound was converted to I with 98% formic acid in 45% overall yield from IV.

Experimental

General Method——Paper chromatography was carried out on Toyo Roshi No. 51, using the ascending technique. The following solvent systems were employed: (A) *n*-BuOH–pyridine–H_2O (10:3:3), (B) *n*-BuOH–AcOH–H_2O (5:2:3). Thin-layer chromatography was carried out on silica gel (Waco Gel) and the following solvent system was employed: chloroform–ethanol (7:1). All melting points were corrected.

5′-O-Tritylcytidine (III)——Cytidine (24.4 g) in 600 ml of anhydrous pyridine was treated with 1.7 equivalents of triphenylmethyl chloride at 45—50° for 3 hours with stirring and the resulting solution was allowed to be kept at 40° for four days. The clear, yellow solution was concentrated to 200 ml under reduced pressure and poured into 4.5 liter of ice water under vigorously stirring. After decantation, the gummy solid was again treated with water, the yellowish solid was filtered and washed with water several times after which the residue was dried. The dried solid was washed with 400 ml of ethyl ether under refluxing for 30 minutes. After filtration, the residue was dissolved in 120 ml of pyridine under refluxing and to the solution was cautiously added 100 ml of methanol. After keeping to stand overnight at room temperature, fine, colorless needles were obtained (42.4 g, 87.2%), mp 244.5—245°. *Anal.* Calcd. for $C_{28}H_{27}O_5N_3 \cdot CH_3OH$: C, 67.30; H, 6.04; N, 8.12. Found: C, 67.49; H, 5.99; N, 8.11.

5′-O-Trityl-2′,3′-di-O-mesylcytidine (IV) from III——Three equivalents of methanesulfonylchloride (9.2 ml) were added dropwise to a cooled solution of 5′-O-tritylcytidine (III, 14.48 g) in 200 ml of anhydrous pyridine at −20° and the reaction was stored at −20° overnight. A crystalline product was formed during this time, but was not separated.

The reaction mixture was treated with 4 ml of H_2O and kept for two more hours. After concentration *in vacuo* below 30° to a sirup, the residue was dissolved in 20 ml of methanol and poured into crushed ice with mechanically stirring. A white, amorphous solid was obtained quantitatively which was filtered swiftly and washed well with crushed ice and water. The solid afforded a single spot (*Rf* 0.27) on thin-layer chromatogram which, after 30% sulfuric acid was sprayed, turned yellow with heating to show the presence of trityl group. UV (λ_{max}^{MeOH} mμ: 267)[6] showed that no sulfonylation occured at N^4. It was used for the subsequent reaction without further purification.

2,2′-Anhydro-3′-O-mesylarabinofuranosylcytosine Mesylate (V) from IV——The amorphous 5′-O-trityl-2′,3′-di-O-mesylcytidine obtained from 12 g of 5′-O-tritylcytidine was dissolved in 50 ml of methanol and 200 ml of water. The solution was heated at 80° for 1.5 hours, followed by refluxing for 20 minutes on oil bath at 120°. After cooling, a white, granular precipitate was filtered and washed with water. The filtrate and washings were combined and evaporated to a sirup. The sirup was treated with ethanol, and crystallization occurred while evaporation. Recrystallization was accomplished from methanol. Yield was 4.88 g, mp 212—213° (decomp). UV $\lambda_{max}^{H_2O,H^+}$ mμ: 232, 263. IR ν_{max}^{KBr} cm^{-1}: 1670—1695 (–N=C–O–C–), 1170 (covalent mesylate),

4) See experimental.
5) M.P. Gordon, O.M. Intrieri and G.B. Brown, *J. Am. Chem. Soc.*, **80**, 5161 (1958).
6) Fox, *et al.* had reported that mesyloxy groups substituted at the 2′ or 3′-positions in the sugar moiety of aldopentofuranosyluracils generally have a hypsochromic effect. We thought the same phenomena might occur in cytidine series.

1330 (sulfonyl ester). Paper chromatography: *Rf* (A) 0.20, *Rf* (B) 0.50, *Anal.* Calcd. for $C_{11}H_{17}O_4N_3S_2$: C, 33.97; H, 4.36; N, 10.88. Found: C, 33.08; H, 4.29; N, 10.52.

1-β-D-Lyxofuranosylcytosine (I) from V (Method A)——1 g of 3′-O-mesyl-2,2′-anhydro-β-D-arabinofuranosylcytosine mesylate was dissolved in 15 ml of aq. pyridine (pyridine–water 2:1 v/v) and the solution was heated at 70°, followed by refluxing for 6 minutes. The cooled solution was concentrated to a sirup *in vacuo* and repeatedly evaporated with water. The residue was dissolved in 5 ml of water and the pH of the solution was adjusted to 1.0 with 1N HCl. The acidic solution was concentrated to a sirup *in vacuo*. The sirup was treated with ethanol and crystallization occured while evapolation under reduced pressure. Yield was 0.365 g (52.1%). Recrystallization was accomplished by dissolving the substance in minimum amount of water and concentration of the solution until crystallization began. After cooling, pure material in the form of hydrochloride salt was obtained.

mp 174° (decomp. and effervesces at 190—191°). UV $\lambda_{max}^{pH7,pH11}$ mμ: 273, λ_{max}^{pH1} mμ: 281. The periodate spray test on paper chromatograph (*Rf*(A) 0.31) was positive, the mobility in the paper electrophoresis[5] was 18.3 cm.[7] *Anal.* Calcd. for $C_9H_{13}O_5N_3 \cdot HCl$: C, 38.65; H, 5.05; N, 15.02. Found: C, 38.86; H, 4.84; N, 15.22.

Method B (from IV *via* VI)——The amorphous 5′-O-trityl-2′,3′-di-O-mesylcytidine obtained from 3 g of 5′-O-tritylcytidine was dissolved in 90 ml of aq. pyridine (pyridine–H_2O 2: 1 v/v) and the refluxing solution was heated at 70° followed by refluxing for 6 minutes. The cooled reaction mixture was evaporated to a sirup *in vacuo* and repeatedly evaporated with aqueous methanol. The residue was dissolved in a minimum amount of methanol, and poured into 1 liter of ice water under stirring. Stirring continued for another hour. A white, granular precipitate was filtered, washed with water and dried over P_2O_5 at 70° under reduced pressure for 10 hours. The further purification of this powder was accomplished by dissolving it in a minimum amount of methanol and pouring into a large amount of ether with shaking. A white granular solid was obtained (2.40 g, 84.17%). *Rf* 0.13, single spot on thin layer chromatograph, consumed metaperiodate by spray test.

0.7 g of the product obtained above, was dissolved in 10 ml of 98% formic acid and allowed to stand overnight at room temperature. The yellow colored solution was concentrated to dryness and repeatedly evaporated with water (10 ml × 3). The nucleoside was extracted with 30 ml of hot water from the residue. The sirup was dissolved in 50% methanol and acidified with 1N HCl. Crystallization occured while evaporation of this acidic solution. Recrystallization from ethanol obtained a white needles (0.216 g, 53.47%). Melting point was consistent with that of the crystal obtained by the method A.

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7) Cytidine, 1-β-D-arabinofuranosylcytosine migrated 13.1 cm, 5.0 cm, respectively in the same condition as used in I.