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Synthesis of 2-Amino-2-deoxy-β-D-arabinofuranosyl Nucleosides

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9-(2-Deoxy-2-amino-β-D-arabinofuranosyl)adenine(IIIa) and guanine (IIIb) were synthesized from the corresponding azide compounds (II) obtained by nucleophilic substitution of tosyloxy groups of 2′-O-*p*-toluensulfonyladenosine (Ia) and guanosine (Ib), respectively. Their antitumor activity against sarcoma-180 solid tumor and cytotoxic activity against HeLa-S_3 cells were determined.

Keywords——aminonucleoside; arabinofuranosyl configuration; adenine derivative; guanine derivative; nucleophilic substituion; antitumor activity; cytotoxic activity

Among many aminonucleosides synthesized over the years, those which have the 2′-amino group unambigously in the "up" (arabino) configuration can hardly be found in the literature. Reist *et al.*[2] introduced the "up" 2′-azide group *via* 9-(2,3-anhydro-5-deoxy-β-D-ribofuranosyl) adenine. However, its conversion to up-amino group has not been stated. Recently, Bobek *et al.*[3] reported the synthesis of 1-(2-amino-2-deoxy-β-D-arabinofuranosyl) uracil and cytosine, which prompted us to publish our study.

The β-D-arabinofuranosyl cytosine and adenine have the potent anticancer activity as antimetabolites and the arabino configuration seems to be an important feature to excert the activity. Moreover, 2′-amino-2′-deoxyguanosine (2AG) from the culture broth of *Aerobacter sp.* was reported to possess antibacterial and antitumor activities.[4] We attempted, therefore, to synthesize 9-(2-amino-2-deoxy-β-D-arabinofuranosyl) adenine (IIIa) and guanine (IIIb).

2′-O-*p*-Toluenesulfonyladenosine (Ia) and guanosine (Ib) were prepared by the same procedure reported by Wagner *et al.*[5] *via* 2′,3′-O-(dibutylstannylene) nucleosides in 57.0% and 48.0% yield, respectively. Selective tosylation of hydroxy group at C-2′ was confirmed by nuclear magnetic resonance (NMR) spectroscopy. Then, nucleophilic substitutions of tosyloxy groups in (Ia) and (Ib) with sodium azide were investigated although Wagner *et al.*[5] reported that (Ia) was labile in treatment with lithium azide in dimethylformamide at 150° to give only adenine. (Ia) and (Ib) were treated with sodium azide in hexamethylphosphoramide (HMPA) at 130° and 140° to give (IIa) and (IIb) with concurrent formation of considerable amounts of adenine and guanine, respectively. Isolation was carried out using Dowex 1×2 (OH) resin by Dekker's method[6] and Diaion HP-10 resin to give a (IIa) and b (IIb) in 45.7% and 10.8% yield, respectively.

Attachment of azide group at C-2′ was confirmed by NMR spectroscopy and spin-decoupling method, where the doublet signal of the secondary hydroxy group was collapsed to singlet under irradiation at 3′-position.

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2) E.J. Reist, D.F. Calkins, and L. Goodman, *J. Org. Chem.*, 32, 2538 (1967).
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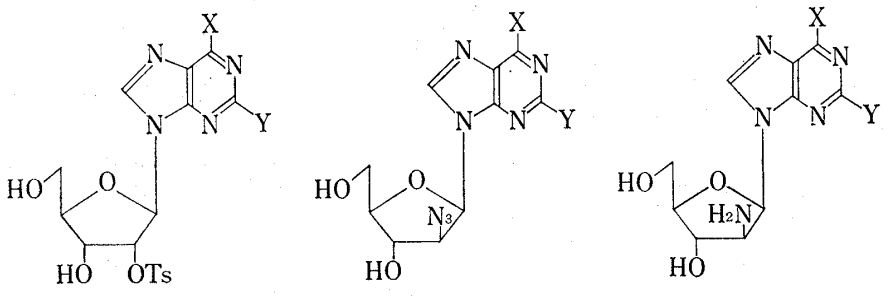
Finally, (IIa) and (IIb) were hydrogenated over palladium charcoal to give amino-nucleosides (IIIa) and (IIIb) in 68.5% and 75.6% yield, respectively. Configuration at C-2′ could not be determined from the NMR spectroscopy since H-1′—H-2′ coupling constant permits no difinitive discrimination between ribo and arabino configurations. However, physical properties of (IIIb) are different from those of authentic 2′-amino-2′-deoxyguanosine[4] suggesting the "up" configuration of the amino group. Furthermore, (IIIa) was hydrolyzed in 1 N HCl to give 2-amino-2-deoxy-pentose hydrochloride whose physical properties agreed with those of 2-amino-2-deoxy-D-arabinose reported by Kuhn *et al.*[7] From the above results, it appeared well established that the structures of (IIIa) and (IIIb) were 9-(2-amino-2-deoxy-β-D-arabinofuranosyl)adenine and guanine, respectively.



Ia, b IIa, b IIIa, b

a: X=NH_2, Y=H b: X=OH, Y=NH_2

Chart 1

As shown in Table I, (IIIa) and (IIIb) showed weak antitumor activity against solid tumor of sarcoma-180 *in vivo* and weak cytotoxicity against HeLa-S_3 cells *in vitro*.

TABLE I. Antitumor Activity against Sarcoma-180 and Cytotoxic Activity against HeLa-S_3 Cells

Compd.	Dose (mg/kg)	Antitumor activity Saroma-180 T/C[a]	Cytotoxic activity IC_{50}[b] (mcg/ml)
IIIa	500 *i.p.* × 1[c]	0.64 (+5.4)[d]	190
IIIb	500 *i.p.* × 1	0.53 (+3.2)	200

a) T/C was calculated by the mean tumor volume of the treated group divided by that of control group.
b) Concentration indicating 50% inhibitions in cell growth.
c) Administered intraperitoneally once 24 hr after implantation.
d) Changes of body weight (g) from 1st to 7th day after implantation. Control group: +3.6 g.

Experimental

Melting points were determined using a Yanagimoto melting points apparatus and are uncorrected. Optical rotations were measured by a Hitachi Perkin-Elmer 141 polarimeter. NMR spectra were determined on a JEOL JNM-PS100 spectrometer using TMS as an internal standard. Ultraviolet (UV) spectra were recorded on a Hitachi EPS-3 spectrophotometer.

9-(2-Deoxy-2-O-*p*-toluenesulfonyl-β-D-ribofuranosyl)guanine (Ib)——By the same procedure reported by Wagner *et al.*,[5] 4.2 g (48.0%) of fine needles were obtained from 5.7 g of guanosine. The compound colored in brown from 235° and then decomposed at 244—247.° $[\alpha]_D^{28}$ −118.9° (*c*=0.37, 50% aq.EtOH). NMR (DMSO-d_6) δ: 2.36 (3H, s, $-CH_3$), 3.60 (2H, br, CH_2-5′), 4.00 (1H, br, H-4′), 4.28 (1H, br, H-3′), 5.24 (1H, t, *J*=6.0 Hz, OH-5′), 5.36 (1H, dd, $J_{1',2'}$=7.0 Hz, $J_{2',3'}$=6.0 Hz, H-2′), 5.88 (1H, d, H-1′), 5.96 (1H, d, $J_{3',OH-3'}$=5.5 Hz, OH-3′), 6.44 (2H, br, NH_2-2), 7.12, 7.48 (4H, two d, *J*=8.5 Hz, aromatic), 7.72 (1H, s, H-8), 10.64 (1H, s, NH-1). *Anal.* Calcd. for $C_{17}H_{19}N_5O_7S$: C, 46.68; H, 4.38; N, 16.01. Found: C, 46.63; H, 4.50; N, 15.93.

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9-(2-Deoxy-2-azido-β-D-arabinofuranosyl)adenine (IIa)——A suspension of (Ia) (600 mg) and sodium azide (560 mg) in anhydrous HMPA (6 ml) was heated at 130° for 6 hr. After evaporation of the solvent the black residue was dissolved in 20 ml of 50% aqueous methanol, applied to a column of Dowex 1×2(OH) (100 ml) and washed with each 200 ml of water, 30% and 60% aqueous methanol. Adsorbed product was eluted with 99% methanol. The solvent was evaporated and residue was crystallized from ethanol to give 190 mg (45.7%) as fine needles, mp 204—205.° $[\alpha]_D^{28}$ −17.0° (c=0.6, H_2O). NMR (DMSO-d_6) δ: 4.44 (1H, m, H-3′; after addition of D_2O, pseudo t, $J_{2',3'}$=8.0 Hz, $J_{3',4'}$=8.0 Hz), 4.60 (1H, pseudo t, $J_{1',2'}$=6.5 Hz, $J_{2',3'}$=8.0 Hz, H-2′), 5.20 (1H, t, J=5.0 Hz, OH-5′), 6.00 (1H, d, $J_{3',OH-3'}$=5.5 Hz, OH-3′), 6.46 (1H, d, H-1′), 7.32 (2H, br, NH_2-6), 8.20, 8.40 (2H, two s, H-2 and H-8). *Anal.* Calcd. for $C_{10}H_{12}N_8O_3$: C, 41.09; H, 4.14; N, 38.34. Found :C, 40.87; H, 4.06; N, 38.12.

9-(2-Deoxy-2-azido-β-D-arabinofuranosyl)guanine (IIb)——A suspension of (Ib) (3.6 g) and sodium azido (3.2 g) in anhydrous HMPA (45 ml) was heated at 140° for 7 hr. After evaporation of the solvent, the black residue was dissolved in 200 ml of 33% aqueous methanol, applied to a column of Dowex 1×2 (OH) (200 ml) and washed with each 800 ml of water, 50% and 80% aqueous methanol. Adsorbed product was eluted with 0.3 M solution of lithium chloride. After the pH of the effluent was adjusted to 6.8 with 1 N HCl, concentrated solution was applied to a column of Diaion HP-10 (Mitsubishi Kasei Co., Ltd. 300 ml) and washed with 600 ml of 10% aqueous methanol. Then adsorbed product was eluted with 20% aqueous methanol. The solvent was evaporated to obtain 220 mg (10.8%) of amorphous white powder, which colored in brown from 210° and then decomposed at 225—228.° $[\alpha]_D^{22}$ +50.2° (c=1.54, H_2O). NMR (DMSO-d_6) δ: 4.36 (1H, m, H-3′; after addition of D_2O, pseudo t, $J_{2',3'}$=8.0 Hz $J_{3',4'}$=7.0 Hz), 4.50 (1H, pseudo t, $J_{1',2'}$=6.0 Hz, $J_{2',3'}$= 8.0 Hz, H-2′), 5.14 (1H, br, OH-5′), 6.04 (1H, br, OH-3′), 6.16 (1H, d, H-1′), 6.56 (2H, br, NH_2-2), 7.92 (1H, s, H-8), 10.75 (1H, br, NH-1).

9-(2-Deoxy-2-amino-β-D-arabinofuranosyl)adenine (IIIa)——(IIa) (1.0 g) in 67% aqueous methanol (150 ml) was catalytically hydrogenated over 10% Pd/C (500 mg) for 5 hr. After removal of catalyst and solvent, the residue was crystallized from ethanol to give 390 mg (68.5%) of a crystalline compound, which colored in brown from 210° and then decomposed at 215—218.° $[\alpha]_D^{28}$ −5.8° (c=0.95, H_2O). NMR (DMSO-d_6) δ: 6.22 (1H, d, $J_{1',2'}$=6.5 Hz, H-1′), 7.22 (2H, br, NH_2-6), 8.12, 8.28 (2H, two s, H-2 and H-8). UV $\lambda_{max}^{pH\,7}$ nm: 260, $\lambda_{max}^{0.1N\ HCl}$ nm: 258, $\lambda_{max}^{0.1N\ NaOH}$ nm: 260. *Anal.* Calcd. for $C_{10}H_{14}N_6O_3$: C, 45.11; H, 5.30; N, 31.57. Found: C, 45.35; H, 5.47; N, 31.12.

9-(2-Deoxy-2-amino-β-D-arabinofuranosyl)guanine (IIIb)——(IIb) (130 mg) in 33% aqueous methanol (45 ml) was hydrogenated and treated similarly in above procedure. The residue was dissolved in hot water and cooled. The resulting precipitate was dried to obtain 90 mg (75.6%) of amorphous white powder, which colored in brown at 190° and then decomposed at 193—196.° $[\alpha]_D^{22}$ −1.9° (c=0.79, H_2O). NMR (DMSO-d_6) δ: 5.98 (1H, d, $J_{1',2'}$=6.0 Hz, H-1′), 6.44 (2H, br, NH_2-2), 7.88 (1H, s, H-8). UV $\lambda_{max}^{pH\,7}$ nm: 253, $\lambda_{max}^{0.1N\ HCl}$ nm: 257, $\lambda_{max}^{0.1N\ NaOH}$ nm: 258—266.

Isolation of the Sugar Moiety——A solution of (IIIa) (300 mg) in 1 N HCl (40 ml) was heated at 100° for 1 hr. After evaporation of the solvent, the residue was dissolved in 10 ml of hot methanol and cooled. The precipitated adenine was removed by filtration, and the filtrate was concentrated. The resulting residue was dissolved in 30 ml of 0.1 N HCl and applied to a column of Dowex 50W×12 (H) (120 ml) and eluted with 0.33 N HCl. The fractions containing the amino sugar were concentrated to give crude crystals. Recrystallization from methanol–acetone afforded fine needles of 2-amino-2-deoxy-D-arabinose hydrochloride (90 mg).[7] mp 154—157° (dec.). $[\alpha]_D^{22}$ −168° (initial, extrapolated)→−113° (c=1.0, H_2O). *Anal.* Calcd. for $C_5H_{12}ClNO_4$: C, 32.36; H, 6.52; N, 7.55; Cl, 19.10. Found: C, 32.55; H, 6.60; N, 7.47; Cl, 19.23.

Biological——Experimental details for determing the activity against sarcoma-180 were described in our preceeding paper.[8]

Cytotoxic activity against HeLa-S_3 cells: 1×10^5 cells of HeLa-S_3 was cultivated at 37° in 1 ml of YLE medium (Earle balanced salt solution containing 0.5% yeast extract and 0.1% lactoalbumin hydrolysate) with 10% of calf serum and 100 units and 100 mcg per mililiter of penicillin and streptomycin, respectively. After incubation for 24 hr at 37,° the test compounds dissolved in PBS (phosphate buffered saline) was added. At 72 hr after addition of the compounds, the medium was removed and 1 ml of 0.1 M solution of citric acid containing 0.02% of crystal violet was added. Then, cells were counted using hemocytometer.

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