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Studies of Nucleosides and Nucleotides. XLII.[1] Purine Cyclonucleosides. (9). Synthesis of Adenine Cyclonucleosides having 8,2′- and 8,3′-O-Anhydro Linkages

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2′-O-Triisopropylbenzenesulfonyl-8-bromoadenosine was converted to 8-oxy derivative either by treatment with sodium acetate in acetic acid or by sodium acetate in acetic anhydride–acetic acid mixture. 2′-Triisopropylbenzenesulfonyl-8-oxy-adenosine or its N^6,3′,5′-triacetyl derivative was cyclized to 8,2′-anhydro-8-oxy-9-β-D-arabinofuranosyladenine with sodium acetate in dimethyl formamide (DMF). Cyclization of 2′- and 3′-triisopropylbenzenesulfonyl-8-oxy derivative was kinetically followed and it was found that the former cyclized easier than the latter compound and decomposition of both cyclonucleosides differed greatly in its rate. Using short cyclization time 8,3′-anhydro-8-oxy-9-β-D-xylofuranosyladenine was first synthesized from 3′-O-triisopropylbenzenesulfonyl-8-oxyadenosine. Optical rotatory dispersion (ORD) and circular dichroism (CD) curves of these cyclonucleosides were described.

In recent years various purine cyclonucleosides having S- and O-anhydro linkages between position 8 of the base and 2′, 3′ or 5′ of the sugar moiety were reported.[3] These cyclonucleosides were shown to be versatile intermediates to obtain deoxynucleosides starting from ribonucleosides[4–6] and were interesting as a model of the nucleoside in which base moiety was fixed by an anhydro linkage in addition to the usual nucleosidic linkage.[7] However a cyclonucleoside having 8,3′-O-anhydro linkage have not yet been synthesized from adenosine, even though 3′-O-tosyl-8-oxyadenosine was rendered cyclization by treatment with sodium benzoate in dimethylformamide.[8] This type of cyclonucleoside could be synthesized from 2′-deoxyadenosine,[9] presumably because a favorable conformation in 2′-deoxyfuranose ring for the 8,3′-O-anhydro linkage formation exists. This paper described a new synthesis of 8,2′-anhydro-8-oxy-9-β-D-arabinoguranosyladenine and the first synthesis of 8,3′-anhydro-8-oxy-9-β-D-xylofuranosyladenine from the intermediate 8-bromo-2′-O- and 3′-O-triisopropylbenzenesulfonyladenosine.[1]

As has been shown in a preceding paper,[1] 8-bromoadenosine was effectively converted to 2′-O- and 3′-O-triisopropylbenzenesulfonyl derivative and these compounds were proved to be suitable intermediates for synthesizing S-cyclonucleosides. We therefore chose these compounds as the starting material of the following reaction. 2′-O-Triisopropylbenzenesulfonyl-8-bromoadenosine (I) was refluxed with anhydrous sodium acetate in glacial acetic acid[10] for 2 hours in order to substitute 8-bromo to 8-oxy function. In this condition partial

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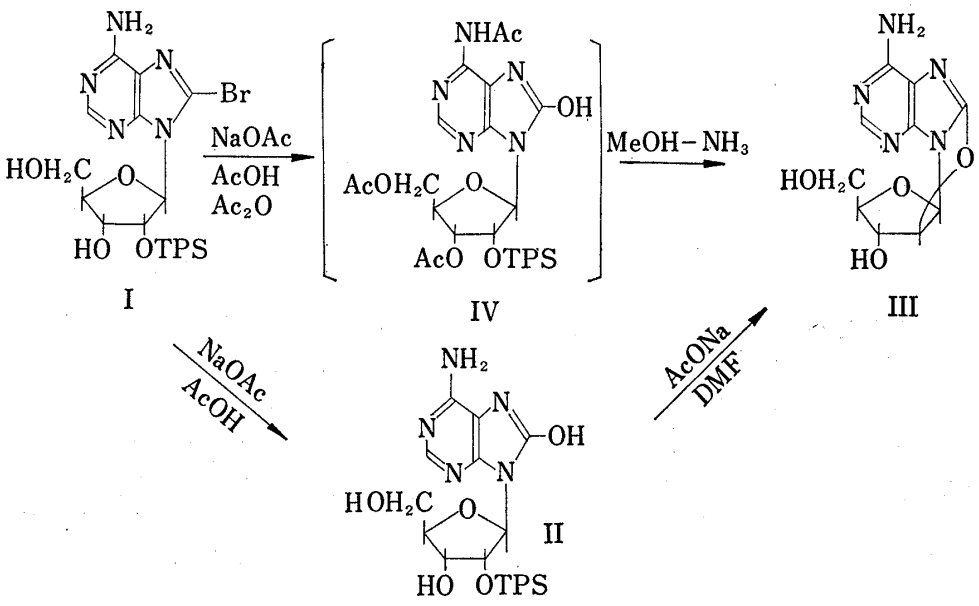
acylation in NH_2 and/or sugar hydroxyl groups occurred. The crude product was then deacylated with methanolic ammonia at room temperature to give 8-oxy-2′-triisopropylbenzenesulfonyladenosine (II) in 44% yield. The structure of compound II was confirmed by elemental analysis and a characteristic ultraviolet (UV) absorption curves, which had $\lambda_{max}^{H^+}$ at 277 nm with several shoulders as described in the experimental. Retention of triisopropylbenzenesulfonyl group was implied by infrared (IR) absorption band at 1185 cm^{-1} and large *Rf* values in paper chromatography. In a large scale experiment for obtaining a sufficient amount of the cyclonucleoside the crude product can be used in subsequent reactions.

Compound II was then cyclized by heating with sodium acetate in dimethylformamide.[9] Although sodium benzoate in dimethylformamide has been used in the cyclization reaction of pyrimidine cyclonucleosides,[11] strong absorption of the benzoate often interfered with the detection of nucleosidic material on chromatograms. As shown in the experimental, sodium acetate in dimethylformamide could be coveniently used for this purpose. Heating of compound II in dimethyl formamide (DMF) for 1.5 hr gave 8,2′-anhydro-8-oxy-9-β-D-arabinofuranosyladenine (8,2′-O-cyclonucleoside) (III) in a yield of 41%. Structure of compound III was confirmed by direct comparison with an authentic sample.[8]



TPS : triisopropylbenzenesulfonyl

Chart 1

In order to improve the yield of compound III, acetolysis and cyclization reactions were investigated further. Holmes and Robins[12] reported that acylation of 6-NH_2 of adenosine enhanced susceptibility of halogen atom in position 8. Therefore, we attempted a total acylation of compound II in the acetolysis reaction of compound I with sodium adetate in acetic acid–acetic anhydride mixture. When the compound I and sodium acetate were refluxed in 1:1 (v/v) mixture of acetic acid and acetic anhydride for 3 hrs, N^6, 3′,5′-triacetyl-2′-triisopropylbenzenesulfonyl-8-oxyadenosine (IV) was obtained. This compound was rendered anhydrous and treated with ammonia in methanol, which has been previously shown to be satisfactory in cyclization of 2′-tosyluridine.[13] By the use of this procedure scission

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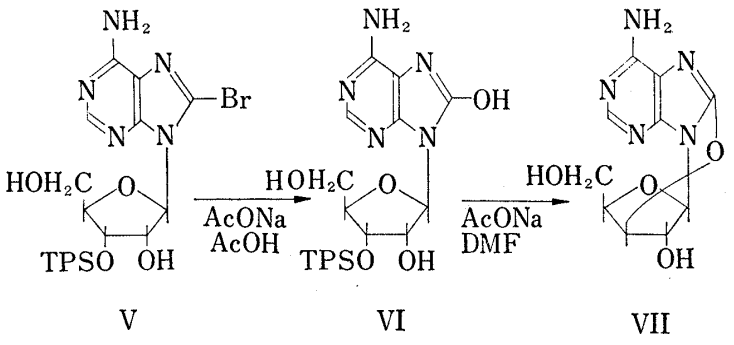
of 8,2′-anhydro bond during cyclization was minimized and the yield of 8,2′-O-cyclonucleoside raised to 52.6% calculated from the starting material (I). Sample obtained by this method was shown to be identical with an authentic sample.[8] From the mother liquor of recrystallization of III, three minor products were detected by paper chromatography. Although structures of these side products have not been confirmed yet, UV absorption and *Rf* values suggested existence of 8-oxy, 8-amino and another O-cyclonucleoside. It is interesting that even in this mild condition fission of the anhydro bond had occurred.

Nevertheless, an alternate synthetic method for 8,2′-O-cyclonucleoside, which is suitable for large scale preparation was thus established.

As it was clarified that 2′-triisopropylbenzenesulfonyl derivative was suitable for the synthesis of 8,2′-cyclonucleoside, 3′-counterpart was similarly subjected to the cyclization. 3′-Triisopropylbenzenesulfonyl-8-bromoadenosine (V) was converted to 8-oxy derivative (VI) by the treatment with sodium acetate in acetic acid. Since in this condition a partial acetylation occurred as described above, the crude 8-oxy compound was purified through treatment with methanolic ammonia and 3′-O-triisopropylbenzenesulfonyl-8-oxyadenosine (VI) was obtained as a colorless crystalline material, which had mp 148—149°. Structure of compound VI was confirmed by elemental analysis as well as UV absorption properties and IR absorption characteristic for aryl sulfonyl derivative. Cyclization of compound VI was attempted then with methanolic ammonia, sodium benzoate in DMF and potassium *t*-butoxide in *t*-butanol. In each case the cyclization was incomplete and the product was a complex mixture. The extent of the cylization was then followed kinetically using 2′-triisopropylbenzenesulfonyl-8-oxyadenosine (II) and its 3′-isomer (VI) as substrates. Result was shown in Fig. 1 and 2. As shown in Fig. 1, in the case of 2′-triisopropylbenzenesulfonyl compound, cyclization was at 80% completion within 2 hr and decomposition of the resulting cyclonucleoside appeared to occur only 8%. Furthermore, decomposition to yield 8-oxyadenine proceeded in the range of 3—4%. In contrast to this ,as shown in Fig. 2, 3′-triisopropylbenzenesulfonyl compound cyclized rather slowly and at the end of 20 hrs' reaction cyclonucleoside appeared only 28%. Decomposition of the resulting cyclonucleoside to the 8-oxy compound was fairly fast and comparable rate of formation and decompositions seems to be the reason of low



V VI VII

Chart 2

yield of cyclonucleoside. Therefore if we want to obtain 8,3′-cyclonucleoside effectively, reaction time should be minimized by using a drastic condition. The cyclization of 3′-triisopropylbenzenesulfonyl-8-oxyadenosine (VI) was performed by heating it with sodium acetate in DMF for 7 minutes. 8,3′-Anhydroy-8-oxy-9-β-D-xylofuranosyladenine (VII) was obtained as colorless crystal having mp 188—190°. The structure of the compound VII was confirmed by UV absorption at 262 nm, loss of IR band of aryl sulfonyl group and elemental analysis. Correctness of the structure was further supported by its nuclear magnetic resonance (NMR) spectrum and optical rotatory dispersion (ORD) and circular dichloism (CD) measurements.

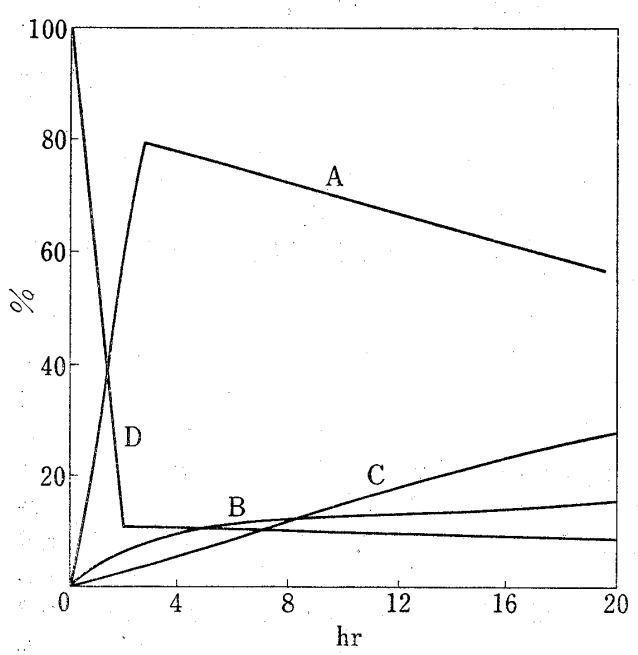


Fig. 1. Kinetic Study of the Formation of 8,2′-Cyclonucleoside

A: 8,2′-cyclonucleoside, B: 8-oxy-arabinofuranosyladenine, C: 8-oxyadenine, D: 2′-TPS-8-bromo-adenosine

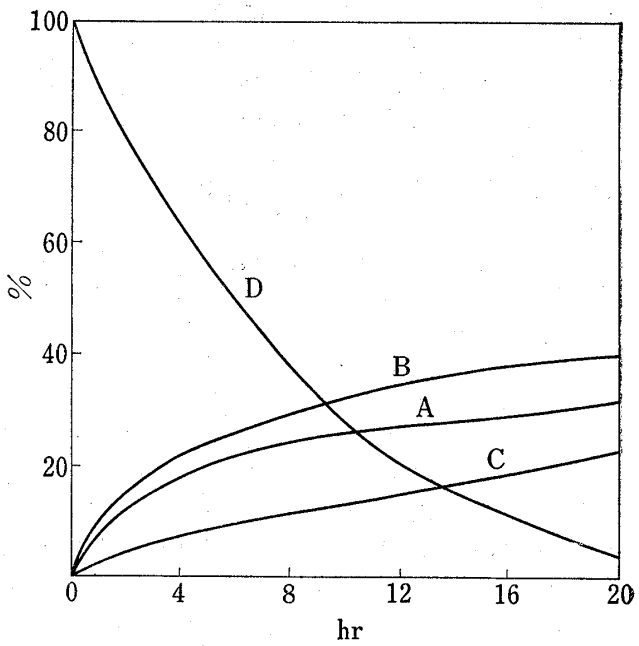


Fig. 2. Kinetic Study of the Formation of 8,3′-Cyclonucleoside

A: 8,3′-cyclonucleoside, B: 8-oxy-xylofuranosyladenine, C: 8-oxyadenine, D: 3′-TPS-8-bromo-adenosine

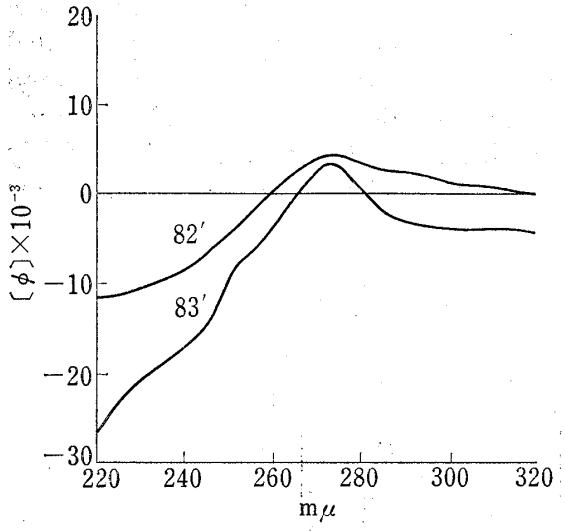


Fig. 3. Optical Rotatory Dispersion Curves of 8,2′- and 8,3′-Cyclonucleosides

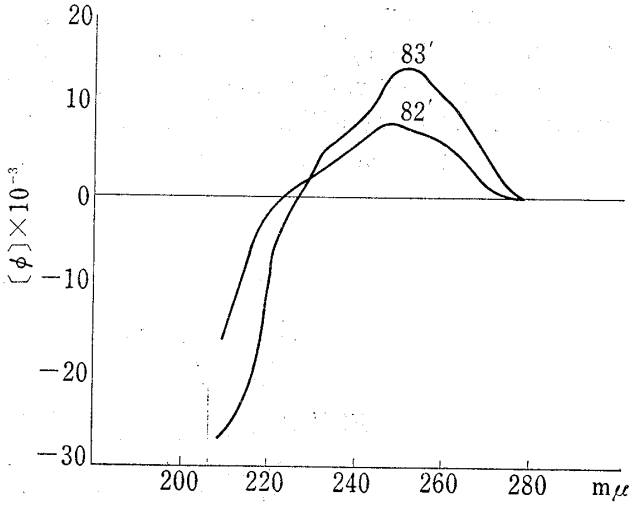


Fig. 4. Circular Dichroism Curves of 8,2′- and 8,3′-Cyclonucleosides

In the NMR spectrum compound VII has four protons which are exchangeable with D in D_2O solution and C_1, proton at 5.74 δ appeared as a singlet, which showed C_3'-endo conformation due to the formation of 8,3′-anhydro bond.[5] As shown in Fig. 3, ORD curve of VII showed close similarity with that of 8,3′-anhydro-8-oxy-9-β-(D-2-deoxythreopentofuranosyl)adenine.[7] In both compounds a positive cotton band around major absorption (260 nm) was found and its magnitude was in the range of 28000—29000. A large cotton effect due to B band suggested the cyclonucleoside structure of compound VII. In the CD curve 8,3′-cyclonucleoside VII showed a large positive cotton band around major absorption and the magnitude is larger than that of 8,2′-O-cyclonucleoside (III) as shown in Fig. 4. All of these optical properties agree with the previous postulation that rotation of the base moiety around nucleosidic linkage

in clockwise direction results in increase of the amplitude of the Cotton effect and its magnitude is depending on the angle of the rotation.[3,7]

8,3′-Anhydro-8-oxy-9-β-D-xylofuranosyladenine thus obtained from adenosine gave a complete set of six possible adenine ribocyclonucleosides together with five cyclonucleosides which has been reported earlier.

Experimental[14]

Paper Chromatography——Solvent A, water adjusted to pH 10 with conc. NH_3; solvent B, *n*-butanol–water, 86:14; solvent C, isopropanol–ammonia–water, 7:1:2. All chromatographies were preformed on Toyo Roshi No. 51A by ascending technique.

Thin-Layer Chromatography——Solvent was $CHCl_3$–ethanol, 19:1, unless otherwise specified. Performed on Merck Kieselgel HF 254.

2′-O-Triisopropylbenzenesulfonyl-8-oxyadenosine——Sodium acetate (4.0 g, freshly dried by fusion) was added in glacial acetic acid (100 ml). The solution was heated in an oil bath at 150° until all solid material dissolved. 8-Bromo-2′-O-triisopropylbenzenesulfonyladenosine[1] (2.0 g, 3.3 mmoles, well dried over P_2O_5 at 50° in 5 mm/Hg) was added into this solution. After refluxing the solution for 2 hr, sodium acetate (1.0 g) was added and the solution was refluxed further for 1 hr. Acetic acid was evaporated under reduced pressure to a volume of 20 ml. Residual solution was added gradually in water (200 ml) with stirring. Precipitates were collected by filtration and dried in a desiccator to give a yellowish powder. The powder was dissolved in a mixture of conc. ammonia (10 ml) and ethanol (10 ml). After kept the solution for 20 hr at room temperature, solvent was removed *in vacuo*. Residual glass was dissolved in a small amount of benzene and passed through a column of alumina (10 g) in order to remove colorized material. Colorless glass which was obtained by evaporation of effuluents was recrystallized from benzene to give white needles having mp 159—160° (800 mg, 44%). *Anal.* Calcd. for $C_{25}H_{35}O_7N_5$: C, 54.62; H, 6.42; N, 12.74. Found: C, 54.32; H, 6.50; N, 12.64. UV: $\lambda_{max}^{H^+}$ 235 nm (sh) (ε 10700), 270 nm (sh) (ε 9700), 277 nm (ε=9800), 285 nm (sh) (ε=9300), 300 nm (sh) (ε=5800); λ_{max}^{MeOH} 234 nm (ε=10800), 260 nm (sh) (ε=8900), 273 nm (ε=11000); $\lambda_{max}^{OH^-}$ 233 nm (sh) (ε=9900), 285 nm (ε=14000). IR: ν_{max}^{KBr} 1185 cm^{-1} (aryl sulfonate), 1725 cm^{-1} (five-membered ureide). TLC[15]: *Rf*: 0.13 ($CHCl_3$–EtOH, 18:2). PPC:[16] *Rf*(B): 0.94, *Rf*(C): 0.96.

8,2′-Anhydro-8-oxy-9-β-D-arabinofuranosyladenine——i) Freshly dried sodium acetate (3.0 g) was dissolved in glacial acetic acid (50 ml) by refluxing in an oil bath. Into this solution was added well-dried 2′-triisopropylbenzenesulfonyl-8-bromoadenosine (3.05 g, 5 mmoles). Reaction mixture was refluxed for 2 hr under exclusion of the moisture. The mixture was added dropwise in water (200 ml) with stirring. Organic material was extracted with $CHCl_3$ (40 ml×3) and the $CHCl_3$-layer was washed throughly with water (100 ml). The solvent was evaporated *in vacuo*, the residue was dissolved in ethanol–benzene mixture, and evaporated to a glass. This procedure was repeated until odor of acetic acid diminished. Into this residual glass was added anhydrous methanol (150 ml) saturated with ammonia at 0°, the solution was tighly stoppered and kept at room temperature for 72 hr. The solvent was completely removed to give a light-brown glass (2.719 g). After drying over P_2O_5 at 100° for 10 hr under vacuum of 10 mm/Hg, the glass was dissolved in dry DMF (200 ml). Sodium acetate (6.0 g) was added into this solution and it was heated in an oil bath at 120° for 1.5 hr. DMF was removed by distillation in a vacuum to *ca.* 5 ml and precipitated sodium acetate was removed by filtration. Sodium acetate was washed with DMF (5 ml), the filtrate and the washings were combined, and evaporated to give a glass. The glass was recrystallized from benzene to give the crystalline 8,2′-cyclonucleoside (555 mg). Mother liquor was evaporated and further 537 mg of the crystalline cyclonucleoside was obtained from the benzene solution. Both crops were combined and recrystallized from water to give a pure material (546 mg, 41%). Sample for analytical purpose was further recrystallized from water. This sample colorized at 190° and decomposed gradually over 190°. *Anal.* Calcd. for $C_{10}H_{11}O_4N_5 \cdot \frac{1}{2}H_2O$: C, 44.57; H, 4.30; N, 25.97. Found: C, 44.23; H, 4.58; N, 25.79. UV: $\lambda_{max}^{H^+}$ 262 nm (ε=146000), 280 nm (sh) (ε=8500); $\lambda_{max}^{H_2O}$ 257 nm (ε=15000); $\lambda_{max}^{OH^-}$ 257 nm (ε=14800). NMR: 8.04 δ (singlet, $J_{1'-2'}$=5.4 cps). PPC: *Rf*(A): 0.46, *Rf*(B): 0.25, *Rf*(C): 0.46. ORD and CD curves are shown in Fig. 3 and 4.

ii) Sodium acetate (3.0 g, freshly dried by fusion) was dissolved in a mixture of acetic acid (25 ml) and acetic anhydride (25 ml) by reflux. 2′-Triisopropylbenzenesulfonyl-8-bromoadenosine (1.53 g, 2.5 mmoles)

14) Ultraviolet absorption was taken with a Hitachi EPS-3T recoding spectrophotometer, infrared absorption was taken with a Hitachi EPI-L spectrophotometer, NMR was taken with a Hitachi H-6013 high resolution spectrometer operated at 60 mc with tetramethylsilane as an internal standard, ORD and CD were taken with a JASCO ORD/UV spectropolarimeter in 10 mm light path at room temperature using water solution of maximum 1.5 OD of nucleosides.

15) Thin-layer chromatography.

16) Paper partition chromatography. *Rf*(A) stands for *Rf* obtained in solvent A.

was added into this solution, which was refluxed for 3 hr under exclusion of the moisture. The solvent was evaporated *in vacuo*, ethanol (30 ml) was added, and the solution was set aside overnight at room temperature. Evaporation and addition of ethanol was repeated until odor of acetic acid diminished. Yellow residue thus obtained was extracted with hot $CHCl_3$ (30 ml×3). $CHCl_3$–layer was washed with water (20 ml×3) and dried over $MgSO_4$ overnight. Desiccant was removed by filtration and washed with $CHCl_3$ (20 ml×3). The filtrate and the washings were combined and evaporated to give N^6,3′,5′-triacetyl-2′-triisopropylbenzenesulfonyl-8-oxyadenosine as yellow glass (1.473 g). The glass was sealed in a steel bottle with anhydrous methanol (30 ml) previously saturated with ammonia at 0° and set aside for 3 days at room temperature. Finally it was heated at 80° for 6 hr to complete the cyclization. The reaction mixture was concentrated under reduced pressure to give a white residue. The residue was dissolved in ethanol (30 ml) and set aside for several hr. 8,2′-O-Cyclonucleoside was obtained as colorless needles (348 mg, 52.6%). This sample was completely identical with the sample obtained in i). *Anal.* Calcd. for $C_{10}H_{11}O_4N_5 \cdot \frac{1}{2}H_2O$: C, 43.79; H, 4.41; N, 25.33. Found: C, 43.50; H, 4.66; N, 25.17.

3′-O-Triisopropylbenzenesulfonyl-8-oxyadenosine——Sodium acetate (3.0 g, freshly dried by fusion) was dissolved in acetic acid (50 ml) by reflux under exclusion of the moisture. 3′-O-Triisopropylbenzenesulfonyl-8-bromo-adenosine (3.04 g, 5.0 mmoles, dried over P_2O_5 at 50° for 3 hr in 5 mm/Hg) was added and the mixture was refluxed for 2 hr. After extent of the reacion was examined by TLC, the solvent was evaporated *in vacuo* until volume of the solution became 10 ml. Concentrated reaction mixture was poured into water (200 ml) with stirring. Precipitates were collected by filtration, washed with water until washings did not show acidic nature, and dried in a desiccator (yield was 2.736 g). The white solid thus obtained was dissolved in anhydrous methanol (50 ml) and the solution was saturated with ammonia at 0°. After setting aside for 41 hr at room temprature, the solution was evaporated to give brown glass (2.75 g). The glass was dissolved in benzene (80 ml) by heating and set aside to precipitate white crystals, which were washed with benzene (20 ml). 8-Oxy compound was obtained in a yield of 1.81 g (66%). Sample (1.7 g) for elemental analysis was recrystallized from benzene (50 ml) and ethanol (2 ml) and colorless fine crystals (861 mg), mp 148—149°, were obtained. *Anal.* Calcd. for $C_{25}H_{35}O_7N_5S$: C, 54.62; H, 6.42; N, 12.74. Found: C, 54.10; H, 6.53; N, 13.10. UV: $\lambda_{max}^{H^+}$ 234 nm (sh) (ε=13000), 271 nm (ε=12100), 286 nm (sh) (ε=10200), 300 nm (sh) (ε=5500); λ_{max}^{MeOH} 235 nm (ε=13800), 260 nm (sh) (ε=11100), 272.5 nm (ε=13500); $\lambda_{max}^{OH^-}$ 232 nm (sh) (ε=11800), 285 nm (ε=16500). IR: ν_{max}^{KBr} 1185 vm^{-1} (aryl sulfonate), 1730 cm^{-1} (five-membered ureide). TLC: $CHCl_3$–EtOH, 18:2, *Rf*: 0.12. PPC: *Rf*(B): 0.94, *Rf*(C): 0.95.

8,3′-Anhydro-8-oxy-9-β-D-xylofuranosyladenine——3′-Triisopropylbenzenesulfonyl-8-oxy-adenosine (1.00 g, 1.82 mmole, dried over P_2O_5 at 50° for 3 hr under 5 mm/Hg) was dissolved in DMF (70 ml), followed by addition of sodium acetate (2.0 g, freshly dried by fusion) and calcium carbonate (5.0 g). The reaction mixture was heated for 5 min until it becomes to refluxing. Reflux was ceased after 2 min and the mixture was set aside for cooling. Precipitated inorganic salts were removed by filtration and the filtrate was concentrated to 2 ml under diminished pressure. DMF (5 ml) was added and precipitated salts were filtered again. Evaporation of the filtrate *in vacuo* gave brownish glass. The glass was dissolved in ethanol (10 ml), decolorized with activated charcoal, and kept in a refrigerator. Precipitated colorless needles (111 mg, 23%) were collected by filtration. From mother liquor 30 mg of crystals were obtained as the second crop. Sample for analytical purpose was further recrystallized twice from water. 8,3′-O-Cyclonucleoside thus obtained colorized at 188° and decomposed at 188—190°. *Anal.* Calcd. for $C_{10}H_{11}O_4N_5 \cdot \frac{3}{4}H_2O$: C, 43.09; H, 4.52; N, 25.12. Found: C, 42.89; H, 4.76; N, 25.22. UV: $\lambda_{max}^{H^+}$ 262 nm (ε=14300), 280 nm (sh) (ε=8600); $\lambda_{max}^{H_2O}$ 262 nm (ε=14900); $\lambda_{max}^{OH^-}$ 265 nm (ε=15200). PPC: *Rf*(A): 0.44, *Rf*(B): 0.29, *Rf*(C): 0.42. ORD and CD curves were shown in Fig. 3 and 4.

Kinetical Study of the Formation of 8,2′- and 8,3′-Cyclonucleosides——8-Oxy-2′- or 3′-triisopropylbenzenesulfonyladenosine (*ca.* 50 mg each) was dissolved in DMSO (5 ml), sodium benzoate (100 mg) was added, and heated at 100—150°. Aliquots were withdrawn from the reaction mixture every 1 hr and applied to a paper electrophoresis in a buffer of pH 7.8 (triethylammonium bicarbonate) at 900 V for 1 hr. Spots were visualized with an ultraviolet lamp, cut and eluted with water (2 ml). Identification of different nucleosides or base were done by their characteristic UV absorption properties and chromatography with authentic samples. Amount of each products was determined from optical density of the eluants. Result were shown in Fig. 1 and 2.

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