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Studies of Nucleosides and Nucleotides. L.[1] Purine Cyclonucleosides. (14). Synthesis and Properties of Cyclonucleosides derived from 9-D-Xylofuranosyladenine[2]

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N^6-Benzoyladenine (II) and 1,2,3,5-tetraacetyl-D-xylofuranose (I) were condensed by fusion method to obtaine α- and β-D-xylofuranosyladenine (IV). Though the β-nucleoside was obtained in a pure crystalline form, the α-anomer was obtained as crystals containing 27% β-anomer as measured by nuclear magnetic resonance. β-D-Xylofuranosyladenine (IVa) was brominated at 8-position and then derived to 3′,5′-O-isopropylidene derivative (VI). Tosylation of VI at 2′-OH followed by the treatment with 40% aqueous NaSH gave a cyclonucleoside, 8,2′-anhydro-8-mercapto-3′,5′-O-isopropylidene-β-D-xylofurnaosyladenine(VII). De-sulfurization with Raney nickel and deprotection with acid gave a novel nucleoside,9-β-(2-deoxy-threo-D-pentofuranosyl)adenine (X).

From α-D-xylofuranosyladenine (IVb), bromination at 8-position with bromine-water in pH 4 gave a crystalline cyclonucleoside, 8,2′-anhydro-8-oxy-9-α-D-xylofuranosyladenine (XV). Optical rotatory dispersion spectrum of XV showed a large negative Cotton effect in contrary to the β-cyclonucleoside, which had the positive effect in B-band region.

We have previously reported on the synthesis of 8,2′-anhydro-α-D-xylofuranosyladenine,[2] which was the only one example of α-type purine cyclonucleoside.[4] As the cyclonucleoside was shown to be useful for transformation of the carbohydrate moiety of Purine nucleosides[5] and for a model of nucleosides having fixed base moieties,[6] synthesis of cyclonucleoside derived from xylofuranosyladenine was attempted.

In order to obtain α- and β-xylofuranosyladenine, 1,2,3,5-tetra-O-acetylxylofuranose[7] (I) was condensed with N^6-benzoyladenine[8] by the fusion method[9] with p-toluenesulfonic acid as catalyst. The anomeric mixture of 6-benzamido-9-(2′,3′,5′-tri-O-acetyl-D-xylofuranosyl)-purine (III) was obtained in a yield of 47%. Without further separation of α- and β-anomer, III was treated with sodium methoxide in methanol to remove protecting groups. The mixture of 9-α- and -β-D-xylofuranosyladenine (IVa,b) was obtained as a glass. When the glass was crystallized from methanol, crystals having mp 225—230° (A) and those having 215—216°(B) were obtained. Examination of the mixture in thin-layer chromatography (solvent, chloroform–ethanol, 15:7) gave two bands corresponding to A (upper band) and B, respectively. As shown in Fig. 1, nuclear magnetic resonance (NMR) spectra of substance A showed a doublet peak of anomeric proton at 6.05 δ having $J_{1'-2'}=3.02$ Hz. Ultraviolet (UV) absorption

1) Part XLIX of this series: M. Ikehara and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **19**, 104 (1971).
2) A part of this study was reported previously: M. Ikehara, M. Kaneko, and Y. Nakahara, *Tetrahedron Letters*, **1968**, 4707.
3) Location: *6-5, Toneyama, Toyonaka, Osaka.*
4) An α-cyclonucleoside has been reported in pyrimidine L-nucleoside: N. Yamaoka, B. A. Otter and J.J. Fox, *J. Med. Chem.*, **11**, 55 (1968).
5) M. Ikehara, *Accounts of Chem. Res.*, **2**, 47 (1969) and references cited therein.
6) M. Ikehara, M. Kaneko, K. Muneyama and H. Tanaka,, *Tetrahedron. Letters*, **1967**, 3977.
7) P.A. Levene and A.L. Raymond, *J. Biol. Chem.*, **102**, 317 (1933).
8) P. Chang and B. Lythgoe, *J. Chem. Soc.*, **1950**, 1992; M.M. Baizer, J.R. Clark, M. Dub and A. Loter, *J. Org. Chem.*, **21**, 1267 (1956).
9) T. Sato, J. Shimadate ,and Y. Ishido, *Nippon Kagaku Zasshi*, **81**, 1440 (1960).

properties of A closely resembled those of adenosine. Although mp of A was different from that reported for 9-β-D-xylofuranosyladenine by Baker and Hewson,[10] mp of picrate, 208—211° (decomp.), coincided with the literature. Also molecular rotation, $[\alpha]_D^{20}$ −16.4° was same with the reported value.[10] Therefore we assigned this substance to 9-β-D-xylofuranosyladenine (IVa).

Substance having mp 215—216° (B) showed almost similar UV absorption properties with those of adenosine. As shown in Fig. 1, NMR of B gave two doublet peaks of anomeric protons at 6.53 δ ($J_{1'-2'}$=3.42) and 6.05 ($J_{1'-2'}$=3.02), respectively. From the integral intensity of these peaks it was shown that the substance B contained 27% of β-anomer (IVa) and 73% of α-anomer (IVb). Further resolution of B to α- and β-anomer by cellulose and silica gel chromatography failed.

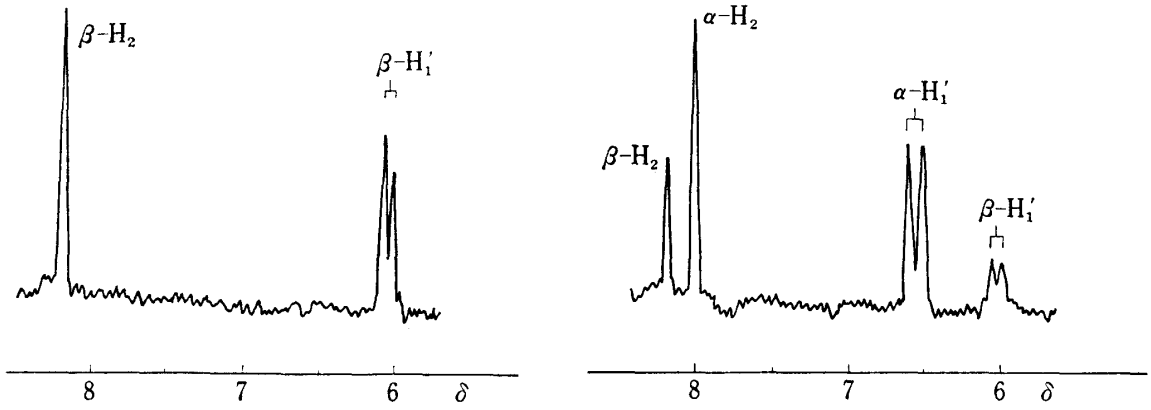


Fig. 1. NMR Spectra of α- and β-D-Xylofuranosyladenine taken in D_2O

9-D-Xylofuranosyladenine (IVa) was then brominated at position 8 with bromine–water in an acetate buffer of pH 4.0.[11] 8-Bromo-9-β-D-xylofuranosyladenine (V) was obtained as crystals having mp 198—199° in a yield of 62.5%. For the large scale experiment, a mixture of anomers was directly brominated and β-bromo derivative was obtained by recrystallization in the yield around 20—30%.

In order to introduce good leaving group to 2'-OH, compound V was converted to 3',5'-O-isopropylidene derivative (VI) by the treatment with di-*p*-nitrophenyl phosphate in acetone-dimethoxypropane mixture[12] and tosylated with tosyl chloride in pyridine to give 2'-O-tosyl-3',5'-O-isopropylidene-8-bromo-9-β-D-xylofuranosyladenine (VII) in a good yield. Although compound VII was obtained as glass, its structure was confirmed by UV absorption properties and infrared (IR) absorption bands at 1336 and 1292 cm^{-1}, which were assigned to tosylate.

When compound VII was treated with 40% aqueous sodium hydrogensulfide in DMF at room temperature for 22 hr, a crystalline compound (VIII) was obtained in a yield of 90%. Ultraviolet absorption spectra of this compound showed λ_{max} around 280 nm, which suggested cyclonucleoside structure as previously shown in adenosine cyclonucleosides.[13] Fast moving behavior in paper chromatography and loss of aryl sulfonate band in IR spectrum also supported this view. The final proof of 8,2'-anhydro bond formation was obtained by desulfurization to afford 2'-deoxy nucleoside (X). Compound VIII was thus confirmed to be 8,2'-anhydro-8-mercapto-9-β-3',5'-O-isopropylidene-D-xylofuranosyladenine. This is the first example of cyclonucleoside derived from xylofuranosyladenine.

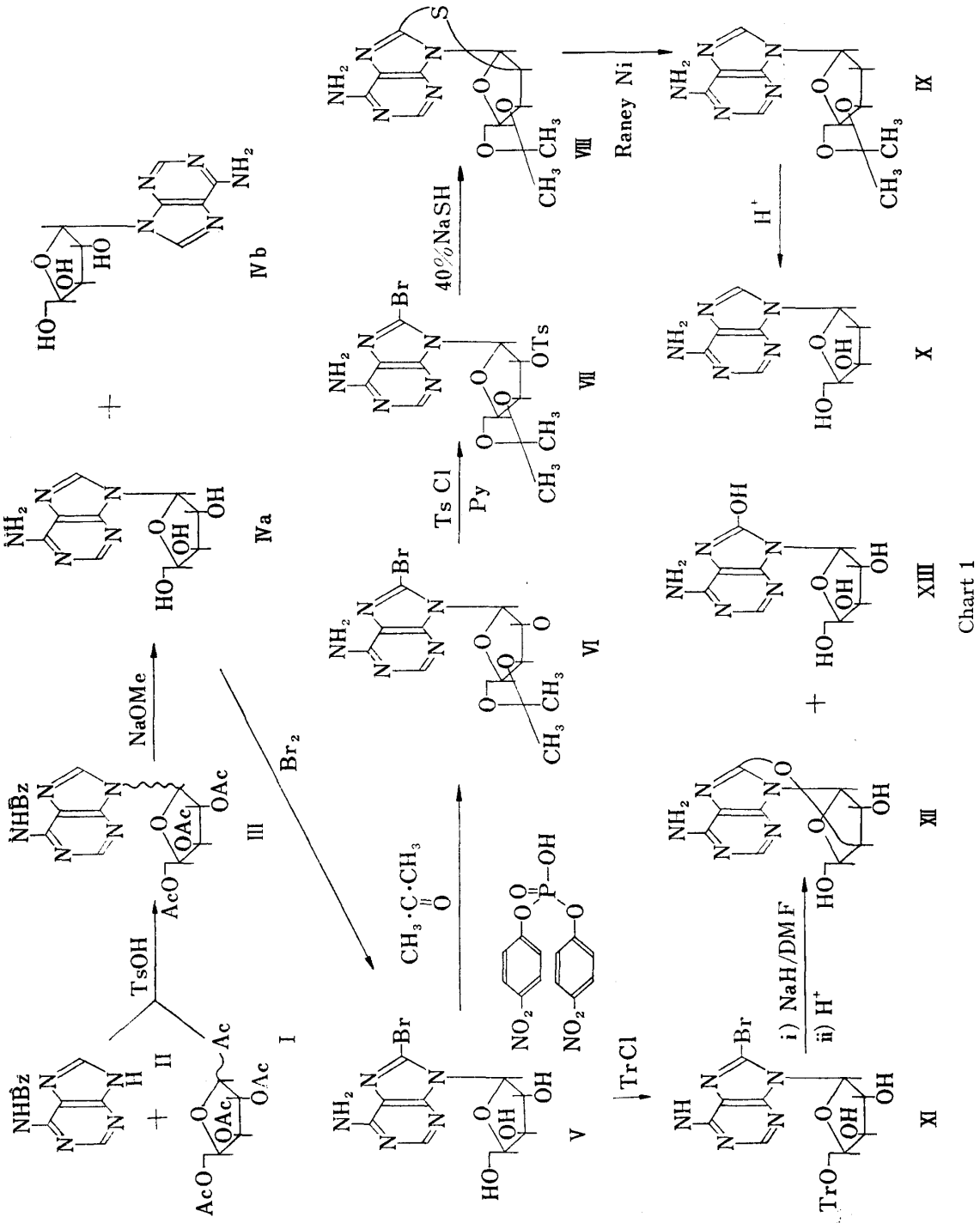
10) B.R. Baker and K. Hewson, *J. Org. Chem.*, **22**, 966 (1957).
11) M. Ikehara, S. Uesugi, and M. Kaneko, *Chem. Commun.*, **1967**, 17.
12) A. Hampton, *J. Am. Chem. Soc.*, **83**, 3640 (1961).
13) M. Ikehara and H. Tada, *Chem. Pharm. Bull.* (Tokyo), **15**, 94 (1967).

The cyclonucleoside VIII was then desulfurized with Raney nickel in 70% aqueous ethanol at reflux temperature for 6 hr. A crystalline nucleoside (IX) was obtained in a yield of 75.5%. Elemental analysis and UV absorption properties, which had λ_{max} around 260 nm showed that the compound IX to be 9-β-(2-deoxy-3,5-O-isopropylidene-*threo*-pentofuranosyl)adenine (IX). Compound IX was then heated in 90% acetic acid at 50° for 3 hr to remove isopropylidene group. Purification of the reaction mixture by paper chromatography in solvent A gave 9-β-(2-deoxy-*threo*-pentofuranosyl)adenine (X). The structure of X was confirmed by UV absorption properties, paper chromatography as well as elemental analysis. The fact that the sugar moiety of compound X revealed red color by sulfuric acid–cystein reagent[14] showed that this carbohydrate should be 2-deoxy sugar.

Goodman, *et al.*[15] previously showed that 8-bromo-9-β-D-xylofuranosyladenine (V) did not give 8,3′-cyclonucleoside, whereas 8-bromo-9-β-D-arabinofuranosyladenine did give 8,2′-cyclonucleoside by a treatment with aqueous alkali. Since we have developed a new method for the cyclization of 8-bromoadenosine using sodium hydride in aprotic solvent,[16] we applied this reaction to compound (V). In order to avoid prior cyclization to 8,5′-cyclonucleoside, compound V was treated with trityl chloride in pyridine to give 8-bromo-9-β-5′-O-trityl-D-xylofuranosyladenine (XI). When compound XI was heated with sodium hydride in DMF at 50° for 13.5 hr, two compounds having *Rf* 0.95[17] and *Rf* 0.80 in thin-layer chromatography (solvent, chloroform–methanol, 8:1) were obtained. Since the latter compound had λ_{max} around 260 nm, which suggested cyclonucleoside structure, this substance was heated with 80% acetic acid to remove trityl group. Separation of the products by thin-layer chromatography gave a compound (XII) having absorption maximum at 260 nm and 8-oxy-9-β-D-xylofuranosyladenine (XIII).[18] Compound XII was identical with 8,3′-anhydro-8-oxy-9-β-D-xylofuranosyl adenine previously synthesized from 2′-O-triisopropylbenzenesulfonyl-8-oxyadenosine.[19] Thus it was proved that 8,3′-O-cyclonucleoside could be obtained from 8-bromo-xylofuranosyladenine by the treatment with sodium hydride in DMF.

When the substance B described above (containing α- and β-xylofuranosyladenine in the ratio of 73:27) was brominated by bromine-water at pH 4.0, a crystalline compound (XV) was obtained as the main product. Compound XV had UV absorption properties closely resembled those of 8,2′-O-cyclonucleoside.[18] Since it was clear that from the β-anomer (IVa) only 8-bromo derivative (V) was formed, the compound XV must arise from 8-bromo-α-D-xylofuranosyladenine (XIV). In order to clarify this point the anomeric mixture of xylofuranosyladenine (IVa,b) was brominated, extracted rapidly with *n*-butanol and applied to a cellulose powder chromatography. The first peak contained 8-bromo-9-β-D-xylofuranosyladenine (V) and the second peak contained its α-anomer (XIV), which had mp 203—204° and UV absorption maximum around 265 nm characteristic for 8-bromo derivative. $[\alpha]_D$ was +17° and an α-configuration was suggested to XIV. When the compound XIV was treated with a buffer solution of pH 4.0 for 25 hr, compound XV was obtained. Therefore, occurrence of XV from the bromination mixture as the main product was interpreted. The conformation in compound XIV might be favorable for such a easy cyclization even in the acidic pH. Structure of compound XV as 8,2′-anhydro-8-oxy-9-α-D-xylofuranosyladenine was confirmed by elemental analysis, NMR spectra, as well as optical rotatory dispersion (ORD) spectrum. In NMR spectra signals appeared at 6.80 δ (2H), 5.65 δ (1H) and 4.71 δ (1H) were exchangeable with deuterium in D_2O and $J_{1'-2'}$ was 5.3 Hz. These data suggested a cyclonucleoside structure having anomeric configuration in α. As shown in Fig. 2, ORD of compound XV showed

14) J.G. Buchanan, *Nature*, **168**, 1091 (1951).
15) E.J. Reist, D.F. Calkins, L.V. Fisher, and L. Goodman, *J. Org. Chem.*, **33**, 1600 (1968).
16) M. Ikehara and M. Kaneko, *J. Am. Chem. Soc.*, **90**, 497 (1968).
17) Compound having *Rf* 0.95 had λ_{max} 314 nm, which suggested N^6-dimethylaminomethylene structure.
18) M. Ikehara, H. Tada, and M. Kaneko, *Tetrahedron*, **24**, 3489 (1968).
19) M. Ikehara and M. Kaneko, *Chem. Pharm. Bull.* (Tokyo), **18**, 2401 (1970).



a large negative Cotton effect around major absorption region. As described previously,[4,5] all β-8-cyclonucleosides had large positive Cotton effects in B region. α-Adenosine has small positive Cotton effect around 260 nm contrary to the naturally occurring β-purine nucleosides.[20] Therefore it is interesting to see an inversion of sign of the Cotton effect in B region by the formation of the anhydro linkages both in α- and β-purine nucleosides.

Compound XV gave 8-oxy-9-α-xylofuranosyladenine (XVI) by the acidic hydrolysis. Regarding a previous investigation that acidic treatment of O-cyclonucleosides always gave 8-oxyadenine nucleosides in which configuration of carbohydrate hydroxyl groups were in retention, the structure of compound XVI was supported.

20) T.R. Emerson, R.J. Swan, and T.L.V. Ulbricht, *Biochemistry*, **6**, 843 (1967).

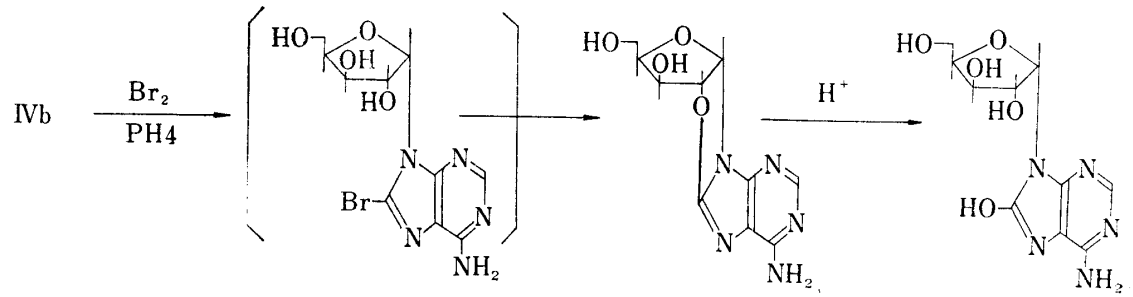


Chart 2

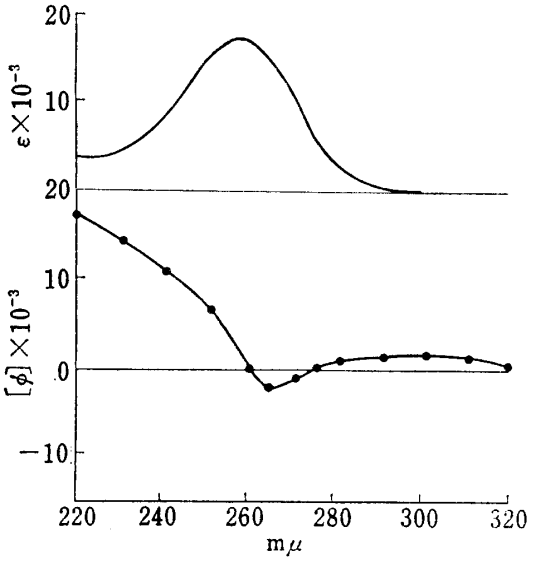


Fig. 2. ORD Spectrum of 8.2'-Anhydro-8-oxy-9-α-D-xylofuranosyladenine

Experimental[21]

Paper Chromatography——All chromatographies were performed by ascending technique unless it was noted. Solvent used were: A, water adjusted to pH 10 by conc. ammonia; B, *n*-butanol–water (86: 14); C, isopropanol–conc.ammonia–water (7: 1: 2); D, isopropanol–conc. ammonia–water (55: 10 :35); E, *n*-butanol–acetic acid–water (5: 2: 3); F, ethanol–1M ammonium acetate (pH7.5) (7: 3).

6-Benzamido-9-α- and β- (2,3,5-tri-O-acetyl-D-xylofuranosyl) purine (III)——1,2,3,5-Tetra-O-acetyl-D-xylofuranose[7] (10 g, 31 mmoles) and N^6-benzoyladenine (7.5 g, 31 mmoles) were mixed in a flask and heated at 150° in a vacuum of 1 mm/Hg. After 10 min *p*-toluenesulfonic acid (286 mg) was added and the mixture was heated at 160—170° with stirring for 30—40 min *in vacuo*. When the reaction mixture became translucent, it was cooled to room temperature. The product was extracted with hot benzene (300 ml), organic layer was washed with saturated aqueous sodium bicarbonate solution, and dried over sodium sulfate. The solvent was evaporated to 30 ml and the solution was applied to a column (6×20 cm) of silica gel, which was previously equilibrated with benzene. Elution with benzene gave unreacted tetraacetylxylose. The column was then eluted with chloroform–ethanol (30: 2, v/v) to give a glass (7.44 g, 47%). UV $\lambda_{max}^{pH\,2}$: 284 nm, $\lambda_{max}^{pH\,7}$: 281 nm, $\lambda_{max}^{pH\,12}$: 316 nm, IR ν_{max}^{film}: 1747 (acetyl), 1705 (sh, N-benzoyl), 1612 (C=C), 1588 (C=N), 1230 (aromatic ring), 1210 cm^{-1} (lactole-O). TLC: *Rf* 0.65 (chloroform–ethanol, 30: 2).

9-α- and β-D-Xylofuranosyladenine (IVb and IVa)——Anomeric mixture of benzamido-9-(2,3,5-tri-O-acetyl-D-xylofuranosyl)purine (10.5 g, 20.7 mmoles) was dissolved in anhydrous methanol (400 ml) containing sodium methoxide (prepared from 714 mg, 51 matoms of sodium metal). After the solution was refluxed for 30 min, it was neutralized with Amberlite IRC-50 (H^+ form). The resin was filtered off and the filtrate was evaporated *in vacuo* to afford a glass (4.64 g, 83.7%). Recrystallization from methanol gave two crops of crystalls, mp 225—230° (A) and mp 215—216° (B) (total yield was 1.54 g). A(9-β-D-xylofuranosyladenine): *Anal.* Calcd. for $C_{10}H_{13}O_4N_5$: C, 43.81; H, 4.71; N, 25.28. Found: C, 43.66; H, 4.52; N, 25.01. UV $\lambda_{max}^{pH\,2}$: 258 nm, $\lambda_{max}^{pH\,7}$: 260 nm, $\lambda_{max}^{pH\,12}$: 260 nm, $[\alpha]_D^{20}$ −16.4° (*c*=1.10, water). NMR: 6.05 (H–1', *d*, 1H, $J=_{1'-2'}=3.02$ Hz). PPC: *Rf*(A) 0.50, *Rf*(B) 0.38, *Rf*(C) 0.64, Picrate: mp 208—211° (decomp.), $[\alpha]_D^{20}$ −41.7° (*c*=0.89, pyridine). B: *Anal.* Calcd. for $C_{10}H_{13}O_4N_5$: C, 43.81; H, 4.71; N, 25.28. Found: C, 43.53; H, 4.87; N, 24.98. UV $\lambda_{max}^{pH\,2}$: 258 nm, $\lambda_{max}^{pH\,7}$: 260 nm, $\lambda_{max}^{pH\,12}$: 260 nm. $[\alpha]_D^{20}$ +14.3° (*c*=1.10, water). NMR: 6.53 (–H–1', *d*, 1H, $J_{1'-2'}=3.42$ Hz), 6.05 (–H–1', *d*, 1H, $J_{1'-2'}=3.02$), ratio of integral intensity α–H–1'/β–H–1'=73/27. PPC: *Rf*(B) 0.23, *Rf*(C) 0.64.

8-Bromo-9-β-D-xylofuranosyladenine (V)——i) 9-β-D-Xylofuranosyladenine (277 mg, 1 mmole) was dissolved in 0.25 M acetate buffer (10 ml, pH 4). Into the solution was added saturated bromine-water (15 ml)

21) Ultraviolet spectra were measured with a Hitachi EPS-3T spectrophotometer, infrared spectra with a Hitachi EPI-L spectrophotometer, NMR spectra with a Hitachi H-6013 high resolution spectrometer operated at 60 mHz with tetramethylsilane as an internal standard, and ORD was taken with a JASCO ORD/UV-5 spectropolarimeter.

and kept at room temperature for 40 hr. The reaction mixture was adjusted to pH 7 with 1N sodium hydroxide and concentrated *in vacuo* to *ca.* 1/3 of its volume. Extraction with *n*-butanol and evaporation *in vacuo* gave amorphous powder (275 mg, 80%). Recrystallization from ethanol gave colorless rods, mp 196—198° (255 mg, 62%). *Anal.* Calcd for $C_{10}H_{12}O_4N_5Br \cdot 1/3C_2H_6O$: C, 35.42; H, 3.87; N, 19.38. Found: C, 35.52; H, 3.85; N, 18.97. UV $\lambda_{max}^{pH\,2}$: 262.5 nm, $\lambda_{max}^{pH\,7}$: 265 nm, $\lambda_{max}^{pH\,12}$: 265 nm. NMR (DMSO-d_6): 5.70 (H-1′, *d*, $J=_{1'-2'}=3.10$ Hz). $[\alpha]_D^{12}$ −39° ($c=0.5$, methanol). PPC: *Rf*(A) 0.49; *Rf*(B) 0.62; *Rf*(C) 0.73.

ii) Anomeric mixture of 9-α- and β-D-xylofuranosyladenine (935 mg, 3.5 mmoles) was dissolved in 0.25M acetate buffer (pH 4, 18 ml). The mixture was added with bromine (−) water (50 ml) and kept at room temperature for 40 hr. The work-up procedure as described in i) gave an amorphous powder (969 mg, 80%). This substance was applied to a column of cellulose powder (6×43 cm) and eluted with solvent B. The first peak was evaporated to give a glass (440 mg), which was recrystallized from ethanl to give colorlessrods, mp 198—199°. This sample was identical with 8-bromo-β-D-xylofuranosyladenine obtained in i).

8-Bromo-9-α-D-xylofuranosyladenine (XIV)——This compound was obtained from the second peak of the column chromatography described in ii) for the synthesis of 8-bromo-9-β-D-xylofuraosyladenine. The second peak was evaporated *in vacuo* and recrystallized from ethanol. Colorless crystals, mp 203—204° were obtained. *Anal.* Calcd. for $C_{10}H_{12}O_4N_5Br \cdot 1/3C_2H_6O$: C, 35.42; H, 3.87; N, 19.38. Found: C, 35.46; H, 3.96; N, 18.90. UV $\lambda_{max}^{pH\,2}$: 263 nm, $\lambda_{max}^{pH\,7}$: 265 nm, $\lambda_{max}^{pH\,12}$: 266 nm, NMR (DMSO-d_6): 5.72 (H-1′, *d*, $J_{1'-2'}=3.87$). $[\alpha]_D^{12}$ +17° ($c=0.5$, methanol).

8-Bromo-9-β-3′,5′-O-isopropylidene-D-xylofuranosyladenine(VI)——8-Bromo-9-β-D-xylofuranosyladenine (980 mg, 2.83 mmoles) was dissolved in anhydrous acetone (30 ml) and dimethoxypropane (2.2 ml) containing di-*p*-nitrophenylphosphate (1.16 g). After stirring ar toom temperature for 6 hr, the solution was made alkaline with methanol–water (1: 1, v/v) (70 ml) containing 0.25M ammonium bicarbonate. Resulting precipitates were removed by filtration and the solvent was evaporated *in vacuo* to give a residue. The residue was dissolved in chloroform and applied to a column of alumina. Elution with chloroform gave a brown oil which was discarded. Ethyl acetate elution gave pale yellow glass, which was recrystallized from ethyl acetate to give colorless needles, mp 216—217° (860 mg, 79%). *Anal.* Calcd. for $C_{13}H_{16}O_4N_5Br$: C, 40.43; H, 4.18; N, 18.14. Found: C, 39.88; H, 4.22; N, 18.05. VU $\lambda_{max}^{pH\,2}$: 264 nm, $\lambda_{max}^{pH\,7}$: 265.5 nm, $\lambda_{max}^{pH\,12}$: 264 nm.

8-Bromo-9-β-2′-O-tosyl-3′,5′-O-isopropylidene-D-xylofuranosyladenine (VII)——Isopropylidene derivative (VI) was dissolved in pyridine (6 ml), tosyl chloride (320 mg, 1.6 mmole) was added and kept at room temperature for 21 hr. The reaction was stopped by addition of water (5 ml) and after 30 min the mixture was extracted with chloroform. The organic layer was washed with saturated aqueous sodium bicarbonate, then with water, and dried over magnesium sulfate. The solvent was evaporated to give a pale yellow glass (400 mg, 98%). UV $\lambda_{max}^{pH\,2}$: 263.5 nm, $\lambda_{max}^{pH\,7}$: 264.5 nm, $\lambda_{max}^{pH\,12}$: 265 nm. IR ν_{max}^{film}: 1336, 1292 cm^{-1} (aryl sulfonate).

8,2′-Anhydro-8-mercapto-9-β-3′,5′-O-isopropylidene-D-xylofuranosyladenine (VIII)——Isopropylideneto-syl (VII) (323 mg, 0.6 mmoles) was dissolved in DMF(50 ml) followed by the addition of 40% aqueous sodium hydrogen sulfide (0.3 ml). The mixture was kept at room temperature for 22 hr. During this time color of the reaction mixture changed from pale yellow to dark green. The solvent was evaporated below 40° in 1—2 mm/Hg vacuum. White crystals (177 mg, 93%), thus obtained, were recrystallized from chloroform to give needles (170 mg, 90%), mp 255—256° (decomp.). *Anal.* Calcd. for $C_{13}H_{15}O_3N_5S$: C, 48.58; H, 4.70; N, 21.79. Found: C, 48.66; H, 4.59; N, 21.59. PPC: *Rf*(A) 0.55; *Rf*(B) 0.55; *Rf*(C) 0.66; *Rf*(E, descending) 0.84.

9-β-(2-Deoxy-3,5-O-isopropylidene-D-threopentofuranosyl)adenine (IX)——Cyclonucleoside (VIII) (58 mg, 0.16 mmole) was dissolved in 70% ethanol (8 ml). To the solution was added ethanol (0.6 ml) containing Raney nickel (W-2, 450 mg) and heated at reflux temperature for 6 hr. The catalyst was removed by filtration and filtrate was evaporated to give white crystals, (40 mg, 75.5%), mp 158—160°. *Anal.* Calcd. for $C_{13}H_{17}O_3N_5$: C, 53.60; H, 5.88; N, 24.03. Found: C, 53.41; H, 5.96; N, 24.01. UV: $\lambda_{max}^{pH\,2}$ 259 nm, $\lambda_{max}^{pH\,7}$ 261 nm, $\lambda_{max}^{pH\,12}$ 260 nm. PPC: *Rf*(C) 0.92; *Rf*(D, descending) 0.89.

9-β-(2-Deoxy-D-threopentofuranosyl)adenine (X)——2′-Deoxy-isopropylidenexylofuranosyladenine (IX) (40 mg) was heated in 90% acetic acid (10 ml) at 50° for 3 hr. Solvent was evaporated *in vacuo* below 35° and a white powder was obtained. Preparative paper chromatography of this powder gave two bands, *Rf* 0.30 and *Rf* 0.53, respectively. The former substance was identical with authentic adenine. The latter compound was recrystallized from water to give colorless tiny needles, mp 191—192°. *Anal.* Calcd. for $C_{10}H_{13}O_3N_5$: C, 46.72; H, 5.07; N, 27.21. Found: C, 46.57; H, 4.92; N, 27.14. UV $\lambda_{max}^{pH\,2}$: 259 nm, $\lambda_{max}^{pH\,7}$: 260 nm, $\lambda_{max}^{pH\,12}$: 260 nm, PPC: *Rf*(D, descending) 0.62; *Rf*(F, descending) 0.60. This sample revealed a pink colorization with cystein-sulfuric acid reagent.[14]

8-Bromo-9-β-5′-trityl-D-xylofuranosyladenine (XI)——8-Bromoxylofuranosyladenine (1.2 g, 3.45 mmoles) was dissolved in pyridine (22 ml) followed by the addition of trityl chloride (25 g, 9 mmoles). The reaction mixture was kept at room temperature for 45 hr. Reaction was stopped by the addition of water (25 ml) and the organic material was extracted with chloroform (30 ml×3). Organic layer was washed with aqueous sodium bicarbonate, then with water, and dried over magnesium sulfate. Chloroform was evaporated *in vacuo*. The residue dissolved in benzene and applied to a column of alumina. Elution with benzene

and chroloform removed trityl alcohol and a small amount of dityrityl derivative. Elution with ethyl acetate-ethanol (2: 1, v/v) gave white crystals (1.56 g, 77%). Recrystallization from ethyl acetate gave colorless needles, mp 218—220°. *Anal.* Calcd. for $C_{29}H_{26}O_4N_5Br$: C, 59.19; H, 4.45; N, 11.90. Found: C, 59.31; H, 4.57; N, 11.78. UV λ_{max}^{EtOH}: 265 nm.

8,3′-Anhydro-8-oxy-9-β-D-xylofuranosyladenine (XII)——Tritylxylosyladenine (XI) (98 mg, 0.17 mmole) was dissolved in DMF (10 ml) followed by the addition of sodium hydride (20 mg, containing 50% mineral oil). The mixture was heated at 50° for 13.5 hr. After cooling ,ice was added to decompose sodium hydride. The solution was evaporated *in vacuo* to dryness, and applied to preparative TLC (chloroform–methanol, 8: 1). Three bands, *Rf* 0.95 (starting material), *Rf* 0.85 and *Rf* 0.80 were revealed. Substance having *Rf* 0.85 showed $\lambda_{max}^{pH\ 2}$ 260, 330 nm, λ_{max}^{EtOH} 260, 314 nm, and $OD_{280}/OD_{260}=0.60$. These properties suggested N^6-dimethylaminomethylene derivative.[22] Substance having *Rf* 0.80 showed $\lambda_{max}^{pH\ 2}$ 261.5 nm, λ_{max}^{EtOH} 260 nm, and OD_{280}/OD_{260} 0.62. These properties suggested cyclonucleoside structure. The latter substance was collected by extraction of silica gel with hot ethanol. White powder, thus obtained, was heated in 80% acetic acid (0.1 ml) at 95° for 10 min. The reaction mixture. was applied to a preparative chromatography on silica gel to give two bands (A and B) in addition to a bond of trityl alcohol. Substance A showed UV $\lambda_{max}^{pH\ 2}$: 266, 287 nm, $\lambda_{max}^{pH\ 7}$: 271 nm, $\lambda_{max}^{pH\ 12}$: 281 nm, PPC: *Rf*(A) 0.56; *Rf*(C,descending) 0.49. These properties were identical with those reported for 8-oxy-9-β-D-xylofuranosyladenine.[18] Substance B showed UV $\lambda_{max}^{pH\ 2}$: 260.5 nm, $\lambda_{max}^{pH\ 7}$: 259 nm, $\lambda_{max}^{pH\ 12}$: 260 nm, $OD_{280}/OD_{260}=0.63$. By the direct comparison with an authentic 8,3′-anhydro-8-oxy-9-β-D-xylofuranosyladenine[19] this compound showed complete identity.

8,2′-Anhydro-8-oxy-9-α-D-xylofuranosyladenine (XV)——i) Anomeric mixture of 9-D-xylofuranosyladenine (7.3 g, 27.3 mmoles) was dissolved in 0.25M acetate buffer (220 ml, pH 4.0) followed by the addition of bromine–water (250 ml). The mixture was kept at room temperature for 25 hr. Color of excess bromine was discharged by 2N sodium bisulfite solution and adjusted to pH 8 with 2N sodium hydroxide solution. When the solution was evaporated *in vacuo* to its half volume, precipitate of crystalline material occurred. Crystals were collected by filtration and recrystallized from water to give colorless rods (975 mg), mp 258—261° (decomp.). *Anal.* Calcd. for $C_{10}H_{11}O_4N_5\cdot H_2O$: C, 42.40; H, 4.63; N, 24.73. Found: C, 42.68; H, 4.57; N, 24.31. UV $\lambda_{max}^{pH\ 2}$: 259.5 nm, (ε 16,570), $\lambda_{max}^{pH\ 7}$: 257 nm, (ε 16,800), $\lambda_{max}^{pH\ 12}$: 260 nm, (ε 16,770). $OD_{280}/OD_{260}=0.57$. $[\alpha]_D^{26}$ +86.9 ($c=0.78$, dimethylsulfoxide). NMR (DMSO-d_6): 8.04 (H–2, s, 1H,) 6.80 (6–NH_2, s, 2H, exchangeable with D), 6.52 (H–1′, d, 1H, $J_{1'-2'}=5.0$ Hz), 5.65 (H–3′, m, 1H, exchangeable with D), 4.71 (H–2, t, 1H, $J_{4'-5'}=5.3$ Hz, exchangable with D). ORD (H_2O at 27°): $[\phi]_{peak}^{220}$ +17,430, $[\phi]_{trough}^{264}$ −2380, amplitude 19800. PPC: *Rf*(A) 0.54; *Rf*(B) 0.36; *Rf*(D, descending) 0.66.

ii) 8-Bromo-9-α-D-xylofuranosyladenine (XIV) (10 mg) was dissolved in a sodium acetate buffer (1 ml, pH 4.0) and kept at room temperature for 3 days. Examination with PPC showed complete cyclization to 8,2′-cyclonucleoside (XV), which was confirmed by comparison with a sample obtained in i).

Acidic Hydrolysis of α-Cyclonucleoside (XV)——The cyclonucleoside XV (18 mg, 0.07 mmole) was dissolved in 2N sulfuric acid (3 ml) and kept at reflux temperature for 1 hr. The reaction mixture was neutralized with conc. ammonia and evaporated to give a residue. The residue was applied to preparative paper chromatography. Results were shown in Table I.

Table I

Compound	Paper chromatography			UV_{max} (nm)		
	Solvent A	B	C	pH 2	pH 7	pH 12
Hydrolysis product I	0.39	0.42	0.56	265 282	271	280
Hydrolysis product II	0.73	0.28	0.65	267 288	272	282
8-Oxyadenine	0.39	0.42	0.57	265 282	271	280
8-Oxy-9-β-D-xylofuranosyl-adenine	0.67	0.25	0.64	266 287	271.5	281

From the slight difference in *Rf* values in three solvent systems and similarity in UV absorption properties with 8-oxy-9-β-D-xylofuranosyladenine, the product II was suggested to be 8-oxy-9-α-D-xylofuranosyladenine. Compound I was identical with adenine.

22) J. Zemlicka and A. Holly, *Collect. Chzech. Chem. Commun.*, **32**, 3159 (1967).