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STUDIES OF NUCLEOSIDES AND NUCLEOTIDES—XXXV[1]

PURINE CYCLONUCLEOSIDES—5. SYNTHESIS OF PURINE CYCLONUCLEOSIDE HAVING 8,2'-O-ANHYDRO LINKAGE AND ITS CLEAVAGE REACTIONS[2]

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Abstract—Starting from 2'(or 3')-O-*p*-toluenesulfonyl-5'-O-acetyl-8-bromoadenosine, introduction of 8-oxy function by the reaction with sodium acetate in acetic acid and cyclization with sodium benzoate in dimethylformamide gave 8,2'-anhydro-8-oxy-9-β-D-arabinofuranosyladenine as the first purine cyclonucleoside having an O-anhydro bond. Configurational similarity of the 8,2'-O- and S-cyclonucleosides was discussed on the basis of UV absorption, NMR and optical rotation studies. Hydrolytic cleavage of the cyclonucleoside with dilute acid gave 8-oxy-9-β-D-arabinofuranosyladenine, showing the attack occurred on the C_8 position. In contrast to this, the reaction with benzoate anion gave 2'-O-benzoyladenosine, which showed the attack by strong nucleophile had occurred on the C_2 position.

THE CHEMISTRY of the cyclonucleoside has been studied extensively as an unique reaction of the pyrimidine nucleosides.[3] By the formation of the anhydro bond, the anomeric configuration of the nucleoside could be established[4] and by the use of suitable reagent, which cleaves the anhydro bond, nucleosides having a variety of sugar moieties have been synthesized.[5] Moreover, recent studies of the ORD of cyclonucleosides[6–9] showed their usefulness as model compounds, in which rotation of the base moiety around nucleosidic linkage was restricted.

We have reported the synthesis of purine cyclonucleosides having S-anhydro linkages formed between 8 and 2'-,[10–12] 3'-[11,13] or 5'-[14] positions. Desulfurization of these S-cyclonucleosides with Raney nickel gave naturally occurring 2'-deoxyadenosine, antibiotic Cordycepin (3'-deoxyadenosine) and 5'-deuoxyguanosine. The properties of these cyclonucleosides are of special interest, because they have base moieties fixed in a definite position by the formation of anhydro linkages and also because of the relatively high stability of the anhydro bond towards hydrolyses.[10] Since an ether linkage may have a tendency to be cleaved much easier than sulfide bond, we attempted to synthesize a purine cyclonucleoside having an O-anhydro bond. This bond could be cleaved by various nucleophiles to give nucleosides having transformed sugar moieties. A hypothetical mechanism[15] on the pathway of purine ribonucleotide to deoxyribonucleotide has been presented assuming 8,2'-O-cyclonucleoside as intermediate.

In order to obtain suitable intermediate for the formation of O-cyclonucleoside, in which 2' (or 3') and 5'-OH group could be selectively blocked, we started with the synthesis of 8-bromo-2',3'-O-isopropylidene-5'-O-acetyladenosine (I). Although this compound has been synthesized,[9] a new method[16] for the bromination of adenosine made the synthesis much easier and increased the yield of the desired product. Thus,

bromination of 2',3'-O-isopropylideneadenosine by bromine-water in a buffered solution gave the 8-bromo compound in the yield of 74%. The usual acetylation of the bromo derivative gave compound I in crystalline form in the over-all yield of 56% from isopropylideneadenosine. 8-Bromo-2',3'-O-isopropylidene-5'-O-acetyladenosine (I) was then deacetonated by formic acid and mono-tosylated at 2' or 3'-position to afford 8-bromo-2' (or 3')-O-tosyl-5'-O-acetyladenosine (II). When II was refluxed in glacial acetic acid in the presence of sodium acetate,[17] the conversion of bromo to oxy function was followed by the change in UV absorption maxima and by the difference in behavior on paper chromatograms. The 8-oxy compound III, thus obtained, was treated with methanolic ammonia to remove the acetyl group and 8-oxy-2' (or 3')-O-tosyladenosine (IV) was obtained.

The cyclization of IV was carried out by the treatment with sodium benzoate-dimethyl formamide,[18,19] which was thought to be one of the powerful nucleophilic reagent. After heating at 100–110° for 2·5 hr, IV changed to a nucleoside having UV absorption max at 260 mμ, which was similar to that of 8-methoxyadenosine.[20] Since the examination of the reaction mixture on paper chromatography showed the existence of several compounds, the mixture was purified by column chromatography on cellulose powder. Elution with 1-propanol–water gave the starting material IV and the cyclonucleoside (V), accompanied by another two compounds having unknown structure. The cyclonucleoside (V) was obtained as a crystalline compound, m.p. 210° (dec). The elemental analysis data suggested the anhydronucleoside structure for this compound. As reported in the previous paper,[11] the UV absorption properties of 8,2'-S-cyclonucleoside are similar to those of 8-methylmercapto-adenosine and 8,3'-isomer has the spectra shifted slightly towards bathochromic region as shown in Table 1. Accordingly, it might be conceivable that the absorption properties of 8,2'-O-cyclonucleoside are similar to those of 8-methoxyadenosine. The NMR spectra (Table 1) of compound V shows that the H_1, proton has a doublet signal at 6·5 δ and coupling constant, $J_{1'-2'}$ 5·4 c/s. This value is consistent with the coupling constant of $H_{1'-2'}$ in the *cis* configuration.[21] The dihedral angle formed by $H_{1'}$—$C_{1'}$—$C_{2'}$—$H_{2'}$ was calculated as 35° according to the Karplus equation.[22] As shown in the case of S-cyclonucleoside, this angle must fall in the range around 30° for 8,2'- and over 100° for 8,3'-anhydronucleoside. This was also supported by the examination of Dreiding molecular models. The optical rotation of V (−121·6°) also suggested the structure of 8,2'-cyclonucleoside rather than 8,3'-compound, because 8,2'-S-cyclonucleoside has the value −214–187° and 8,3'- has −32°. From these chemical and physical properties the structure of V was concluded to be 8,2'-anhydro-8-oxy-9-β-D-arabinofuranosyladenine.

The cleavage reaction of this O-anhydro linkage in V was then investigated. As reported in the pyrimidine cyclonucleoside,[23] cleavage by acidic hydrolysis was presumed to give an *arabino* nucleoside. When V was refluxed in 0·1N H_2SO_4 for 2 hr, a nucleoside having the 8-oxyadenine chromophore (VI) and 8-oxyadenine[24] (VII) were obtained in addition to a sugar derivative. In order to determine the structure of VI, a comparison was made with the authentic samples of 8-oxyadenosine (VIII) and 8-oxy-9-β-D-xylofuranosyladenine (IX). Compound VIII was synthesized from 8-bromoadenosine by sodium acetate–acetic anhydride treatment, followed by ammoniacal removal of N^6-acetyl group. Compound IX was obtained by the condensation of 8-bromoadenine[25] chloromercuri salt with 2,3,5-tri-O-acetyl-D-

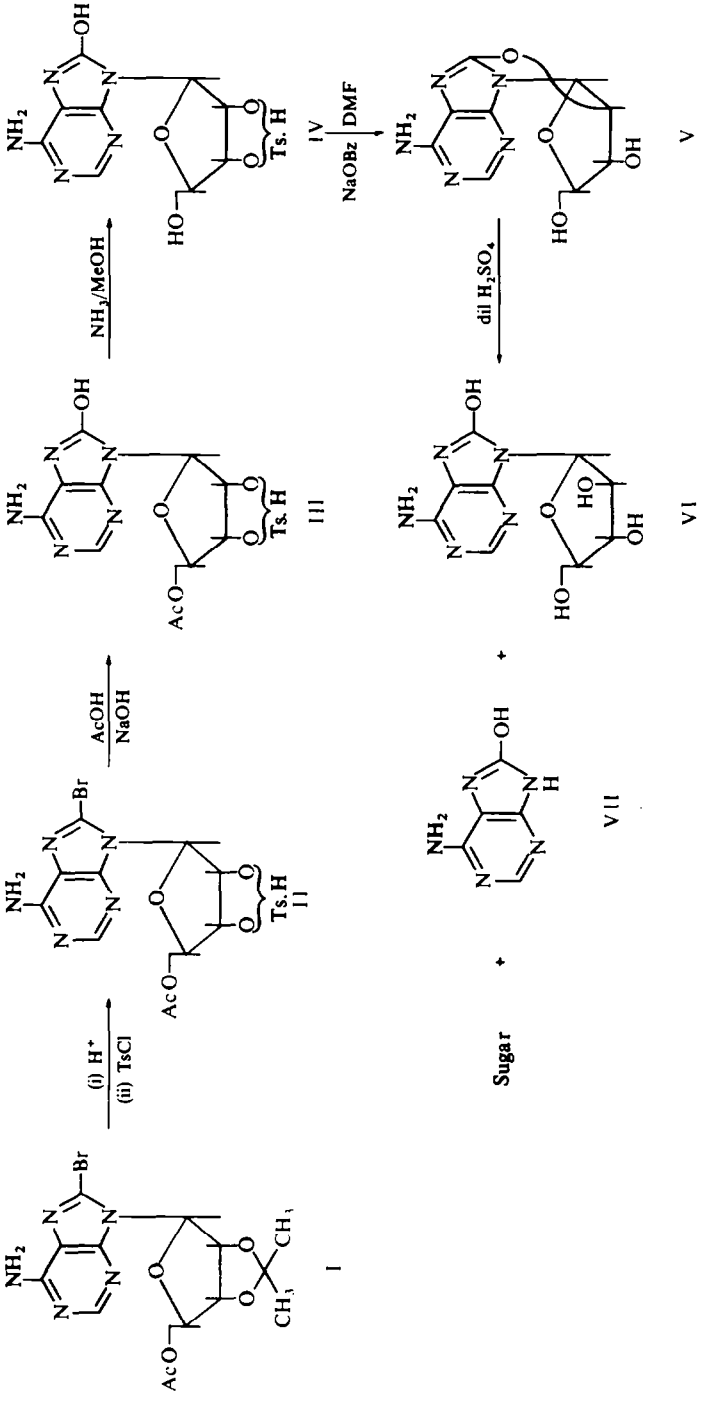
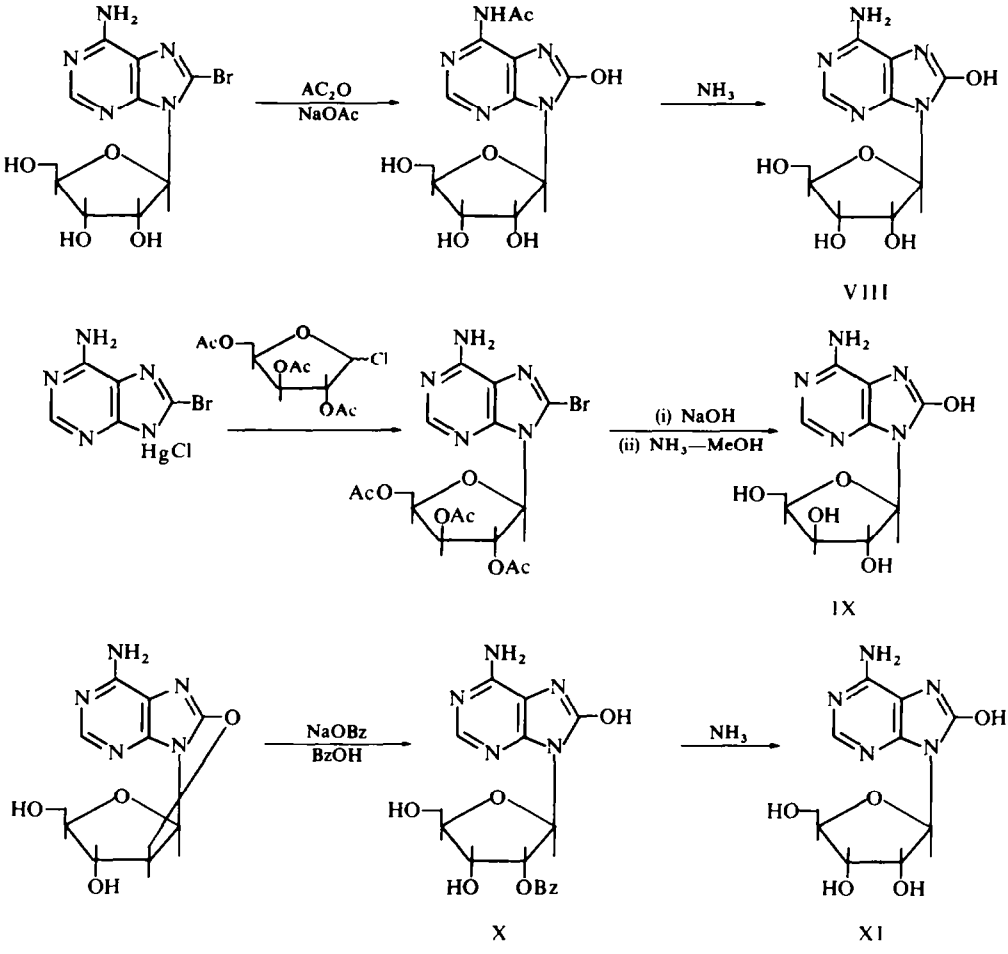

(i) H+
(ii) TsCl
AcOH
NaOH
NH3/MeOH
NaOBz | DMF
dil H2SO4
Sugar
+
+
I
II
III
IV
V
VI
VII

TABLE 1. PHYSICAL CONSTANTS OF VARIOUS PURINE CYCLONUCLEOSIDES AND RELATED COMPOUNDS

Compound	λ_{max} (mμ) in ultraviolet absorption			$[\alpha]_D$	$H_{1'}$ (δ)	NMR $J_{1'-2'}$ (c/s)	Angle[a]	Ref.
	pH 1	H_2O	pH 14					
8,2'-Anhydro-8-mercapto-9-β-D-arabinofuranosyladenine	276	276	277	−187·2°[b]	6·51	6·6	25·6°	11
2-Chloro-8,2'-anhydro-8-mercapto-9-β-D-arabinofuranosyladenine	281	281	282·5	−214°[c]	6·82	7·0	21·8°	10
8,3'-Anhydro-8-mercapto-9-β-D-xylofuranosyladenine	284	283	285	−32·0°[d]	6·39	2·5	121°	11
8,2'-Anhydro-8-oxy-9-β-D-arabinofuranoslyladenine	259	260	260	−121·6°[e]	6·50	5·4	35·4°	
8-Methylmercaptoadenosine	281		279					20
8-Methoxyadenosine	261		259					20

[a] Calculated value.
[b] Measured at 23·5°, c = 1·0 in water.
[c] Measured at 20·5°, c = 1·0 in water.
[d] Measured at 20°, c = 0·75 in pyridine.
[e] Measured at 19°, c = 0·85 in pyridine.

xylofuranosyl chloride[26] in refluxing dichloroethane, followed by the removal of protecting groups with ammonia and successive conversion to 8-oxy derivative with sodium acetate. The structure of VIII and IX was confirmed by chemical and physical properties as well as elemental analyses. The comparison VI with these two isomers was achieved by the paper chromatography in various solvent systems as summarized in Table 2. The R_f values of VI clearly differ from either VIII or IX. Since VI was assumed to be 8-oxy-9-glycosyladenine as described above, the structure of VI was determined as 8-oxy-9-β-D-arabinofuranosyladenine by this comparison. The alternate possibilities that VI has the pyranoside or acyclic sugar moiety were neglected, because VI did not consume metaperiodate.



In order to confirm the structure of the sugar obtained in the above acidic hydrolysis of V and the sugar attached to VI, the total acidic hydrolysate was analyzed by gas chromatography.[27] As shown in Figs. 1 and 2, the chromatogram of the sugar from V closely resembled that of D-arabinose (which was previously treated under acidic conditions) and not D-ribose also treated with acid. From these analyses, the structure of VI assigned as 8-oxy-9-β-D-arabinofuranosyladenine was shown to be correct.

TABLE 2. R_f VALUES OF 8-OXYADENINE PENTOFURANOSIDES

Solvent[a]	8-Oxyadenosine	8-Oxy-9-β-D-Arabino-furanosyladenine	8-Oxy-9-β-D-xylo-furanosyladenine
A	0·48	0·39	0·48
B	0·23	0·14	0·20
G	0·43	0·44	0·42
H	0·50	0·41	0·50
IO_4^- spray	+	−	−

[a] Composition of the solvent, see text.

From this experiment it was found that the hydrolysis with sulfuric acid of the 8,2′-O-anhydro linkage in V occured by the attack on C_8 to give the *arabino* rather than on $C_{2'}$ from the rear side to give the *ribo* configuration to the sugar. This is in accordance the report on the pyrimidine cyclonucleoside.[21]

The introduction of an OH function in the *ribo* configuration into the sugar moiety by the cleavage of the 8,2′-O-anhydro linkage was then investigated. As shown in the pyrimidine nucleoside, a powerful nucleophile such as the alkali salt of an organic acid in a polar aprotic solvent[16] could be utilized for this purpose. When V was heated with sodium benzoate in DMF in the presence of benzoic acid, a nucleoside (X) having the 8-oxyadenine chromophore and a benzoyl group in the sugar moiety was obtained. The structure of X was confirmed by elemental analyses as well as UV and IR absorption properties. When X was deacylated with ammonia, 8-oxyadenosine (VIII) was obtained. This result showed that an attack of benzoate anion had occurred on $C_{2'}$ from the rear side and formed the *ribo* configuration in 2′ and 3′-position.

From this evidence, the O-anhydro linkage between the C_8 of adenine and $C_{2'}$ of ribose moieties was shown to be easily cleaved to give rise to various nucleosides substituted either at C_8 or on $C_{2'}$ positions.

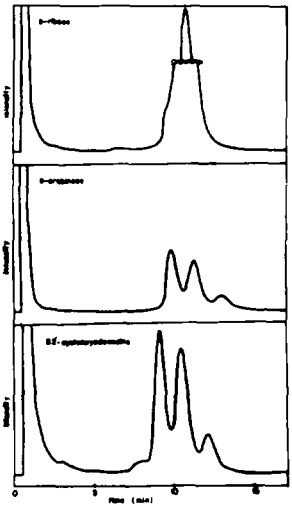


FIG. 1 Gas Chromatography of Acid-treated D-Ribose, D-Arabinose and 8,2′-Anhydro-8-oxy-9-β-D-arabinofuranosyladenine on Chromosorb-W coated with 1·5% SE-30.

The reason why the counterpart 8,3′-O-anhydronucleoside could not be obtained may be due to the large steric distortion required for the formation of the 8,3′-O-anhydro linkage[28] in the ribonucleoside or by the extreme instability of the 8,3′-cyclonucleoside.

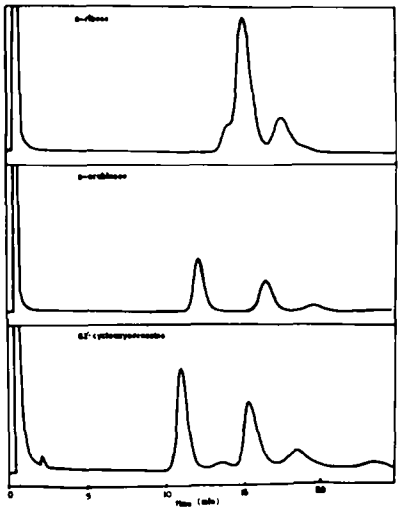


FIG. 2. Gas Chromatography of Acid-treated D-Ribose, D-Arabinose and 8,2′-Anhydro-8-oxy-9-β-D-arabinofuranosyladenine on Chromosorb-W coated with 1·5% Neopentyl-glycol succinate.

EXPERIMENTAL

UV absorption spectra were taken with Hitachi EPS-2U automatic recording spectrophotometer. IR absorption spectra were taken with JASCO DS-301 spectrophotometer. NMR were taken with Varian V-4300 C high-resolution spectrometer operating at 60 Mc. TMS was used as an internal reference. Gas chromatography was carried out by Shimadzu GC-1B apparatus with hydrogen flame ionizing detector.

Paper chromatography. All chromatography was performed by the ascending technique on Toyo filter paper No. 51A. Solvent A: 1-BuOH–AcOH–water, 4:1:5 (upper layer was used); B: 1-BuOH–water, 86:14; C: solvent B-conc ammonia, 100:1; D: water adjusted to pH 10 with ammonia; E: 1-propanol–water 3:1; G: 2-propanol–conc ammonia–water, 7:1:2; H: solvent E containing 1M sodium borate; F: 1-BuOH–AcOH–water, 5:2:3. R_f (A) stands for R_f value in solvent A.

2′,3′-O-*Isopropylidene*-8-*bromoadenosine.* 2′,3′-O-Isopropylideneadenosine (3·05 g, 10 mmoles) was dissolved in a mixture of dioxan and 10% disodium hydrogen phosphate buffer (300 ml, 1:1, vol/vol), followed by the addition of Br_2 (1·9 g, 12 mmoles). The reaction mixture was shaken for 5 hr and then kept overnight at room temp. The soln was extracted throughly with $CHCl_3$, which was washed with 2N $NaHSO_3$ and finally with water. After drying over Na_2SO_4 $CHCl_3$, was evaporated to afford a solid material, which recrystallized from EtOH as needles, m.p. 221–222°, yield 2·85 g, 74·2%). (Found: C, 40·59; H, 4·33; N, 18·26. Calc for $C_{13}H_{16}O_4N_5Br$: C, 40·7; H, 4·18; N, 18·1%). UV: pHl, 263 mμ, H_2O, 265 mμ, pHl4, 265·5 mμ; paper chromatography: R_f (A) 0·80, R_f (B) 0·73. This material was identical with an authentic sample.[9]

2′,3′-O-*Isopropylidene*-5′-O-*acetyl*-8-*bromoadenosine* (I). Acetylation of 2′,3′-O-isopropylidene-8-bromoadenosine was carried as described in Ref. 9, p 98, procedure (a).

5′-O-*Acetyl*-8-*bromoadenosine.* Hydrolytic removal of the isopropylidene group by formic acid was performed as described in Ref. 9. The product was identical with the authentic sample.[9]

2′(*or* 3′)-O-p-*Toluenesulfonyl*-5′-O-*acetyl*-8-*bromoadenosine* (II). Following the tosyaltion procedure,[9] the mixture of products was obtained as an amorphous solid, which was used for the subsequent reaction without crystallization.

2'(or 3')-O-p-Toluenesulfonyl-5'-O-acetyl-8-oxyadenosine. Compound II (3·022 g) was added to a soln of glacial AcOH (150 ml) containing anhyd NaOAc (freshly fused, 4·5 g). After refluxing for 2 hr, the solvent was removed *in vacuo* with repeated addition and evaporation of EtOH. The residue thus obtained was extracted with hot EtOAc, which was washed with a sat $NaHCO_3$ aq. EtOAc was evaporated *in vacuo* and the residue was dried over P_2O_5 in 3 mm at room temp (yield 2·045 g).

2'(or 3')-O-p-Toluenesulfonyl-8-oxyadenosine (IV). Monotosyl-acetyl-8-oxyadenosine, obtained as above, was dissolved in 40 ml anhyd MeOH previously saturated with ammonia at 0°. The mixture was stored at 0° for 21 hr and the solvent was evaporated *in vacuo*. The residual caramel was washed with hot benzene and evacuated over P_2O_5 to afford a glass (yield 1·876 g). UV: pH1, 264, 284 (shoulder) mμ; pH14, 282 mμ; IR (Nujol); 1186 cm^{-1}, 1172 cm^{-1} (covalent tosylate); 1715 cm^{-1} (8-CO); paper chromatography: R_f (G) 0·66 accompanied with a very thin spot 0·77 (presumably ditosylate).

8,2'-Anhydro-8-oxy-9-β-D-arabinofuranosyladenine (V). 8-Oxyadenosine monotosylate (1·813 g), thus obtained, was heated in DMF (90 ml) containing sodium benzoate (4·5 g) at 100–105° in an oil bath for 2 hr with mechanical stirring. After cooling to 20°, the mixture was filtered, the ppt was washed with DMF and filtrate and washings were combined and evaporated *in vacuo*. A reddish residue (2·30 g) was obtained. The residue was dissolved in 1-propanol (50 ml) and applied to a column (4 × 57 cm) of cellulose powder (220 g). By the elution with 1-propanol–water (3:1, vol/vol) fractions having H_2O 260 mμ were collected. Evaporation of the solvent gave a glass (333 mg), which was crystallized from EtOH, m.p. < 190° (dec), yield 124 mg. (Found: C, 45·08; H, 4·24; N, 26·54. Calc for $C_{10}H_{11}O_4N_5$: C, 45·28; H, 4·18; N, 26·4%); UV: pH 1, 259 mμ (ε 10,800), H_2O, 260 mμ (ε 11,000), pH 14, 259 mμ (ε 10,700); IR: no band at 1170 cm^{-1} (tosyl) and 1715 cm^{-1} (8-CO); $[\alpha]_D^{19}$ −121·6° (*c*, 0·75, pyridine); Paper chromatography: R_f (D) 0·47, R_f (C) 0·14, R_f (E) 0·35; NMR: H_1, 6·50 δ (doublet), $J_{1'-2'}$ = 5·4 c/s (taken in DMSO).

8-Oxy-9-β-D-arabinofuranosyladenine (VI). Compound V (124 mg) was refluxed in 0·1N H_2SO_4 (20 ml) for 2·5 hr. The reaction mixture was neutralized with $NaHCO_3$ and examined by paper chromatography in solvent G. Three UV bands having R_f's 0·54, 0·44 and 0·37 were detected. In addition, a spot revealed only by metaperiodate spray[29] was detected at R_f 0·26. The substance having R_f 0·37 was identical with 8-oxyadenine by the comparison with an authentic sample.[24] Since substance having R_f 0·54 was the starting material, the band having R_f 0·44 was excized and extracted with water. Evaporation of water *in vacuo* gave a white solid, which had UV bands: pH 1 264, 281 mμ; pH 14 279 mμ, which were similar to those of 8-oxyadenosine described below. This substance did not consume metaperiodate on the paper chromatogram. Comparison of this material with the authentic 8-oxyadenosine and 8-oxy-9-β-D-xylofuranosyladenine, synthesized as described below, side by side on the paper chromatogram showed distinct differences (see Table 2).

Determination of the structure of the sugar obtained in acidic hydrolysis of 8,2'-anhydro-8-oxy-9-β-D-arabinofuranosyladenine. Compound VI (4·1 mg) was dissolved in 0·1N H_2SO_4 (2 ml) and refluxed for 4·5 hr. Examination of the reaction mixture by paper chromatography in solvent D showed complete hydrolysis of the starting material (R_f 0·52) to 8-oxyadenine (R_f 0·41). A spot of sugar derivative (R_f 0·64) was also revealed by metaperiodate spray. The reaction mixture was passed through a little column of Dowex 1 × (OH^- form) resin (1 ml). Washing (water, 20 ml) and effluent were combined and evaporated to dryness *in vacuo*. Traces of water were removed by several co-distillations with anhyd pyridine (1 ml × 3). The dried residue was further evacuated over P_2O_5 under 1 mm at room temp for 10 hr. The hard oil, thus obtained, was dissolved finally in pyridine (1 ml), followed by the addition of bis-monomethylsilyldisilazane (0·2 ml) and trimethylsilyl chloride (0·1 ml). The mixture was shaken at room temp for 10–20 min (exclusion of the moisture) and evaporated *in vacuo* to remove pyridine and unreacted reagents. The residue was further dried over P_2O_5 at 1 mm vacuum for 30 hr and finally taken up in n-hexane (1 ml). Insoluble material was removed by centrifugation and the supernatant was concentrated to ca. 0·5 ml. D-Ribose or D-arabinose were treated with 0·1N H_2SO_4 and worked up as desdribed above for the sample of gas chromatography.

Chromatography was carried out on the solumn: (i) 1·5% SE 30 on Chromosorb-W (60–80 mesh), 1·5 cm × 4 mm, inner diameter, U-shape stainless steel tube, (ii) 1·5% neopentyl glycol succinate on Chromosorb-W (60–80 mesh), 1·5 cm × 4 mm, inner diameter, U-shape stainless steel tube. Temperature in (i) was 142° ± 1° and (ii) 123·5° ± 0·5°. Flow rate of N_2 as carrier was 37 ml/min. H_2 (64 ml/min) and air (0·8 l/min) were used for detector. Results were shown in Figs. 1 and 2.

8-Oxyadenosine (VIII). 8-Bromoadenosine[16] (1·73 g, 5 mmoles) was dissolved in Ac_2O (40 ml) containing anhyd NaOAc (2·05 g, 50 mmoles). The mixture was refluxed for 1·5 hr, during which the soln colorized slightly. EtOH (50 ml) was added to the reaction mixture and the solvent was removed by vacuum distil-

lation after 30 min. The evaporation was repeated until the odour of AcOH was not detected. $CHCl_3$ (50 ml) was added to the residue, insoluble material removed by filtration and the $CHCl_3$ soln dried over Na_2SO_4. Evaporation of $CHCl_3$ gave a white solid, which was deacylated by treatment with MeOH ammonia. Evaporation of the solvent gave a solid, which was recrystallized from 50% EtOH. N^6-Acetyl-8-Oxyadenosine (196 mg), m.p. > 220°, was obtained as white crystals. (Found: 3, 43·53; H, 4·92; N, 21·59. Calc for $C_{12}H_{15}O_6N_5 \cdot 0{\cdot}5H_2O$: C, 43·15; H, 4·81; N, 20·97%); UV: pH 1 240, 289 mμ; pH 14 270 (shoulder), 299 mμ.

The mother liquor from the above crystallization was evaporated to give a caramel, which was dissolved in conc ammonia. The mixture was kept at room temp for 2 days and ammonia was evaporated *in vacuo*. The residue was extracted with $CHCl_3$ to remove acetamide and was recrystallized from water. 8-Oxyadenosine (900 mg; 72·2%), m.p. > 220°, was obtained as colorless fine needles. (Found: C, 42·22; H, 4·73; N, 24·94. Calc for $C_{10}H_{13}O_5N_5$: C, 42·44; H, 4·63; N, 24·75%); UV: pH 1, 265, 282 mμ; H_2O, 260 (shoulder), 270 mμ; pH 14, 280 mμ; IR (KBr): 1740 cm^{-1} (8-CO); paper chromatography: R_f (B) 0·17, R_f (D) 0·49, R_f (G) 0·44, R_f (F) 0·29.

8-*Oxy*-9-β-D-*xylofuranosyladenine* (IX)

(i) 8-*Bromoadenine chloromercuri salt*. 8-Bromoadenine[25] (25·57 g, 0·12 mole) was dissolved in NaOH (4·8 ml, 0·12 mole) containing $HgCl_2$ (32·5 g, 0·12 mole) in EtOH (100 ml). The ppt was collected by centrifugation, washed with EtOH and dried over P_2O_5 (51·1 g, 95%).

(ii) 2',3',5'-*Tri*-O-*acetyl*-8-*bromo*-9-β-D-*xylofuranosyladenine*. 8-Bromoadenine chloromercuri salt (44·9 g, 0·1 mole) and Celite (20·0 g) were suspended in dichloroethane (1000 ml, freshly distilled from P_2O_5). The mixture was concentrated to about ¾ of its volume to remove traces of water. After the addition of 1,2,3,5-tetra-O-acetyl-D-xylofuranose[26] (38·1 g, 0·12 mole) dissolved in 100 ml dichloroethane, soln of titanium tetrachloride (18·9 g) in 50 ml dichloroethane was added dropwise during 2 hr at reflux temp. The mixture was further refluxed for 10 hr and set aside for cooling to 40°. Addition of 600 ml $NaHCO_3$ aq and stirring for 8 hr decomposed the titanium chloride. The solid material was removed by filtration, the filtrate and washings ($CHCl_3$, 200 ml) were combined and the organic layer was separated. Washing of the organic phase with 30% KI aq and water, followed by drying over $MgSO_4$ and evaporation of the solvent, gave a yellow oil (yield 18 g). The oil was purified by the column chromatography on alumina (400 g) to give 11·2 g of pale yellow caramel (yield 26%). UV: pH 1, 265 mμ, EtOH, 265 mμ, pH 14, 266 mμ; IR (KBr): 1745 cm^{-1} (ester CO), 1045 cm^{-1} (sugar lactol), 1645, 1600 cm^{-1} (adenine); TLC: R_f 0·56 ($CHCl_3$–EtOH, 35:5).

(iii) 8-*Oxy*-9-β-D-*xylofuranosyladenine*. NaOAc (11·5 g, 140 mmoles, freshly fused) was dissolved in glacial AcOH (60 ml) by slight warming, followed by the addition of 8-bromo-9-β-(2',3',5'-tri-O-acetyl)-D-xylofuranosyladenine (6 g, 13 mmoles). The reaction flask was immersed in a preheated oil bath at 140° and refluxed for 2 hr. Examination of an aliquot by TLC ($CHCl_3$–EtOH, 35:5) showed 2 spots having R_f 0·67 and 0·32, both revealed by H_2SO_4. AcOH was removed by vacuum distillation and the residue was dissolved in anhyd MeOH (150 ml) previously saturated with dry ammonia at 0°. After storing overnight in an ice-box, the solvent was evaporated *in vacuo*. The residue was taken up in 200 ml water and treated with Amberlite IRC 50 (H^+ form) resin to remove Na ion. The resin was removed by filtration, the filtrate was treated with activated charcoal, and the solvent was evaporated under reduced press to 50 ml. Storing in a refrigerator for 2 days gave a fluffy ppt (500 mg), which was removed by filtration. The filtrate was chromatographed on a column of cellulose powder (200 g), which was eluted with 1-BuOH–water (86:14). The third peak was pooled and evaporated to give 530 mg of 8-oxy-9-β-D-xylofuranosyladenine as white crystalline residue, which could be crystallized from EtOH–water, m.p. 233° (dec). From the mixture of the first and the second peaks 100 mg of the same substance was obtained. (Found: C, 41·93; H, 4·93; N, 25·00. Calc for $C_{10}H_{13}O_5N_5$: C, 42·40; H, 4·59; N, 24·73%); UV: pH 1, 264 mμ (ε 10,400), 286·5 mμ (ε 10,200); pH 14, 281 mμ (ε 14,800); IR (KBr): 1740 cm^{-1} (8-CO), 1000 cm^{-1} (sugar lactol); paper chromatography: R_f (D) 0·68, R_f (G) 0·42, R_f (B) 0·20. The β-configuration of the nucleoside linkage could be deduced from the 1,2-*trans* rule.[30] The fluffy crystal obtained above was recrystallized from 20% EtOH to give m.p. > 230°. (Found: C, 42·57; H, 4·89; N, 20·37. Calc for $C_{12}H_{15}O_6N_5 \cdot 1{\cdot}5H_2O$: C, 42·73; H, 4·85; N, 20·77%); UV: pH 1, 291 mμ; H_2O, 290 mμ; pH 14, 265 mμ (shoulder), 320 mμ; IR (Nujol); 1700 cm^{-1} (amide CO), 1714 cm^{-1} (8-CO), 1050 cm^{-1} (sugar lactol); paper chromatography: R_f (G) 0·29. R_f (D) 0·86. Since deacetylation of this compound with conc HN_4OH gave a substance identical with that obtained above, it was N^6-acetyl-8-oxy-9-β-D-xylofuranosyladenine.

2'-O-*Benzoyl*-8-*oxyadenosine*. Compound V (106 mg) was refluxed in a mixture of sodium benzoate

(300 mg), benzoic acid (103 mg) and DMF (30 ml, freshly distilled) for 70 min. Examination of the reaction mixture by paper chromatography (solvent D) showed a spot having R_f 0·40, which had UV maxima at 237 mμ and 270 mμ. After cooling, the precipitated benzoate was removed by filtration, the ppt was washed with DMF, the filtrate and washings were combined and evaporated *in vacuo*. The residue was taken up in a small amount of water and applied to preparative paper chromatography in solvent D. The band having R_f 0·36–0·40 was cut out and extracted with EtOH. Evaporation of EtOH gave a white solid (27 mg), which was recrystallized from EtOH to afford colorless needles, m.p. 119–121·5°. (Found: C, 52·47; H, 4·62; N, 18·14. Anal. calc for $C_{17}H_{17}O_6N_5$: C, 52·71; H, 4·42; N, 18·08%); UV: pH 1, 231 mμ, 265 mμ, 280 mμ (shoulder); pH 14, 280 mμ; IR (KBr): 1740 cm^{-1} (benzoyl), 1725 cm^{-1} (8-CO); paper chromatography: R_f (D) 0·40, R_f (G) 0·62, R_f (A) 0·69.

A small amount of 2'-O-benzoyl-8-oxyadenosine was dissolved in 20% ammonia and kept for 2 hr at room temp. Paper chromatography of this material in three solvent systems with authentic 8-oxyadenosine side by side showed complete identity: R_f (E) 0·41, R_f (D) 0·51, R_f (H) 0·36.

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