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STUDIES OF NUCLEOSIDES AND NUCLEOTIDES—LIII
PURINE CYCLONUCLEOSIDES-18[1]. SELECTIVE TOSYLATION OF ADENINE NUCLEOTIDES.
SYNTHESIS OF 8,2′-ANHYDRO-8-MERCAPTO-9-β-D-ARABINOFURANOSYLADENINE 5′- AND 3′,5-CYCLIC PHOSPHATE[2]

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Abstract—Adenosine 5′-monophosphate (AMP) and 8-bromo-AMP were tosylated with tosyl chloride in aqueous alkaline solutions to give 2′-tosyl nucleotides selectively. 8-Bromo-2′-tosyl AMP was easily cyclized to 8.2′anhydro-8-mercapto-9-β-D-arabinofuranosyladenine (8.2′-S-cycloadenosine) 5′-MP by treatment with H_2S in pyridine or NaSH in water-DMF mixture. Desulfurization of the cyclonucleoside MP gave 2′-deoxy-AMP. 3′.5′-Cyclic 8-bromo AMP could also be tosylated and cyclized to give 8.2′-S-cycloadenosine 3′.5′-MP by the same procedure.

Snake venom 5′-nucleotidase. E. coli and sheep intestine alkaline phosphatases hydrolyzed 8.2′-S-cycloadenosine 5′-phosphate to give 8.2′-S-cyclonucleoside.

PHOSPHATE esters of purine 8-cyclonucleosides[3] are of interest because of their unique conformation and chemical properties. Two routes might be considered for their preparation; first, the phosphorylation of preformed cyclonucleosides by means of various phosphorylating agents[4], and second by the cyclization of purine nucleotides having the necessary substituents for subsequent cyclization. This paper describes synthesis by the latter method, involving the selective tosylation of adenosine 5′-phosphate(AMP) and 8-bromo-AMP.

The synthesis of cyclonucleosides generally utilizes sulfonyloxy leaving groups[5] in the carbohydrate moiety. However, there have been few reports of sulfonylation of nucleotides. Michelson *et al.*[6] reported mesylation of the 5′-OH of adenosine 2′, 3′-cyclic phosphate using its trialkylammonium salt in dioxane. We attempted the tosylation of AMP and UMP in pyridine, but this was unsuccessful, possibly because of interaction of phosphate group with tosyl chloride, prohibiting attack of the latter at the 2′ and 3′-OH groups.

We therefore investigated tosylation by the Schotten–Baumann procedure. A solution of the disodium salt of AMP (I) in aqueous sodium hydroxide was added dropwise to a solution of tosyl chloride. The mixture became first turbid but clarified after 2 hr. After being neutralized with NaOH, the reaction mixture was examined by paper chromatography in solvent A. The results are summarized in Table I. The starting material (AMP) migrated at R_f 0·10 and it shows a UV absorption maximum at 260 mμ (pH 7). The compound eluted at R_f 0·60 and 0·26 both showed λ maxima at 230 and 262 nm, the ratios A 230/260 being *ca* 2 and 1, respectively. The former compound was therefore assigned as ditosylate (III) and the latter as monotosylate (II). A fraction of R_f 0·36 remaining at the origin of paper electrophoreogram was assigned as non-

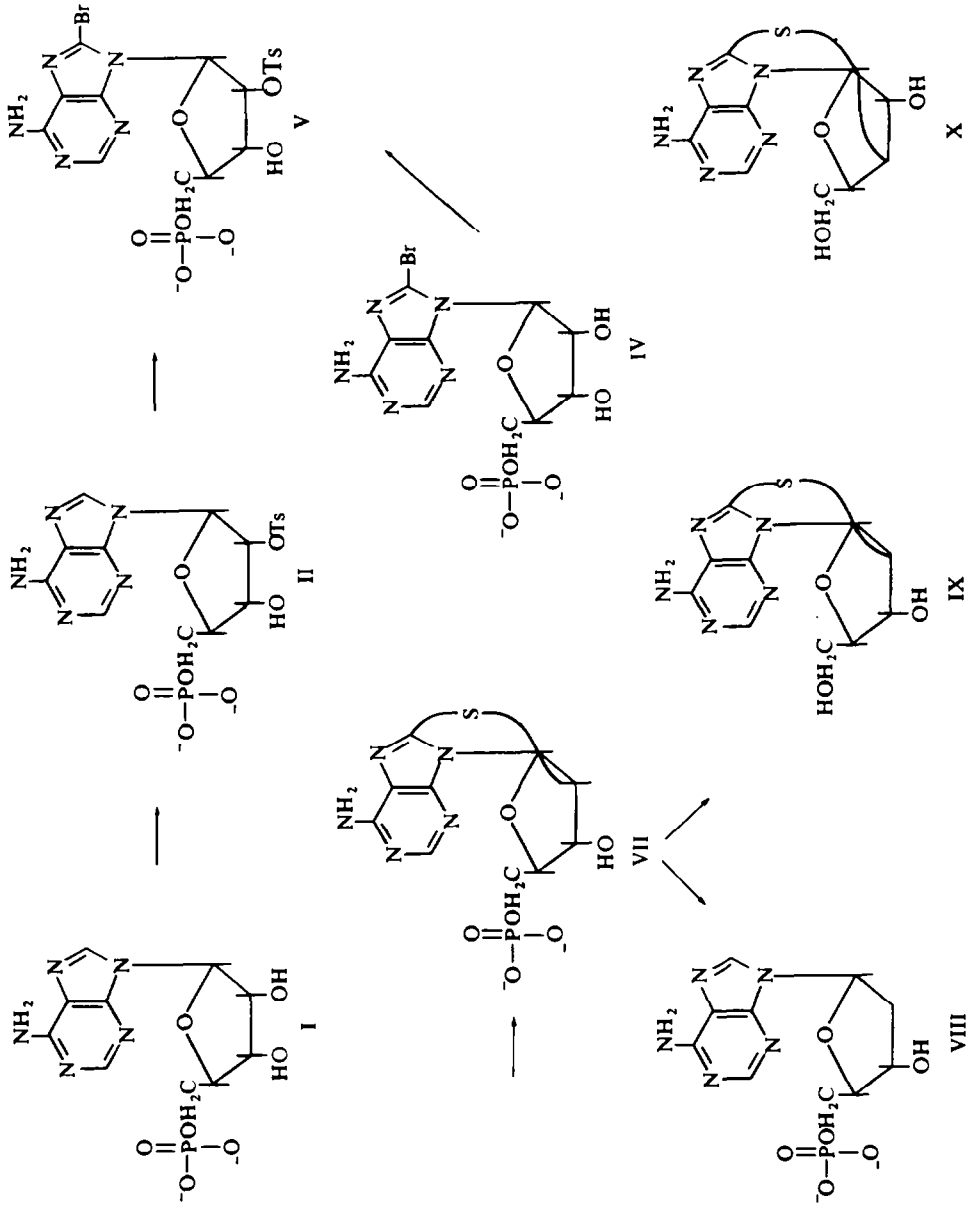
nucleotidyl material. Table 1 shows that reactions using 2N NaOH with 3 equivalents of tosyl chloride, or N NaOH with 3·5–4 equivalents TsCl, gave the highest yields of both mono- and di-tosylate; 55–60% of II, *ca* 20% of III. Lesser yields of tosylates were obtained on reducing the amounts of NaOH and tosyl chloride used. Compound II was isolated by preparative paper chromatography and characterized as monotosyl-AMP by phosphate analysis and UV absorption properties. The position of the tosyl group was determined as 2′ by the following series of reactions leading to 8,2′-S-cycloadenosine monophosphate.

Compound II was easily brominated with bromine-water in acetate buffer of pH 4 to give 8-bromo-2′-tosyl AMP (V) according to the procedure previously reported for AMP.[7] Tosylation of 8-bromo-AMP (IV) by the procedure used for AMP gave the corresponding monotosylate (V, R_f 0·33), ditosylate (VI, R_f 0·58) and a byproduct which remained at the origin in paper chromatography in solvent A. Conditions for this reaction and yields of products are summarized in Table II. Compared with the tosylation of AMP, this reaction gave rather a low yield of ditosylate.

Cyclization of monotosylated 8-bromo-AMP (V) was effected by treatment with either H_2S in pyridine-water mixture or NaSH in DMF-water mixture. The latter procedure is preferable because it gives no byproduct. The cyclization products were purified on a Dowex 1 (formate) column. In each case, cyclonucleoside phosphate (VII) appeared in one symmetrical peak as shown in Fig 1. Over-all yield calculated from AMP was 21–27%. The structure of the monophosphate (VII) was characterized as follows. Elemental analyses gave values corresponding to cyclonucleoside monophosphate dihydrate. UV absorption properties suggested the presence of 8,2′-anhydro-8-mercapto-9-β-D-arabinofuranosyladenine chromophore.[8] On paper chromatography compound VII migrated to 0·93 as compared to the migration of AMP (1·00). Its NMR spectrum showed an H-2 proton at 8·24δ as a singlet and an H-1′ proton at 6·13δ as a doublet. The coupling constant $J_{H1'\text{-}H2'}$ was 6·8 c/S, which showed an 8,2′- and not an 8,3′-cyclonucleoside structure.

The final proof of the structure of compound VII was obtained by the following experiments. Compound VII was desulfurized with Raney nickel to give 2′-deoxyadenosine 5′-monophosphate (VIII), which was identical with an authentic sample. The compound VII was then treated with alkaline phosphatase[9] to remove the 5′-phosphate group. The resulting cyclonucleoside was applied to a Dekker's alkaline Dowex column[10] and eluted with methanol. A comparative experiment (Fig 2a) with authentic 8,2- (IX) and 8,3′-cyclonucleoside[11] (X), showed that the former could be eluted by methanol, but the latter only by 0·5% acetic acid. Fig 2b clearly shows that the nucleoside obtained by alkaline phosphatase was 8,2′- and not 8,3′-cyclonucleoside. Therefore the structure of compound VII was confirmed as 2′-O-tosyl-S-cycloadenosine 5′-MP.

As the monotosylate (V) was confirmed as 2′-tosyl AMP by these experiments, the reason for the attack of tosyl group selectively at 2′-OH must be interpreted. Examples of the introduction of arylsulfonyl chloride[12] or benzyl chloride[13] into unprotected nucleosides using NaH have been reported, also the introduction of a phosphate group to a nucleoside in sodium hydroxide solution.[14] In each of these cases attack occurred at the 2′- or 3′-OH group and it was suggested that these groups dissociate before the 5′-OH does under these conditions. When the 5′-OH of adenosine was



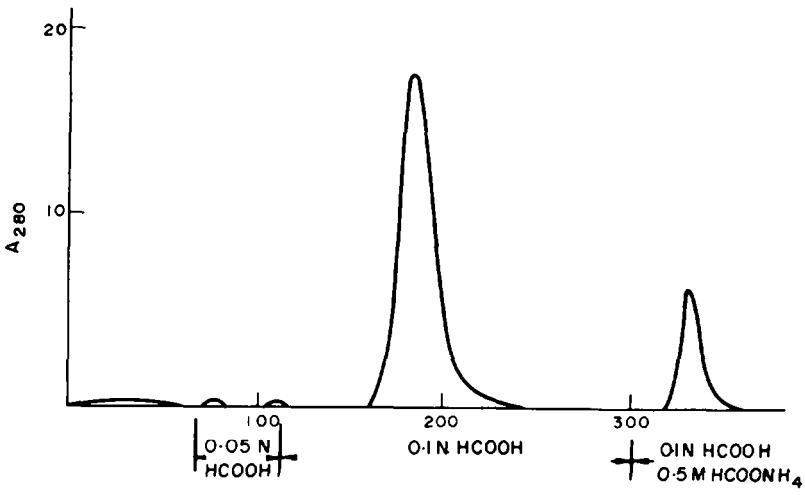


FIG 1. Chromatographic separation of 8,2-S-cyclo-AMP.

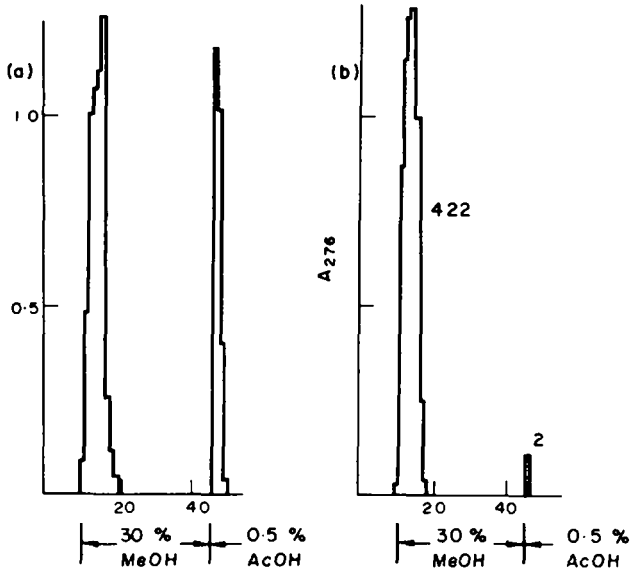


FIG 2. Chromatography of cyclonucleosides on Dowex (OH^-) column

-blocked with a bulky group such as a trityl group, the attacking species of triisopropylbenzene-sulfonyl chloride can approach the 2′-OH group more easily than the 3′-OH[11]. Therefore, although a bulky substitution like phosphate on 5′-OH will exert some effect on the entry of an attacking species, this cannot be the only reason for the complete selectivity. Considering the pKa of sugar OHs of adenosine (12·35),[15] the 2′- and 3′-OH groups should be dissociated in sodium hydroxide solution. If the 5′-phosphate group has two negative charges, the dissociation of 3′-OH must

be more difficult than that of 2'-OH due to repulsion between the nearer negative charges. Furthermore, puckering of the furanosering to the 2'-endo conformation[16] may put the 2'-OH in a more favorable position than 3'-OH for the attack of tosylate. Both these effects might be responsible for the selectivity of attack of the tosyl group at 2'-OH.

8-Bromoadenosine 3',5'-cyclic monophosphate (IX) was also tosylated in alkaline conditions as described above. The tosylated compound (X) was cyclized by treatment with H_2S in pyridine. The resulting cyclonucleoside 3',5'-monophosphate (XI) was purified on a Dowex-I (formate) column. The yield was 46%.

Finally, the properties of 8,2'-S-cycloadenosine 5'-monophosphate (VII) as a substrate of several phosphatases were investigated. When compound VII was hydrolyzed with snake venom 5'-nucleotidase[17] the rate of cleavage was very slow relative to that of AMP, but using a large excess of nucleotidase VII was completely digested. The relative rate of hydrolysis was faster than that of 8-bromo-AMP.[18] *Escherichia coli* alkaline phosphatase[9] hydrolyzed compound VII as fast as it did AMP. Hydrolysis of VII by sheep intestine alkaline phosphatase[19] was accompanied by deamination at N^6 by contaminating deaminase to give 8,2'-anhydro-8-mercapto-9-β-D-arabinofuranosylhypoxanthine.[20]

From these experiments, it was found that AMP and 8-bromo-AMP are tosylated selectively at the 2'-OH group by tosyl chloride in alkaline conditions. 3',5'-Cyclic 8-bromo-AMP can also be tosylated without cleavage of cyclic phosphate linkages. 8,2'-S-Cyclonucleoside phosphates are easily obtained from these tosylated nucleotides using H_2S in pyridine or NaSH in DMF-water mixture.

The tosylation of other nucleotides by this method will be reported in a subsequent paper.

TABLE 1. TOSYLATION OF AMP

Na	OH ml.	TsCl mmole	dioxane ml.	H_2O ml.	AMP %	mono Ts %	R_f^a 0·36	di Ts %
N	1·5	3	7·5	0·5	39	45	5%	11
2N	0·75	3	4·5	0·5	17	59	4	20
N	1·5	3·5	7·5	0·5	14	61	5	20
N	1·5	3·5	4·5	0	23	54	6	17
N	1·5	4	7·5	1·0	21	56	5	17
N	1·5	4	4·5	0	11	60	6	22
N	1.0	1	4·5	0·5	67	22	—	8
N	1·5	1	4·5	0·5	56	27	—	13
N	1·5	2	4·5	0·5	34	43	—	18

[a] R_f 0·36 means a product run at R_f 0·36 in paper chromatography

TABLE 2. TOSYLATION OF BrAMP

N NaOH ml.	TsCl mmoles	di	mono
1·5	3	2·4%	56%
1·5	4	4·7%	68

EXPERIMENTAL*

2'-O-Tosyladenosine 5'-monophosphate

AMP (free acid, 1 mole) was dissolved in N NaOH (2 ml) and the soln was evaporated to dryness *in vacuo*. The residue was dissolved in N NaOH (1·5 ml) and added dropwise with stirring into a soln of tosyl chloride (665 mg, 3,5 mmoles) in dioxane (7·5 ml) and water (0·5 ml). The soln became turbid a few min after the start of the addition and by 30 min has become a gel. When stirring was continued to 1·5 hr, the mixture again became clear, at which time it was acidic. After neutralization with N NaOH, the extent of the reaction was examined by paper chromatography in solvent A. Monotosylated AMP was detected in 61% yield, and was isolated by preparative paper chromatography as a white powder. UV absorption properties (ε was obtained by phosphorus analysis[21]); λ_{max}^{pH2} 230 nm (ε 1·27 × 10^4), 258,5 nm (1.29 × 10^4); λ_{max}^{pH7} 231 nm (1·31 × 10^4), 261·5 nm (1·26 × 10^4); λ_{max}^{pH13} 261·5 nm; λ_{max}^{pH2} 244·5 nm; λ_{min}^{pH7} 245 nm. PPC: R_f(A) 0·25 (AMP 0·07), R_f(B) 0·55 (AMP 0·19), R_f(D) 0·37 (AMP 0·16). PEP: R_{pA-A} 0·95.

2'-O-Tosyl-8-bromoadenosine 5'-monophosphate

8-Bromoadenosine 5'-monophosphate (2 Na salt, 0·5 mmole) was dissolved in N NaOH (0·75 ml) and the soln was added dropwise with stirring into a dioxane-water (3·75 ml–0·25 ml) mixture containing tosyl chloride (380 mg, 4 equiv.). The mixture turned a brown-red colour and became turbid during the addition. Stirring was continued for 2 hr then the mixture was gradually neutralized with N NaOH. Examination by PPC in solvent A showed the existence of monotosylate in a yield of 68%. The monotosylate was isolated by preparative PPC as a white powder. UV absorption properties: λ_{max}^{pH2} 229 nm (ε 1·24 × 10^4), 264 nm (1·61 × 10^4); λ_{max}^{pH7} 266 nm (1·40 × 10^4); λ_{max}^{pH13} 266 nm; λ_{min}^{pH2} 245 nm, λ_{min}^{pH7} 245·5 nm. PPC: R_f(A) 0·33 (AMP 0·12). PEP: R_{pA-A} 0·94.

8,2'Anhydro-8-mercapto-9-β-D-arabinofuranosyladenine 5'-monophosphate

(i) *From* 2'-O-*Tosyl*-AMP: AMP (5 mmoles) was tosylated as above. Examination of the mixture showed the presence of 50% monotosylate and 19% ditosylate. Solvent was evaporated and the residue was dissolved in water (100 ml). Into this soln was added 2 M acetate buffer (pH 4, 100 ml) and saturated bromine water (50 ml). After 30 min at room temp, more saturated bromine water (50 ml) was added. The mixture was kept at room temp for 2 days, then unreacted bromine was removed by aeration for 1–2 hr. The solvent was evaporated *in vacuo* and the residue was dissolved in water, insoluble material was filtered off and filtrate was evaporated. The residue was dissolved in water (10 ml) and decolorized by addition of a small amount of $NaHSO_3$. (When a large excess of $NaHSO_3$ was used, debromination of tosyl-8-bromo-AMP occurred.) The yield of 2'-tosyl-8-bromo-AMP estimated by paper chromatography was 14,700 OD_{260} units (21%) from AMP.

Pyridine (50 ml) was added to this soln then H_2S was bubbled through for 7 min, on which the soln turned green. The tightly stoppered mixture was kept at room temp for 2 days, then H_2S was removed by bubbling N_2 gas through the soln. Solvent was evaporated and the residue was dissolved in water. The soln was again evaporated and the final residue was dissolved in water. (2 1) and applied to a column (3 × 30) cm) of Dowex 1 × 8 (formate form) resin. The column was washed with water then eluted with 0·05 N formic acid (Fraction No. 68–104), 0·1 N formic acid (Fraction No. 105–265), 0·1 M ammonium formate and 0·01 N formic acid (Fraction No. 266–299), successively. 50 ml of each fraction was collected at a flow rate of 100–150 ml/hr. Fraction No. 168–217 corresponding to a major peak was pooled and evaporated *in vacuo* to a small volume. Water (*ca* 200 ml) was added and evaporated. This was repeated twice and the final soln (*ca* 100 ml) was lyophilyzed. The residue was triturated with MeOH (5 ml) and acetone (30 ml) to give a powder, which was collected by centrifugation. Supernatant and washings (acetone-ether) were concentrated *in vacuo*, dissolved in MeOH, and precipitated with acetone (30 ml). The ppt was collected by centrifugation, washed with acetone and ether, and dried over P_2O_5. The yield was 421 mg

* UV absorption spectra were taken with a Hitachi EPS-3T spectrophotometer, IR spectra with a Hitachi EPI-L spectrophotometer, and NMR spectra with a Hitachi H-6013 high resolution spectrometer operated at 60 mHz with TMS as internal standard. Paper chromatography (PPC) was performed in the following solvents: A, isopropanol-conc. NH_3-water (7:1:2); B, n-butanol-acetic acid-water (5:2:3); C, n-propanol-conc. NH_3-water (55:10:35). Paper electrophoresis (PEP) was performed in a triethylammonium bicarbonate (0·1 M, pH 7·5) buffer. R_{pA-A} stands for migratory distance relative to AMP (1·0) and adenosine (0·0).

(21%, calculated from AMP as free acid $2H_2O$). An analytical sample was obtained by dissolving the product in a small amount of water and precipitating with EtOH. (Found: C, 30·31; H, 4·11; N, 16·65, calcd. for $C_{10}H_{12}O_6N_5PS \cdot 2H_2O$: C, 30·23; H, 4·06; N, 17·63). Other properties are described in section (ii).

(ii) *From* 8-BrAMP: AMP disodium salt (5 mmoles) was dissolved in 1 M barium acetate buffer (pH 4·0, 200 ml). Saturated Br_2 water (50 ml) was added and the soln was kept at room temp overnight. Completion of the reaction was confirmed by paper chromatography in solvent B, and excess Br_2 was removed by aeration. Solvent was evaporated *in vacuo*, then the residue was suspended in water (20 ml) and EtOH (20 ml) was added. The ppt which formed was collected by centrifugation, washed twice with each of 50% EtOH and EtOH, and dried over P_2O_5 *in vacuo* to give 8-bromo-AMP barium salt in a yield of 2·6–2·9 g (70%). This product was suspended in water (250 ml) and the mixture heated to dissolve as much solid as possible. The soln was cooled then Na_2SO_4 (equiv to the Ba salt) dissolved in water (20 ml) was added. The soln was shaken well and precipitated $BaSO_4$ was removed by centrifugation. The filtrate was neutralized with N NaOH (0·5 ml) and evaporated to give 8-Br-AMP disodium salt. The sodium salt was dissolved in N NaOH (7·5 ml) and added gradually with stirring to a dioxane soln (22·5 ml) containing tosyl chloride (3·8 g). After addition was complete (1 hr), the mixture was stirred further until it became clear. The mixture was neutralized with N NaOH and evaporated *in vacuo*. The tosylate thus obtained was dissolved in DMF (5 ml) and water (·5 ml) and 40% aqueous NaSH (1·4 ml, 10 mmoles) was added. The mixture was kept at room temp for 2 days then neutralized with N HCl. Solvent was evaporated *in vacuo* and the residue was dissolved in water. Insoluble material was filtered off and the volume was adjusted to 1:1 with water. This soln contained 30,500 OD_{276} units of 8,2′-S-cycloadenosine 5′-MP (yield calculated from AMP was 27%). (Found: C, 30·33; H, 4·45; N, 17·15. Calcd. for $C_{10}H_{12}O_6N_5PS \cdot 2H_2O$: C, 30·23; H, 4·06; N, 17·63). UV absorption properties: λ_{max}^{pH1} 278 nm (ε 2·15 × 10^4), λ_{max}^{pH7} 220·5 nm (2·19 × 10^4), λ_{max}^{pH13} 277 nm (2·28 × 10^4): λ_{min}^{pH1} 237·5 nm (2·8 × 10^3), λ_{min}^{pH7} 238 (4·2 × 10^3), λ_{min}^{pH13} 239 nm (4·4 × 10^3). PPC: R_f(A) 0·06 (AMP 0·07), R_f(B) 0·29, (AMP 0·24, BrAMP 0·32), R_f(C) 0·44 (AMP 0·48), R_f(D) 0·12 (AMP 0·15). PEP: R_{pA-A} 0·93. NMR (taken in 0·1 M D_2O soln): 8·24 δ (s, 1 H, H-2), 6·73 δ (d, 1 H, H-1′, $J_{1'-2'}$ 6·8 cs).

8,2′-Anhydro-8-mercapto-9-β-D-arabinofuranosyladenine 3′, 5′-cyclic monophosphate

8-Br-3′,5′ cyclic AMP (0·2 mmole) was dissolved in 2 N KOH (0·3 vol) and the soln was added dropwise with stirring to a dioxane (1 ml) soln containing tosyl chloride (152 mg, 4 equiv). During the addition (25 min) a brownish oil which first appeared redissolved to give a clear soln. Stirring was continued until the soln became acidic. The soln was neutralized with 2 N KOH. Examination with paper chromatography showed the absence of starting material (R_f(A) 0·49, R_f(B) 0·61) and the presence of a product having R_f(A) 0·73 and R_f(B) 0·86. UV absorption properties: $\lambda_{max}^{H^+}$ 265 nm, $\lambda_{max}^{H_2}$ 267 nm: $\lambda_{min}^{H^+}$ 261·5 nm, $\lambda_{min}^{H_2}$ 246·5 nm.

The solvent was evaporated *in vacuo* and the residue was dissolved in water (6 ml) and pyridine (6 ml). H_2S gas was bubbled through the soln for 10 min. The stoppered mixture was kept at room temp for 2 days. Solvent was evaporated *in vacuo* and the residue was dissolved in water. Insoluble material was filtered off and the filtrate was applied to a column (1 × 20 cm) of Dowex 1 × 8 (chloride form). The column was washed with water and eluted with 0–0·1 M NaCl and 0·003 N HCl (1 1–1 1) by a linear gradient technique. 10 ml of each fraction was collected, at a flow rate of 50 ml/hr. Fractions No. 70–111 corresponding to a major peak were pooled, treated with active charcoal and eluted with alcohol-water containing 2% ammonia. Eluants were evaporated carefully to obtain the product. The yield was 1970 OD_{280} (46% from 8-bromo-3′,5′-cyclic-AMP). UV absorption properties: $\lambda_{max}^{H^+}$ 279 nm, $\lambda_{max}^{H_2O}$ 278 nm, λ_{min}^{OH} 278 nm: $\lambda_{min}^{H^+}$ 246 nm, $\lambda_{min}^{H_2O}$ 246 nm. PPC: R_f(A) 0·43 (BrA-3′,5′ MP 0·47), R_f(B) 0·34 (BrA-3′,5 MP 0·29). PEP: R_{pA-A} 0·50 (BrA-3′,5′ MP 0·53).

2′-Deoxyadenosine 5′-monophosphate

8,2′-S-Cycloadenosine 5′-MP (free acid, 5 mg) was neutralized with N NaOH and dissolved in water (5 ml). Total OD_{276} units at this stage was 270. Two micro-spoonfuls of Raney nickel (W-7) was added and the soln was heated at reflux temp for 3 hr. The mixture was submitted to preparative paper chromatography in solvent D. The band having R_f 0·16 was cut out and eluted with water. The yield was 31·8 OD_{260} (18%). UV absorption: $\lambda_{max}^{H_2O}$ 260 nm, $\lambda_{min}^{H_2O}$ 228 nm. Cochromatography with an authentic sample of 2′-deoxy AMP showed the same R_f(D) 0·23 (S-Cyclo AMP 0·16, AMP 0·19).

A portion of the 2′-deoxy AMP (5·0 OD) thus obtained was incubated with crude snake venom[17] (containing 5′-nucleotidase) for 5 hr. By this treatment 2′-deoxy AMP was dephosphorylated by as much as

98% to give 2'-deoxyadenosine and inorganic phosphate. PPC: R_f(A) 0·59 (authentic 2'-deoxyadenosine 0·59, S-cycloadenosine 0·48).

Analysis of cyclonucleoside derived from 5'-MP. Cyclonucleoside MP (free, 5 mg) was neutralized with N NaOH and incubated with *E. coli* alkaline phosphatase[9] (0·4 mg/ml, 0·5 ml) and 0·5 M ammonium bicarbonate (0·5 ml) in a total volume of 5 ml (adjusted with water) at 37° for 8 hr. Solvent was evaporated and a small amount of water was added. Pale yellow crystals which appeared were collected by filtration. Filtrate and washings were combined and subjected to paper chromatography in solvent C. The band at R_f 0·57 corresponding to cyclonucleoside was cut out and eluted with water. The eluant and the crystals obtained above were dissolved in water (10 ml) (TOD_{276} = 246). The soln was applied to a column (0·6 × 2 cm) of Dowex 1 × 2 (OH^- form, 100–200 mesh). Fractions of 20 ml each were collected at a flow rate of 80 ml/hr. By washing the column with water 8,2'-S-cyclonucleoside (Fraction No. 4–17) was obtained (TOD_{267} = 247). Elution with 30% MeOH (Fraction No. 22–29) and 1% acetic acid (Fraction No. 30–100) gave no 8,3'-cyclonucleoside. The same type of column chromatography of a soln containing 8,2'- (3 mg) and 8,3'-cyclonucleoside (2 mg) showed complete separation and recovery of both compounds as shown in Fig 2a.

Enzymatic reaction of 8,2'-*S-cyclonucleoside derivatives*

(i) *Snake venom 5'-nucleotidase.*[17] The incubation mixture (100 µl) contained 8,2'-Cyclonucleoside MP (5 OD units), 0·25 M Tris-HCl buffer (pH 8,7,20 µl), 0·1 M $MgCl_2$ (10 µl) and crude snake venom (20 mg/ml, 10 or 30 µl). Incubation was performed at 37° for 4 hr. Analysis by paper electrophoresis (pH 7·5) showed 52% (10 µl enzyme) and 92% (30 µl enzyme) dephosphorylation, respectively. Under the same conditions AMP was hydrolyzed completely in 2 hr.

(ii) E. coli *alkaline phosphatase.*[9] The incubation mixture (100 µl) contained 8,2'-cyclonucleoside MP (5 OD), 1 M NH_4HCO_3 (10 µl) and enzyme (0·4 mg/ml, 5 ml). The mixture was incubated at 37° for 30 min. Analysis of the mixture by paper electrophoresis showed that the substrate was 98% dephosphorylated. Under the same conditions AMP was hydrolyzed to 96%.

(iii) *Sheep intestinal alkaline phosphatase.*[19] The incubation mixture (300 µl) contained 8,2'-cyclonucleoside MP (40 OD), 1 M $(NH_4)_2CO_3$ (30 µl) and enzyme (0·5 mg/ml, 60 µl). Incubation was performed at 37° for 4·5 hr. The mixture was analyzed by paper chromatography in solvent A. The substrate was dephosphorylated and converted to 8,2'-S-cycloinosine by a deaminase contained in the enzyme preparation. The latter compound was identical with an authentic sample of 8,2'-S-cycloinosine. PPC: R_f(A) 0·30 (inosine 0·31, 8,2'-S-cycloadenosine 0·49).

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