Tetrahedron. Vol. 28, pp. 3695 to 3704. Pergamon Press 1972. Printed in Great Britain

STUDIES OF NUCLEOSIDES AND NUCLEOTIDES—LIV[1]

PURINE CYCLONUCLEOSIDES—19. FURTHER INVESTIGATIONS ON THE CLEAVAGE OF THE 8,2′-O-ANHYDRO LINKAGE. A NEW SYNTHESIS OF 9-β-D-ARABINOFURANOSYLADENINE[2]

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(*Received in Japan* 8 *January* 1972; *Received in the UK for publication* 6 *March* 1972)

Abstract—Reaction of 8,2′-anhydro-8-oxy-9-β-D-arabinofuranosyladenine (I) with various nucleophiles is reported. I reacts with liquid ammonia to give 8-amino-9-β-D-arabinofuranosyladenine (IV) and 8,5′-anhydro-8-oxy-9-β-D-arabinofuranosyladenine (VI). The latter is also obtained by heating I at 60° in 0·01 N sodium hydroxide with which it is in equilibrium.

I gives 8-mercapto-9-β-D-arabinofuranosyladenine (VIII) by heating in liquid H_2S and anhydrous pyridine. Desulphurization of VIII either with Raney nickel or oxidation with iodine or hydrogen peroxide gives 9-β-D-arabinofuranosyladenine (ara A) (IX). I with hydrogen chloride in methanol gives 8-chloro-9-β-D-arabinofuranosyladenine (X) and 2-(adenyl-8)-D-arabinose (XI), and in DMF gives 2′-chloro-2′-deoxy-8-oxyadenosine (XII). I with sodium ethylmercaptide yields 8-ethylthio-9-β-D-arabinosyladenine (XIII) and 2′-deoxy-2′-ethylmercapto-8-oxyadenosine (XIV). UV, NMR and CD spectra are reported.

WE HAVE PREVIOUSLY reported on the reactions of 8,2′-anhydro-8-oxy-9-β-D-arabinofuranosyladenine[3] (8,2′-O-cycloadenosine) (I), but in view of difficulties in the large scale synthesis of I, the investigation has been limited. Recently, a new route to 8,2′-O-cycloadenosine was found[4] and further investigations on its cleavage by various nucleophiles became possible. A new method for the synthesis of 9-β-D-arabinofuranosyladenine (ara A), known to be active against virus infection,[5] is also reported here.

In the course of the cylization of 2′-O-(2,4,6-triisopropylbenzenesulfonyl)-8-oxyadenosine (II)[4] or its N^6,3′,5′-tri-O-acetyl derivative[4] (III) by methanolic ammonia, we found that in addition to the desired cyclonucleoside (I), three other compounds (IV, V and VI) were formed and separated by paper chromatography in solvent A.* UV absorption spectra of IV, V and VI resembled those of 8-aminoadenosine,[6] 8-oxyadenosine[3,6] and 8,5′-anhydro-8-oxyadenosine,[7,8] respectively. All these compounds did not consume periodate[9] on paper chromatograms and migrated slower than the corresponding *ribo*-nucleosides in electrophoresis performed in borate buffer.[10] We tentatively assign IV, V and VI to 8-amino-9-β-D-arabinofuranosyladenine, 8-oxy-9-β-D-arabinofuranosyladenine, and 8,5′-anhydro-8-oxy-9-β-D-arabinofuranosyladenine, respectively. The ratio of compounds I, IV and VI in the mixture analyzed by paper chromatography are summarized in Table 1. It was found that if the reaction mixture did not contain traces of water, compound V was not produced.

When I was heated in a mixture of liquid NH_3 and MeOH (1:1, vol/vol) at 100° for 6 hr, we obtained IV and VI. IV had a UV absorption maximum at 275 nm, which

* See footnote on page 8.

TABLE 1. PERCENT YIELD OF PRODUCTS IN CYCLIZATION OF 8-OXY-2'-TPS-ADENOSINE

Reaction conditions	Compound I	IV	V
60°, 6 hr	94·1%	6·0%	0 %
60°, 12 hr	80·6	10·6	8·8
80°, 6 hr	79·0	12·5	8·3
100°, 1 hr	74·8	13·8	11·3

resembled that of 8-aminoadenosine.[6] This compound (Table 2), showed its H-1 signal in the NMR spectrum as a doublet at δ 6·08 having $J_{1'-2'} = 5{\cdot}0$ Hz. The large coupling constant was consistent with the assignment[11] that 1'- and 2'-H were *cis*. It was also shown that IV migrated slower than the *ribo* isomer in paper electrophoresis performed in borate buffer, as expected for the *arabino* configuration which is unable to form a complex with borate. The structure was also supported by elemental

TABLE 2. CHEMICAL SHIFTS (δ) AND COUPLING CONSTANTS (Hz) IN NMR OF ADENOSINE, ARABINOSYLADENINE AND THEIR DERIVATIVES

	8-H	2-H	6-NH_2	1'-H	2'-H	3'-H	4'-H	5'-H	2'-OH or 3'-OH	5'-OH
Adenosine	8·820	8·73								
Ara A (XI)	8·12	8·18	7·18	6·30 6·22 $J_{1'-2'}$, 4·8						
8-SH-Ads	8·3	8·07	6·89	6·28 2·34 $J_{1'-2'}$, 6	4·90 4·96 5·02 $J_{2'-3'}$, 6	4·18 4·21 4·24 4·27 $J_{3'-4'}$, 3	3·92 3·95	3·50 3·54 3·58 3·62	5·0	3·3
8-SH-Ara A (VIII)	8·72	8·06	6·83	6·74 6·64 $J_{1'-2'}$, 6	4·38 4·48	3·72	3·72	3·18	5·3	4·40
8-NH_2-Ara A (IV)	8·28	7·80	6·28	6·06 6·11 $J_{1'-2'}$, 5	4·09 4·14					
8,5'-O-cyclo-Ads		8·10	7·04	6·05	5·07 5·13 $J_{2'-3'}$, 6	4·84 4·90	4·72 4·74 $J_{4'-5'}$, 2	4·05 4·18 4·56 4·58 4·69 4·71 $J_{5'-5'}$, 13		
8,5'-O-cyclo-Ara A (VI)		8·02	6·82	6·22 6·28 $J_{1'-2'}$, 6		4·32[a]	4·19 4·21 $J_{4'-5'}$, 2	4·00 4·13 4·43 4·45 4·56 4·58 $J_{5'-5'}$, 13		

[a] This signal could not be assigned either to 2'- or 3'-H.

analyses. This compound was synthesized previously by Reist *et al.*,[12] but our sample had a different m.p. We first thought that compound VI was an 8-methoxy derivative in view of the similar UV absorption at 260 nm. However, the spectrum in acidic media differed in shape and resembled that of 8,5′-anhydro-8-oxyadenosine.[8] Compound VI gave also a coupling constant $J_{1'-2'} = 6{\cdot}0$ Hz, consistent with the *arabino* configuration. As shown in Fig. 1, a large ($[\theta] = 29{,}000$) positive Cotton band at 260 nm suggested the 8,5′-O-cyclonucleoside structure for VI according to a general rule concerning the magnitude of Cotton effects of cyclonucleosides.[13] Elemental analyses

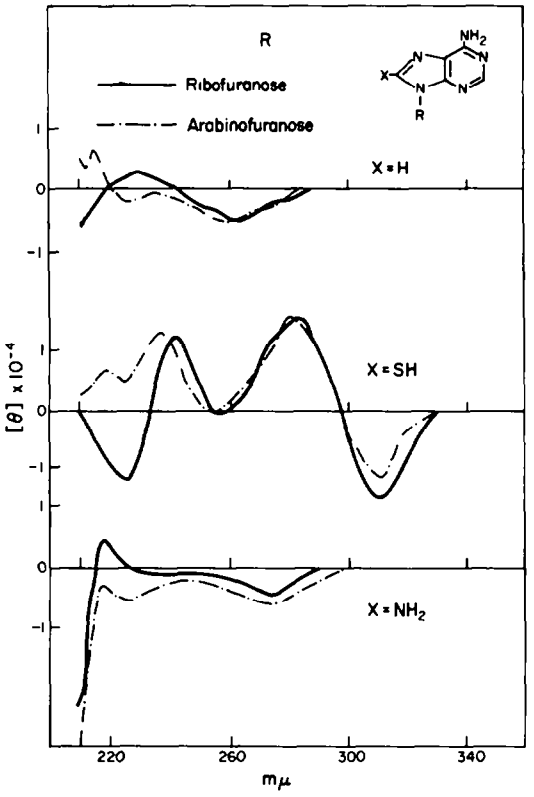
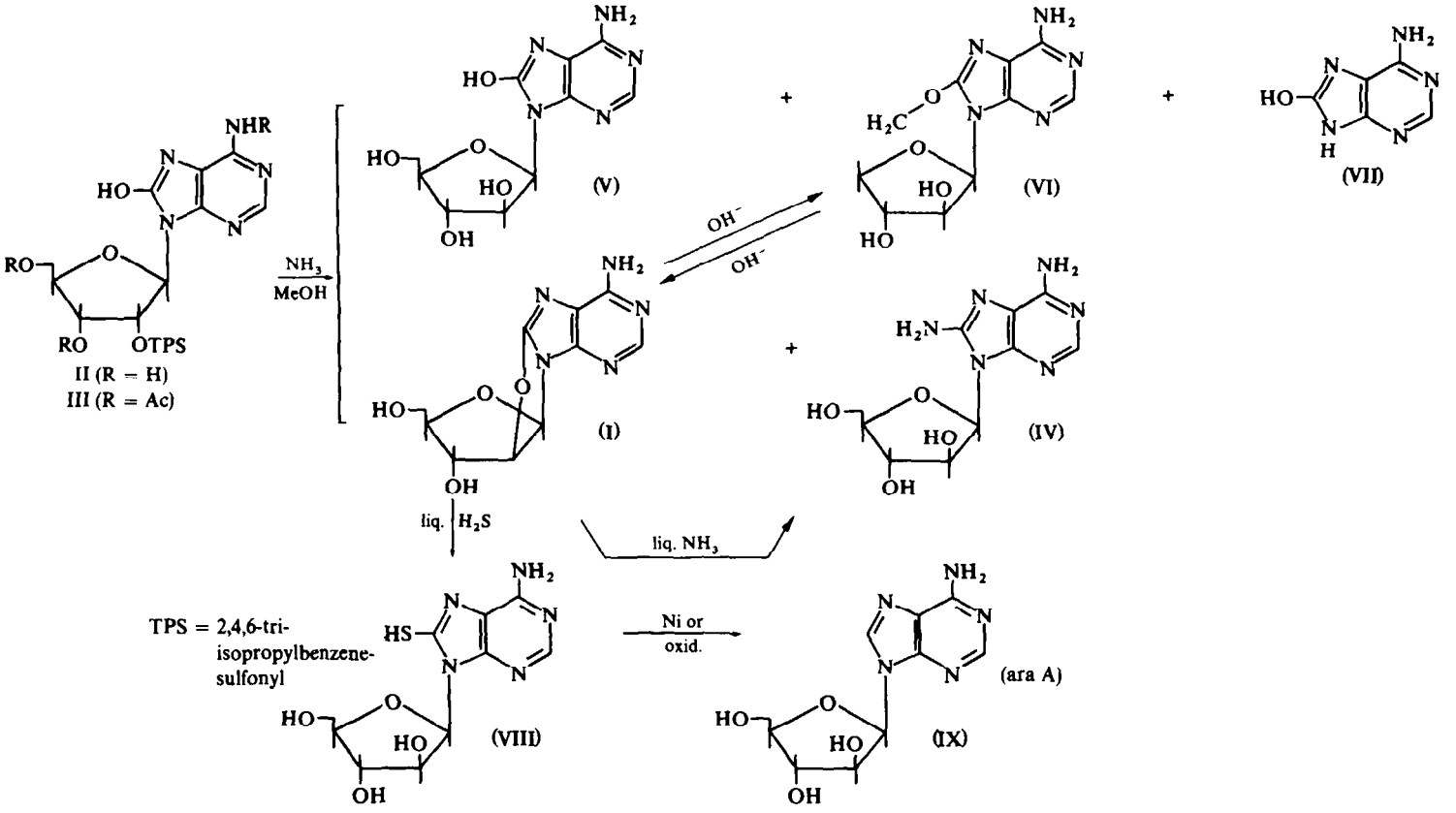


FIG 1. CD spectra of adenosine, arabinosyladenine and 8-substituted derivatives

and properties on paper chromatography also supported the structure. When the reaction with liquid NH_3 was performed in anhydrous pyridine (1:1, vol/vol) at 130° for 16 hr, only 8-amino-9-β-D-arabinofuranosyladenine (IV) was obtained in moderate yield. However, in the presence of a trace of water or in large scale experiments, we often obtained 8-oxyadenine (VII) in addition to IV. Whether VII arose from scission of 8-oxy-arabinosyladenine (V) directly or *via* cyclonucleoside VI as intermediate, must be clarified by further investigation.

When I was heated with H_2S in pyridine (1:1, vol/vol) at 100° for 14 hr, 8-mercapto-9-β-D-arabinofuranosyladenine (VIII) was obtained in 74·4% yield. It showed a UV absorption spectrum similar to that of 8-mercaptoadenosine,[6] but migrated slower in paper electrophoresis in borate buffer. Compound VIII has been reported by Reist *et al.*[12] and our sample had identical properties. Compound VIII gave 9-β-D-

CHART 1


II (R = H)
III (R = Ac)
NH3
MeOH
(V)
(VI)
(VII)
(I)
(IV)
OH⁻
OH⁻
liq. H2S
liq. NH3
TPS = 2,4,6-tri-isopropylbenzene-sulfonyl
Ni or oxid.
(VIII)
(IX)
(ara A)

arabinofuranosyladenine (IX) on desulfurization with Raney Ni in 36·7% yield. UV absorption properties, NMR signals, and paper chromatography behavior performed with several solvent systems were consistent with the description by Reist *et al.*[14] and with an authentic sample of arabinosyladenine.[14] The m.p. of our sample was higher than the authentic one, which was prepared enzymatically.* Because of a relatively low yield in the Raney Ni desulfurization, we attempted the oxidation of VIII with I_2 or H_2O_2. When VIII was dissolved in 0·1 M phosphate buffer (pH 7·4) and oxidized with 1 N I_2-KI solution at room temperature, arabinofuranosyladenine (IX) was obtained almost quantitatively. Oxidation with 3% H_2O_2 on 0·1 N HCl aq gave similar results. In the latter case no N^1-oxidation of the adenine[15] moiety was detected. Therefore, the procedure described here constitutes a new and versatile method for the synthesis of arabinofuranosyladenine starting from naturally occurring adenosine. It may be emphasized that by this route only the biologically active β-anomer is obtainable, instead of the α,β-mixture formed by condensation of the base with the sugar moieties.

In order to discover how the 8,5′-cyclonucleoside VI was formed in the reaction of I with NH_3, we treated I with dilute alkali solution. When I was heated in 0·01 N NaOH, at 60°, almost half the starting material changed to VI in 3 hr. Compounds I and VI could be obtained by fractional crystallization from water in 37 and 38%, respectively. If we treated VI (isolated in a pure state) the same way as above, about 50% of compound I was again produced. Therefore, conversion between I and VI seems to be equilibrium reaction *via* a common intermediate. This interpretation is plausible, because the relative geometrical arrangement of 5′-OH to C-8 in 8,2′-cyclonucleoside (I) and 2′-*arabino*-OH to C-8 in 8,5′-cyclonucleoside (VI) is quite similar when Corey-Pauling-Kortun models[16] of I and VI were investigated. Further work with other cyclonucleosides must be performed for a total understanding of the mechanism.

Now we consider the reaction of 8,2′-O-cyclonucleoside (I) with HCl in MeOH. Compound I was heated at 90° for 2·5 hr in anhydrous MeOH containing 1% dry HCl by weight and gave 8-chloro-9-β-D-arabinofuranosyladenine (X), 8-oxy-adenine (VII), and a compound of unknown structure (XI), in 10, 68 and 24%, respectively. When the same mixture was kept at 30° overnight the ratio changed to 5, 37 and 58%. Compound X had a UV absorption spectrum similar to that of 8-bromoadenosine,[17] although the maxima shifted hypsochromically. Paper electrophoresis in a borate buffer, as well as coupling constant $J_{1'-2'}$ *ca.* 6 Hz, showed the *arabino* configuration of the sugar moiety. Although elemental analysis of this compound was not satisfactory presumably because of crystallization difficulties, we assigned X to 8-chloro-arabinofuranosyladenine. Compound XI showed a UV absorption similar to that of a compound obtained by acidic hydrolysis of 8,5′-O-cycloadenosine,[8] which had the 5-(adenyl-8)-D-ribose structure. Furthermore, XI rapidly consumed metaperiodate on a paper chromatograpm and reduced Fehling's solution showing it to be a reducing sugar. On this evidence we assigned the 2-(adenyl-8)-D-arabinose structure to compound XI. This compound may give rise to 8-oxyadenine by cleavage of the 8,2′-ether linkage. The cleavage reaction of the glycosidic bond prior to the anhydro linkage was observed also in the hydrolysis of I in 0·1 N H_2SO_4 and 10% HCl aq.

When I was heated in anhydrous 5% HCl-DMF solution at 20° for 1 hr, a single

* Produced and supplied from Upjohn Co., Kalamazoo, Michigan U.S.A.

CHART 2

(XI)
(VIII)
Et I
+
(X)
HCl
MeOH
(XIII)
+
(I)
EtSNa
(XIV)
+ XI + unknown compound
HCl/DMF
NaOAc/DMF
Ni
(XII)
(XV)

product (XII) was obtained in 62% yield. Compound XII had a similar UV absorption spectrum to that of 8-oxyadenosine[3] and contained halogen by the Beilstein or $AgNO_3$ test. The $H_{2'}$ signal appeared at 5·36, being downfield relative to the $H_{2'}$ signal of adenosine. The coupling constant of $H_{1'}$ due to $H_{2'}$ was 8 Hz. This indicated a $C_{2'}$-*endo* puckering of the furanose ring due to the introduction of chlorine at the 2'-position. Furthermore XII reverted to 8,2'-cycloadenosine (I) by treatment with NaOAc in DMF as reported for the 2'-deoxy-2'-chlorouridine derivatives.[18] These facts suggest that XII should be 2'-chloro-2'-deoxy-8-oxyadenosine. From these acidic cleavage reactions, it was suggested that attack of the chloride ion occurred either at the C_8- or N_9-position in a protic solvent but at $C_{2'}$ in an aprotic solvent.

Compound I was further allowed to react with sodium ethylmercaptide in absolute MeOH. Reaction at 150° for 3 hr gave 8-ethylmercapto-9-β-D-arabinofuranosyladenine (XIII), 2'-deoxy-2'-ethylmercapto-8-oxy-9-β-D-arabinofuranosyladenine (XIV), 2-(adenylyl-8)-D-arabinose (XI) and an unknown compound. The ratios of these compounds was changed slightly with change of reaction conditions. The structure of XIII was confirmed by its UV absorption spectrum which resembled that of 8-methylmercaptoadenosine[19] and Raney Ni desulfurization to give arabinosyladenine. The structure of XIV was deduced also by its UV absorption properties and desulfurization to give 8-oxy-2'-deoxyadenosine (XV).[20] When I was heated with sodium ethylmercaptide in DMF at 70° for 1 hr, the 2'-deoxy-2'-ethylmercapto compound (XIII) was the sole product. These experiments showed that the same reagent could react at both C-8 and C-2' by changing the solvent and temperature. This may be caused by a different dissociation of sodium ethylmercaptide under these conditions.

Finally, we compared the CD spectra of unsubstituted, 8-mercapto and 8-amino-

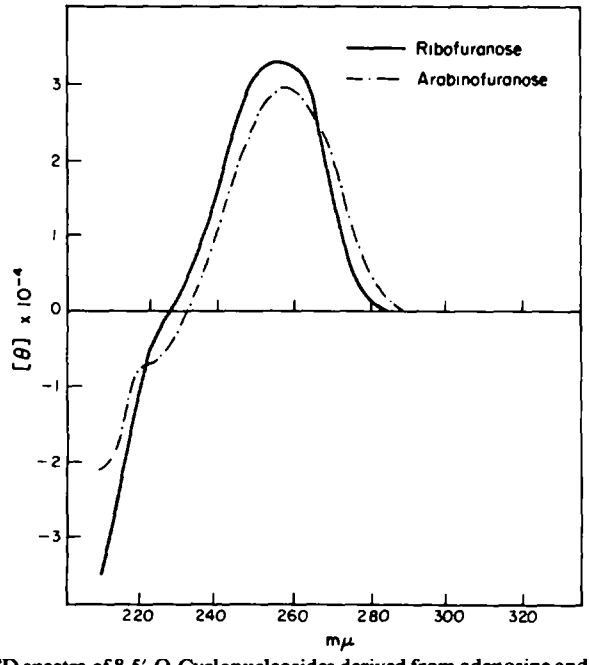


FIG 2. CD spectra of 8,5'-O-Cyclonucleosides derived from adenosine and arabinosyladenine

adenine nucleosides, which have a *ribo* or *arabino* configuration in the sugar moieties. As shown in Fig. 2, all the corresponding nucleosides had similar CD profiles except in the E-region.[21] This might be interpreted by assuming almost the same torsion angle[22] of *ribo* and *arabino* isomers and an effect due to the *up* 2'-OH group on the puckering of the sugar moieties.

EXPERIMENTAL*

NMR data are summarized in Table II. CD spectra are recorded in Figs 1 and 2.

2'-Triisopropylbenzenesulfonyl-3',5',N⁶-triacetyl-8-oxyadenosine (III) *with methanolic ammonia.* III (0·1 mmole) was heated in MeOH (10 ml) containing 0·1 mM liquid NH_3 at 60°–100° for 1–12 hr as in Table 1. After reaction part of the mixture was spotted on paper and developed in solvent A. Each spot was cut and eluted with water. Product ratio was obtained from the UV absorption of each spot. The structures of compounds I, IV, V and VI corresponding to each spot was elucidated by direct comparison with authentic samples (I, IV, V and VI) obtained in the following large scale experiments.

8-Amino-9-β-D-arabinofuranosyladenine (IV). 8,2'-O-Cycloadenosine (I) (265 mg) was dissolved in a mixture of pyridine (30 ml) and liquid NH_3 (30 ml). The mixture was heated at 130° in a sealed steel tube for 16 hr. After cooling the mixture was evaporated *in vacuo* to give a colorless residue, which was applied to a prep TLC and developed with $CHCl_3$–EtOH (7:2). The band corresponding to 8-aminoarabinosyladenine was removed and eluted with $CHCl_3$–MeOH. The residue obtained by evaporation of the solvent *in vacuo* recrystallized from EtOH–H_2O as colorless needles, m.p. 245–246°, (yield 95 mg, 33·7%). (Calc. for $C_{10}H_{14}N_6O_4$ 1·5 H_2O: C, 39·65; H, 5·66; N, 27·75. Found: C, 39·43; H, 5·97; N, 27·69%). UV: $\lambda_{max}^{H^+}$ 273 nm (ε 14,600), 290 nm (sh); $\lambda_{max}^{H_2O}$ 275 nm (16,200); $\lambda_{max}^{OH^-}$ 276 nm (15,900). CD ([θ]): λ peak 245 (−4,000), 218 nm (−3,000); λ trough 273 (−5,750), 227 nm (−5,250). 8-Oxyadenine was obtained in a yield of 70 mg (44·6%). (Calc. for $C_5H_5N_5O$: C, 39·73; H, 3·33; N, 46·34. Found: C, 39·77; H, 3·32; N, 46·36%).

8-Mercapto-9-β-D-arabinofuranosyladenine (VIII). 8,2'-O-cycloadenosine (I) (530 mg) was dissolved in pyridine (20 ml) and liquid H_2S (obtained by cooling with dry ice–ether, 20 ml). The mixture was sealed in a steel tube and heated at 100° for 14 hr. The tube was cooled with dry ice–ether and N_2 gas bubbled through to remove H_2S completely. Pyridine was removed by distillation *in vacuo* and the residue recrystallized from MeOH as white needles, m.p. 154° (decom.†), 445 mg (74·4%). (Calc. for $C_{10}H_{13}N_5O_5 \cdot S \cdot CH_3OH$: C, 39·62; H, 5·15; N, 21·03; S, 9·63. Found: C, 39·73; H, 4·90; N, 21·24; S, 9·54%). UV: $\lambda_{max}^{H^+}$ 223, 243, 298 (sh), 308 nm (ε 21,900); $\lambda_{max}^{H_2O}$ 238, 298, 305·5 nm (24,800); $\lambda_{max}^{OH^-}$ 228, 296 (21,100), 303 nm. CD: λ peak 280 (16,000), 237 (13,500), 219 nm (7,000); λ trough 311 (−11,000), 254 (0), 225 nm (4,500). PPC: R_f(A) 0·43, R_f(B) 0·36, R_f(C) 0·25. Paper electrophoresis: (0·05 M borate buffer, pH 6·8, 75· v, 4·5 mA): $R_{adenosine}$ 0·72.

9-β-D-Arabinofuranosyladenine (IX). (i) By Raney Ni desulfurization: 8-Mercaptoarabinosyladenine (VIII) (300 mg) was dissolved in MeOH (20 ml) and EtOH (7·4 ml) containing Raney Ni (w-2, 2·8 g) added. The mixture was refluxed for 3 hr and completion confirmed by electrophoresis performed in a triethylammonium bicarbonate buffer (0·05 M, pH 7·5). The nickel was removed by filtration and the filtrate and washings (hot MeOH) combined and evaporated *in vacuo* giving a white amorphous powder. Recrystallization (MeOH) gave 110 mg (41%) of ara A, m.p. 260° (decomp.). (Calc. for $C_{10}H_{13}N_5O_4$: C, 44·94; H, 4·90; N, 26·21. Found: C, 45·10; H, 48·2; N, 26·51%). UV: $\lambda_{max}^{H^+}$ 258 nm (ε 15,100); $\lambda_{max}^{H_2O}$ 260 nm (15,400); $\lambda_{max}^{OH^-}$ 260 nm (15,400). CD: λ peak 236 (−1,000), 214 nm (6,500); λ trough 258 (−5,700), 226 (−2,000), 212 nm (3,000). PPC: R_f(A) 0·43, R_f(B) 0·09, R_f(C) 0·42. Paper electrophoresis (0·05 M borate buffer, pH 6·8, 75· v, 4·5 mA): $R_{adenosine}$ 0·63. This sample was identical with a sample synthesized enzymatically, except that the latter one had m.p. 253° (decomp.).

(ii) By I_2-oxidation: 8-mercaptoarabinosyladenine (30 mg) was dissolved in 0·1 M phosphate buffer

* UV absorption spectra were measured with a Hitachi EPS-3T spectrophotometer, IR spectra were with a Hitachi EPI-L spectrophotometer, and NMR spectra with a Hitachi H-6013 and a Varian HA-100 high resolution spectrometer in DMSO-d_6 with TMS as internal standard. CD was measured with a JASCO ORD/UV-5 spectropolarimeter installed with a CD attachment.

Paper chromatography (PPC) was performed on Toyo filter paper No. 51A with following solvents: Solvent A, water adjusted to pH 10 with ammonia; solvent B, n-butanol–water (86:14); solvent C, isopropanol–conc. ammonia–water (7:1:2).

† M.p. reported as 199·5–202·5°.[12]

(pH 7·4, 1 ml) and water (1 ml). To the solution was added NI_2 solution (0·3 ml) and the mixture kept at room temp overnight. Separation by prep TLC and recrystallization (MeOH) gave arabinosyladenine, 22 mg (82%) identical with that obtained in (i).

(iii) By H_2O_2-oxidation: VIII (15 mg) was dissolved in 50% MeOH (1 ml) and 3% H_2O_2 (0·2 ml) and 0·1 N HCl (0·05 ml) added. The mixture was kept at room temp overnight and neutralized with N NaOH. Evaporation of the mixture and recrystallization of the residue (MeOH) gave ara A, m.p. 239–243°, 9·5 mg (73%) identical with that obtained in (i).

8,5'-*Anhydro*-8-*oxy*-9-β-D-*arabinofuranosyladenine* (VI). 8,2'-O-Cycloadenosine (I) (265 mg) was heated in 0·01 N NaOH (30 ml) at 60° for 3 hr then neutralized with 0·1 N HCl and evaporated *in vacuo* to *ca.* 5 ml precipitating crystalline material. The crystals were collected by filtration and washed with MeOH, which dissolved the starting material (38%). The residual crystals were recrystallized from water to give white prisms (88 mg, 37%). (Calc. for $C_{10}H_{11}N_5O_4$: C, 45·28; H, 4·18; N, 26·40; O, 24·13. Found: C, 45·56; H, 3·92; N, 26·75; O, 24·09%). UV: $\lambda_{max}^{H^+}$ 261 nm (ε 17,400), $\lambda_{max}^{H_2O}$ 261·5 nm (18,300), $\lambda_{max}^{OH^-}$ 261·5 nm (17,900). CD: λ peak 258 nm (29,000), λ trough 221 nm (7,500). PPC: R_f(A) 0·44, R_f(C) 0·39. NMR data are in Table II. CD curve is in Fig. 2.

The same reaction of 8,5'-anhydro-9-β-D-arabinosyladenine (VI) (25 mg) gave 8,2'-O-cycloadenosine (I) in *ca.* 50% yield.

8-*Oxy*-2'-*deoxy*-2'-*chloroadenosine* (XII). 8,2'-O-Cycloadenosine (I) (520 mg, 2 mmoles) was dissolved in DMF (30 ml) containing 0·05% HCl gas. The solution was sealed in a glass tube and heated at 100° for 1 hr, completion confirmed by PPC (A) and the mixture then neutralized with N NaOH. DMF was evaporated *in vacuo*, precipitated NaCl filtered off, and the filtrate concentrated. Addition of MeOH to the concentrated solution precipitated further NaCl, which was removed. The filtrate was evaporated to dryness *in vacuo* and the residual glass applied to TLC. The 8-oxy-2'-chloro compound was obtained as a glass (m.p. 130°) 370 mg (61·2%). (Calc. for $C_{10}H_{12}N_5O_4Cl$: C, 39·81; H, 4·01; Cl, 11·75. Found: C, 39·79; H, 4·51; Cl, 12·17%). UV: $\lambda_{max}^{H^+}$ 265, 283 nm; $\lambda_{max}^{H_2O}$ 270 nm; $\lambda_{max}^{OH^-}$ 280 nm. PPC: R_f(A) 0·42, R_f(C) 0·46. NMR: 5·92 δ (d, 1H, $H_{1'}$, $J_{1'-2'}$ = 8 Hz), 5·36 δ (q, 1H, $H_{2'}$, $J_{2'-3'}$ = 5 Hz). When this compound (10 mg) was heated in DMF (5 ml) containing NaOAc (10 mg), it was converted to 8,2'-O-cycloadenosine.

8,2'-O-*Cycloadenosine* (I) *with* HCl *in* MeOH. I (133, 0·5 mmole) was dissolved in anhydrous MeOH (5 ml) containing 1% HCl. The mixture was heated at 90° for 2·5 hr then applied to a PPC in solvent A. Band at R_f 0·33 gave 8-oxyadenine (68%). UV: $\lambda_{max}^{H^+}$ 265, 280 nm; $\lambda_{max}^{H_2O}$ 255 (sh), 270 nm; $\lambda_{max}^{OH^-}$ 279 nm. Band at R_f 0·51 gave 24% of 2-(adenyl-9)-D-arabinose (XI). UV: $\lambda_{max}^{H^+}$ 272 nm, $\lambda_{max}^{H_2O}$ 266 nm, $\lambda_{max}^{OH^-}$ 273 nm. This sample reduced Fehling's solution showing the presence of reducing sugar. Band at R_f 0·60 gave 8-chloroara A (IX) (10%). UV: $\lambda_{max}^{H^+}$ 263 nm, $\lambda_{max}^{H_2O}$ 265 nm, $\lambda_{max}^{OH^-}$ 267 nm. Values resembled to those of 8-chloroadenosine. This sample contained halogen by $AgNO_3$ test after fusion with Na.

When the same reaction was performed at 30° overnight, 8-oxyadenine, XI and X were obtained in 37, 58 and 5% yield, respectively.

8-*Oxy*-2'-*deoxy*-2'-*ethylmercapto-adenosine* (XIV). NaH (58 mg, containing 50% mineral oil) washed with dry benzene, and ethylmercaptan, (0·6 ml) were dissolved in DMF (10 ml). 8,2'-O-Cycloadenosine (I) (265 mg) was added to mixture which was heated at 70° for 1 hr. After all starting material disappeared (confirmed by PPC) the mixture was neutralized with NHCl whilst bubbling through with N_2. DMF was removed by distillation *in vacuo* and the residue applied to TLC. Nucleoside was eluted from the appropriate band and recrystallized (MeOH) to give colorless prisms, m.p. 206–210° (60%). (Calc. for $C_{12}H_{17}O_4N_5S$: C, 44·21; H, 5·36; N, 20·85; S, 9·51. Found: C, 40·03; H, 5·24; N, 21·40; S, 9·77%). UV: $\lambda_{max}^{H^+}$ 265, 285 (sh) nm; $\lambda_{max}^{H_2O}$ 258 (sh) 271 nm; $\lambda_{max}^{OH^-}$ 281·5 nm.

A small amount of 2'-ethylmercapto nucleoside (XIV) was refluxed in MeOH with Raney Ni (w-2) for 30 min. The product was isolated by paper chromatography in solvent A. Band having R_f 0·59 was cut and water extracted. This compound colored with cystein-H_2SO_4 reagent.[23] UV: $\lambda_{max}^{H^+}$ 265, 283 nm, $\lambda_{max}^{H_2O}$ 270 nm, $\lambda_{max}^{OH^-}$ 280. PPC: R_f(A) 0·58, R_f(C) 0·48. These values coincided with authentic 8-oxy-2'-deoxyadenosine.[20]

8,2'-O-*Cycloadenosine* (I) *with ethylmercaptan in methanol.* I (26 mg, 0·1 mmole) was dissolved in anhydrous MeOH (8 ml) containing ethylmercaptan (1 ml). The mixture was heated at 150° for 3 hr in a steel tube then applied to TLC performed in $CHCl_3$–EtOH (15·5:4·5, vol/vol). Spots appeared corresponding to 8-ethylmercapto-9-β-D-arabinofuranosyladenine (XIII) (20%), 8,5'-anhydro-8-oxy-9-β-D-arabinofuranosyladenine (VI) (34·4%) and starting material (45·1%). The structure of XIII was confirmed by comparison with an authentic sample obtained from 8-mercapto-ara A.

8-*Ethylmercapto*-9-β-D-*arabinofuranosyladenine* (XIII). 8-Mercapto-9-β-D-arabinofuranosyladenine (VIII) (260 mg, 1 mmole) was dissolved in DMF (10 ml). To this solution was added EtI (0·4 ml, 5 equiv.).

The mixture was kept at room temp for 5 days in the dark. When the starting material (which migrated in paper electrophoresis) had disappeared, the mixture was evaporated gently *in vacuo*. The solution was neutralized with N NaOH and evaporated. Recrystallization of the residue (MeOH–H_2O) gave colourless needles, m.p. 107–110° (223 nm, 68·2%). (Calc. for $C_{12}H_{17}O_4N_5S$ 1 5 H_2O: C, 42·85; H, 5·39; N, 20·82; S, 9·53. Found: C, 42·65; H, 5·09; N, 20·48; S, 9·70%). UV: $\lambda_{max}^{H^+}$ 286·5 (ε 19,200); $\lambda_{max}^{H_2O}$ 223, 282·5 (19,000); $\lambda_{max}^{OH^-}$ 223, 282·5 (19,000).

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