Purine Nucleosides. XII. The Preparation of 2′,3′-Dideoxyadenosine, 2′,5′-Dideoxyadenosine, and 2′,3′,5′-Trideoxyadenosine from 2′-Deoxyadenosine*

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ABSTRACT: General synthetic routes have now been devised for the chemical preparation of a number of new deoxyadenosines *via* the requisite intermediate 3′- or 5′-alkylthionucleosides. 5′-*O*-Trityl-3′-*O*-tosyl-2′-deoxyadenosine (II) has been converted in good yield to 3′-*O*-tosyl-2′-deoxyadenosine (III) which in turn has been treated with ethanethiol to yield 6-amino-9-(3′-*S*-ethyl-3′-thio-2′,3′-dideoxy-β-D-*threo*-pentofuranosyl)-purine (IV). Raney nickel desulfurization of IV yielded 2′,3′-dideoxyadenosine (V). Selective 5′-*O*-tosylation of 2′-deoxyadenosine has been successfully accomplished.

Treatment of 5′-*O*-tosyl-2′-deoxyadenosine (VII) with ethanethiol yielded 5′-*S*-ethyl-5-thio-2′,5′-dideoxyadenosine (VIII). The structure of VIII was verified by an unambiguous synthesis from 3′-*O*-acetyl-2′-deoxyadenosine (XI) *via* 5′-*O*-tosyl-3′-*O*-acetyl-2′-deoxyadenosine (XII). Raney nickel desulfurization of VIII gave a good yield of 2′,5′-dideoxyadenosine (IX). Similarly, 3′,5′-di-*O*-tosyl-2′-deoxyadenosine (XIII) yielded 2′,3′,5′-trideoxyadenosine (XVI) *via* the 3′,5′-diethylthio derivative. The significance of these compounds as potential inhibitors of deoxyribonucleic acid biosynthesis is discussed.

Reasons for the synthesis and study of various deoxyadenosines have been briefly outlined in a preliminary communication (Robins and Robins, 1964) describing a portion of the present work. Rich *et al.* (1965) have shown that 3′-deoxyadenosine (cordycepin) depresses the incorporation of adenosine into ribonucleic acid (RNA). These workers have suggested that the inhibitory effects of 3′-deoxyadenosine are *not* due to the blocking of early stages of purine biosynthesis, since adenosine does not reverse the inhibition. It has recently been shown that 3′-deoxyadenosine in the form of the triphosphate inhibits RNA at the terminal step involving RNA polymerase (Shigeura and Gordon, 1965). Shigeura and Boxer (1964) demonstrated that this inhibition by 3′-deoxyadenosine was due to 3′-deoxyadenosine triphosphate which, once incorporated in place of adenosine triphosphate (ATP) in a growing polynucleotide chain, prevented the further elongation of the RNA. Owing to the absence of the 3′-hydroxyl group, the attachment of the next incoming nucleotide was prevented, thus the synthesis of RNA was abruptly terminated. Such a selective inhibition of deoxyribonucleic acid (DNA) biosynthesis was postulated for 2′,3′-dideoxyadenosine (Robins and Robins, 1964) which should act as a chain terminator of deoxypolynucleotide synthesis in a similar manner. In the investigation of a practical synthesis of 2′,3′-dideoxyadenosine (V), 2′-deoxyadenosine was selected as starting material since the stereochemistry is determined as the desired β-anomer. Synthetic procedures involving the attachment of a 2′-deoxy sugar to a heterocyclic base are known to give a mixture of anomers (Bowles and Robins, 1964; Robins *et al.*, 1964; Iwamoto *et al.*, 1962; Robins and Robins, 1965; Whittle and Robins, 1965).

5′-*O*-Trityl-2′-deoxyadenosine (I) (Anderson *et al.*, 1954) was treated with *p*-toluenesulfonyl chloride in the presence of pyridine to give 5′-*O*-trityl-3′-*O*-*p*-toluenesulfonyl-2′-deoxyadenosine (II) which was obtained as a chromatographically homogeneous solid in 65% yield (see Scheme I). When 5′-*O*-trityl-3′-*O*-tosyl-2′-deoxyadenosine (II) was heated for 12 min at 100° in 80% acetic acid, an essentially quantitative yield of 3′-*O*-tosyl-2′-deoxyadenosine (III) was obtained. Replacement of the *p*-toluenesulfonate group of III was effected by ethyl mercaptide ion to yield 6-amino-9-(3′-*S*-ethyl-3′-thio-2′,3′-dideoxy-β-D-*threo*-pento furanosyl)purine (IV). The D-*threo* configuration of IV has been tentatively assigned assuming direct displacement. Raney nickel desulfurization of IV gave 6-amino-9-(2′,3′-dideoxy-β-D-*glycero*-pentofuranosyl)purine (V, 2′,3′-dideoxyadenosine). The proton magnetic resonance (pmr)[1] spectrum of V (Figure 1) confirms this structural assignment. An alternative synthesis of 2′,3′-

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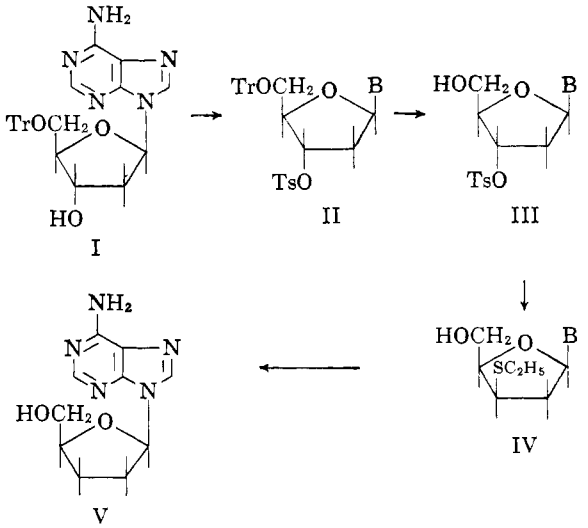
[1] Abbreviations used: pmr, proton magnetic resonance; PRPP, 5-phosphoribosyl pyrophosphate.



SCHEME I

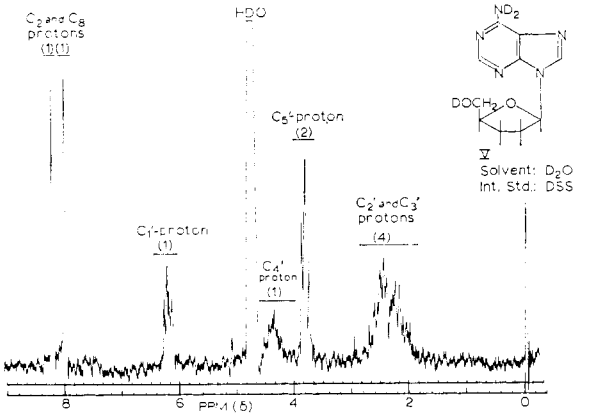
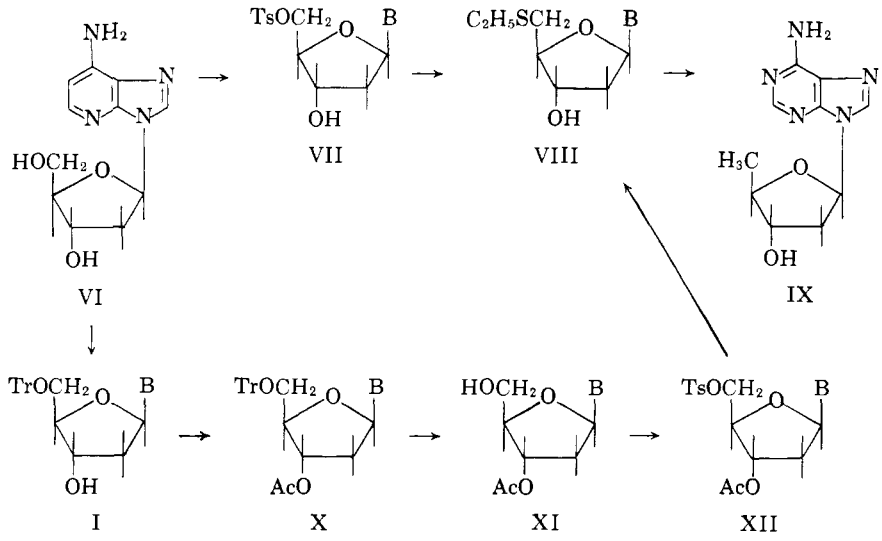


FIGURE 1: The pmr spectrum of 6-amino-9-(2′,3′-dideoxy-β-D-*glycero*-pentofuranosyl)purine (2′,3′-dideoxyadenosine, V).

dideoxyadenosine has recently been reported (Tong *et al.*, 1965).

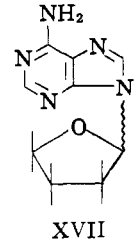
A related compound of interest is 2′,5′-dideoxyadenosine (XVI). Since this nucleoside does not possess a 5′-hydroxyl function, it should act as 2′-deoxyadenosine at the nucleoside level *only*. This compound could provide a test of the possible specific action of purine-2′-deoxynucleosides as feed back inhibitors in DNA biosynthesis. The inhibition of DNA synthesis by 2′-deoxyadenosine has already been amply demonstrated (Klenow, 1959; Maley and Maley, 1960; Morris and Fischer, 1963; Morris *et al.*, 1963) presumably due to 2′-deoxyadenosine acting as a phosphorylated derivative. Although the synthesis of 2′,5′-dideoxyadenosyl cobalamin has recently been described (Hogenkamp and Oikawa, 1964), the preparation of 2′,5′-dideoxyadenosine itself is unreported. Adenine 5′-deoxynucleosides have recently received increased attention due to the structural elucidation of the antibiotic decoynine (angustmycin A) which has been shown to be a 9-(5′-deoxy-β-D-furanosyl)adenine derivative (Hoeksema *et al.*, 1964) related to psicofuranine. The antitumor activity of decoynine (Tanaka *et al.*, 1961) would appear to be due to the inhibition of nucleotide formation in the condensation of 5-phosphoribosyl pyrophosphate (PRPP) with purines (Savel and Handschumacher, 1965). Decoynine has also been shown to inhibit the conversion of xanthosine 5′-monophosphate to guanosine 5′-monophosphate (Tanaka, 1963; Bloch and Nichol, 1964). Thus it is quite possible that 2′,5′-dideoxyadenosine (IX) could similarly provide selective inhibition in the biosynthesis of the purine 2′-deoxynucleotides required for DNA synthesis.

The preparation of 2′,5′-dideoxyadenosine (IX) is outlined in Scheme II. 2′-Deoxyadenosine was treated with *p*-toluenesulfonyl chloride under carefully controlled conditions to give 5′-*O*-tosyl-2′-deoxyadenosine (VII) in 40% yield. Treatment of VII with ethanethiol gave a 60% yield of crystalline 5′-*S*-ethyl-5′-thio-2′,5′-dideoxyadenosine (VIII). Conversion of VIII to 2′,5′-dideoxyadenosine (IX) was accomplished in 47% yield by the use of Raney nickel catalyst. The pmr spectrum of 2′,5′-dideoxyadenosine (Figure 2) confirmed the assigned structure. A rigorous proof of the structure of 5′-*S*-ethyl-5′-thio-2′,5′-dideoxyadenosine (VIII) was provided by an alternate route. 5′-*O*-Trityl-2′-deoxyadenosine (I) was converted to 5′-*O*-trityl-3′-*O*-acetyl-2′-deoxyadenosine (X). Successful removal of the 5′-*O*-trityl group was accomplished with 80% acetic acid to give 3′-*O*-acetyl-2′-deoxyadenosine (XI) in greater than 60% yield. This provides a new practical synthetic route to XI which previously had been prepared by partial acylation of 2′-deoxyadenosine followed by separation of XI from the other products by countercurrent distribution (Anderson *et al.*, 1954). Anderson *et al.* (1954) were unable to effect acidic removal of the trityl group in 5′-*O*-trityl-N^6,3′-*O*-diacetyl-2′-deoxyadenosine without concomitant cleavage to adenine. *N*-Acylation of a heterocyclic base has been shown to have a substantial effect on the rate of acidic cleavage of the glycosidic bond (Khorana *et al.*, 1961). The striking influence of a 6-*N*-acetylamino group has recently been demonstrated in the case of 8-bromo-2′,3′,5′,N^6-tetracetyladenosine (Holmes and Robins, 1965). 5′-*O*-Trityl-2′-deoxyadenosine (I) was therefore carefully treated with acetic anhydride in pyridine at 15° to avoid *N*-acetylation. It is unnecessary to use di-*p*-anisylphenylmethyl chloride as a blocking agent for the 5′-hydroxyl group in the synthesis of 3′-*O*-acetyl-2′-deoxyadenosine (Hogenkamp and Oikawa, 1964). Tosylation of 3′-*O*-acetyl-2′-deoxyadenosine (XI) gave 5′-*O*-tosyl-3′-*O*-acetyl-2′-deoxyadenosine (XII) (Anderson *et al.*, 1954) which was converted to 5′-*S*-ethyl-5′-thio-2′,5′-dideoxyadenosine (VIII) with ethanethiol in sodium ethoxide. This established VII as the 5′-*O*-tosyl derivative and left no doubt as to the structure of VIII.



SCHEME II

The activity of 9-(tetrahydro-2′-furyl)adenine (XVII) (Lewis *et al.*, 1961) against solid Friend virus leukemia (Bowles *et al.*, 1963) suggested that the compound 2′,3′,5′-trideoxyadenosine (XVI) should be prepared and studied because of its closer structural relationship to 2′-deoxyadenosine. Certain 9-(tetrahydro-2′-pyranyl)- (Robins *et al.*, 1961) and 9-(tetrahydro-2′-furyl)adenine derivatives have recently been shown to

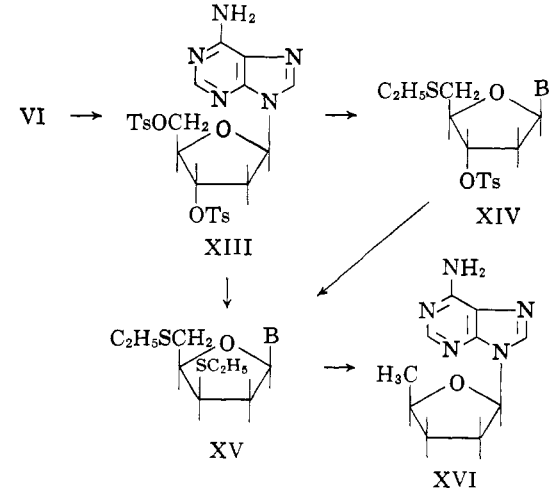


XVII

act as kinins in the rapid stimulation of fruit growth (Crane and Van Overbeek, 1964).

3′,5′-Di-*O*-*p*-toluenesulfonyl-2′-deoxyadenosine (XIII) was prepared from 2′-deoxyadenosine in good yield. Treatment of XIII with ethanolic ethanethiol in sodium ethoxide at 0° for 4 hr followed by refluxing the solution for 5 hr gave a 30% yield of 6-amino-9-(3′,5′-di-*S*-ethyl-3′,5′-dithio-2′,3′,5′-trideoxy-β-D-*threo*-pentofuranosyl)purine (XV) (see Scheme III).

Treatment of 3′,5′-di-*O*-*p*-toluenesulfonyl-2′-deoxyadenosine (XII) with sodium ethyl mercaptide in an ethanol solution at 5° resulted in a 50% yield of 5′-*S*-ethyl-3′-*O*-*p*-toluenesulfonyl-5′-thio-2′,5′-dideoxyadenosine (XIV). Correct analytical data for XIV was obtained and the compound was shown to contain one *S*-ethyl and one *p*-toluenesulfonyl group by pmr spectra. The presence of a covalent *p*-toluenesulfonate group was also verified by an infrared absorption band at 1170 cm^{-1}. The position of replacement was suggested by the absence of cyclonucleoside formation upon recrystallization of XIV from acetone or ethanol–



SCHEME III

water. Similar replacement of the primary tosyl group in *p*-toluenesulfonyl-2′,3′-*O*-isopropylideneguanosine by ethyl mercaptide ion at 0° has previously been noted (Reist *et al.*, 1961).

Treatment of 5′-*S*-ethyl-3′-*O*-*p*-toluenesulfonyl-5′-thio-2′,5′-dideoxyadenosine (XIV) with ethanethiol in a refluxing ethanolic solution of sodium ethoxide gave 6-amino-9-(3′,5′-di-*S*-ethyl-2′,3′,5′-trideoxy-β-D-*threo*-pentofuranosyl)purine (XV) identical with that prepared directly from 3′,5′-di-*O*-*p*-toluenesulfonyl-2′-deoxyadenosine (XII). This represents the second example in the present work of secondary *p*-toluenesulfonate replacement by ethyl mercaptide ion and suggests the application of this relatively unexplored procedure for the synthesis of 2′- and 3′-deoxynucleosides. 6-Amino-9-(3′,5′-di-*S*-ethyl-2′,3′,5′-trideoxy-β-D-*threo*-pentofuranosyl)purine (XV) was characterized

by elemental analysis and the presence of two *S*-ethyl groups was verified by pmr spectra. The *threo* configuration, however, remains tentatively assigned assuming direct nucleophilic displacement. The desired 2′,3′,5′-trideoxyadenosine (XVI) was obtained from XV *via* Raney nickel dethiation. The pmr spectrum of XVI is shown in Figure 3 and confirms the assigned structure.

Experimental Section

5′-O-Triphenylmethyl-3′-O-p-toluenesulfonyl-2′-deoxyadenosine (II). 5′-*O*-Triphenylmethyl-2′-deoxyadenosine (Anderson *et al.*, 1954) (20 g, 0.041 mole) was dissolved in 100 ml of AR pyridine (dried over $CaSO_4$) and *p*-toluenesulfonyl chloride (11.5 g, 0.061 mole) was added. The resulting yellow solution was allowed to stand at room temperature for 1 week. The brown solution was poured into a mixture of sodium bicarbonate (5.2 g, 0.062 mole) and ice (500 g) and the ensuing emulsion was extracted with four 150-ml portions of chloroform. The combined phase was washed with water and dried over sodium sulfate. The drying agent was removed by filtration, and solvent was removed *in vacuo* (oil pump) to give a solid tan foam. This solid was dissolved in 50 ml of chloroform and applied to a column of neutral alumina (908 g). The column was washed with chloroform (1 l.) and ethyl acetate (1 l.) and these washes were discarded. Elution with ethyl acetate–ethanol (1:1) (1.5 l.) and removal of the solvent *in vacuo* provided 5′-*O*-triphenylmethyl-3′-*O*-*p*-toluenesulfonyl-2′-deoxyadenosine (II) (17.3 g, 65%) as a colorless solid foam of 95–100% purity as judged by ultraviolet spectroscopy. In subsequent runs, the product was eluted by ethyl acetate or chloroform–ethyl acetate mixtures presumably depending on the extent of solvent removal before chromatography. A small sample was dissolved in ethyl acetate, passed through alumina, and treated with charcoal. Solvent removal gave the analytically pure solid foam. Spectral data showed: λ_{max}^{MeOH} 259 mμ (ε 15,500), 226 mμ (ε 24,400); infrared bands at 705 (OTr) and 1170 cm^{-1} (OTs).

Anal. Calcd for $C_{36}H_{33}N_5O_5S$: C, 66.8; H, 5.14; N, 10.8. Found: C, 66.8; H, 5.43; N, 10.7.

3′-O-p-Toluenesulfonyl-2′-deoxyadenosine (III). Compound II (17 g, 0.026 mole) was dissolved in 250 ml of 80% acetic acid and placed in a preheated oil bath at 110°. The solution was heated for 12 min at 100° (inside temperature) and then cooled in ice. Triphenylcarbinol (5.5 g) was removed by filtration and solvent was removed at 25° (bath temperature) *in vacuo* (oil pump) to give a solid foam. This solid was triturated several times with ether and dried to give a chromatographically homogeneous solid which contained no adenine as judged by paper chromatography in several systems. A small sample was crystallized from ether–ethanol to give pure III, mp 184–184.5°. Spectral data showed: λ_{max}^{MeOH} 259 and 228 mμ (ε 15,900 and 13,500); strong infrared band at 1170 cm^{-1} (OTs), no absorption at 705 cm^{-1} (OTr).

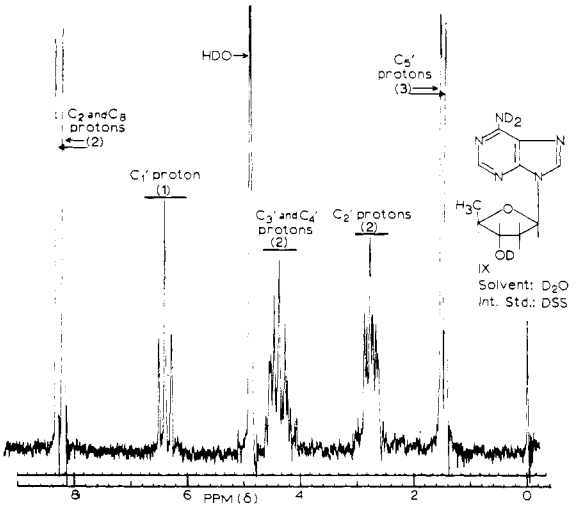


FIGURE 2: The pmr spectrum of 6-amino-9-(2′,5′-dideoxy-β-D-*erythro*-pentofuranosyl)purine (2′,5′-dideoxyadenosine, IX).

Anal. Calcd for $C_{17}H_{19}N_5O_5S$: C, 50.4; H, 4.70; N, 17.3. Found: C, 50.6; H, 4.59; N, 17.3.

6-Amino-9-(3′-S-ethyl-3′-thio-2′,3′-dideoxy-β-D-threo-pentofuranosyl)purine (IV). Compound III (6.5 g, 0.016 mole) was dissolved in an ethanethiol (14.4 g, 0.24 mole) containing ethanolic sodium ethoxide (3.7 g of sodium, 0.16 g-atom) solution (185 ml). The yellow solution was placed in an oil bath at 80° and heated at reflux (approximately 65° inside temperature) for 5 hr. The solution was cooled at 2° for 15 hr and the sodium *p*-toluenesulfonate (1.7 g) which separated, was collected by filtration. The filtrate was carefully neutralized to pH 7 with 0.5 N HCl at 5° and the neutral solution was evaporated to dryness *in vacuo*. The solid material remaining was extracted with six 15-ml portions of boiling absolute ethanol. The combined ethanol extracts were treated with charcoal and concentrated to about 20 ml. The solution was cooled at 0° for 3 days and 1.35 g (28.6%) of colorless needles crystallized. Recrystallization of this product from water gave IV (1.18 g, 25%), mp 210–212°. Spectral data showed: λ_{max}^{MeOH} 259 mμ (ε 15,600), pmr triplet centered at δ 1.20 ($-SCH_2CH_3$).

Anal. Calcd for $C_{12}H_{17}N_5O_2S$: C, 48.8; H, 5.76; N, 23.7. Found: C, 48.7; H, 5.72; N, 23.6.

6-Amino-9-(2′,3′-dideoxy-β-D-glycero-pentofuranosyl)purine (2′,3′-Dideoxyadenosine) (V). Compound IV (1.5 g, 0.0051 mole) was dissolved in 75 ml of Methyl Cellosolve, and enough ethanol was added to give a 100° internal reflux temperature. The solution was heated at reflux for 6 hr with 30 g of Raney nickel. The catalyst was removed by filtration and was washed with 200 ml of boiling ethanol. The combined filtrate was evaporated to dryness *in vacuo* (oil pump) at 30° (bath temperature). Crystallization of the colorless solid from a minimum volume of absolute ethanol gave 0.25 g (21%) of 2′,3′-dideoxyadenosine (V), mp 184–186°,

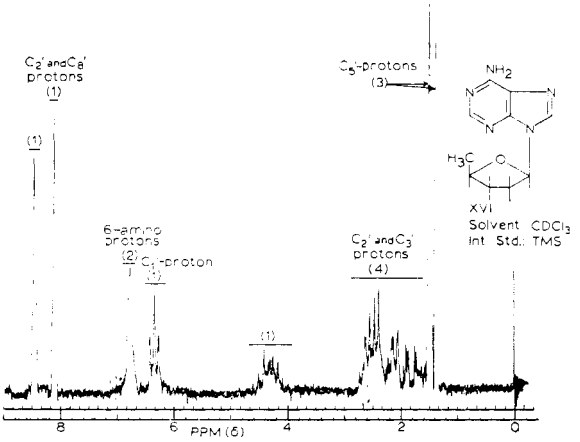


FIGURE 3: The pmr spectrum of 6-amino-9-(2′,3′,5′-trideoxy-β-D-*glycero*-pentofuranosyl)purine (2′,3′,5′-trideoxyadenosine, XVI).

TABLE I: Paper Chromatographic Data.[a]

Compound	Solvent System[b] A	B	C	D	E
2′,3′,5′-Trideoxyadenosine (XVI)	2.4	2.1	1.3	1.3	
2′,3′-Dideoxyadenosine (V)	1.9	1.2	1.1	1.2	
3′-*O*-Acetyl-2′-deoxyadenosine (XI)	2.4	1.4	1.2	1.2	
5′-*O*-*p*-Toluenesulfonyl-2′-deoxyadenosine (VII)	2.4		1.2	1.3	1.2
5′-*S*-Ethyl-5′-thio-2′,5′-dideoxyadenosine (VIII)	2.3		1.2	1.3	1.2
2′,5′-Dideoxyadenosine (IX)	1.9		1.1	1.3	1.1

[a] All data are $R_{Adenine}$ values run descending on Whatman No. 1 paper. [b] Solvent systems are: A, DMF–*i*-PrOH–concentrated aqueous NH_3 (25:65:10); B, *n*-BuOH–H_2O (86:14); C, *t*-BuOH–HOAc–H_2O (5:4:1); D, MeOH–H_2O (7:3); E, *i*-PrOH–concentrated aqueous NH_3–H_2O (14:1:5); all proportions v:v. R_F of adenine: A, 0.25; B, 0.29; C, 0.60; D, 0.53; E, 0.58.

$[\alpha]^{25}D$ − 25.2° (*c* 1.01, H_2O). Spectral data showed: λ_{max}^{MeOH} 259.5 mμ (ε 14,400); pmr (Figure 1) multiplet at δ 2.0 to 2.8 corresponding to four protons (2′ and 3′ protons), multiplet at 3.75 to 3.95 (5′ protons), multiplet at 4.22 to 4.58 (4′ proton), and quartet centered at 6.23 (1′ proton). See Table I for paper chromatographic data.

Anal. Calcd for $C_{10}H_{13}N_5O_2$: C, 51.1; H, 5.54; N, 29.8. Found: C, 50.9; H, 5.32; N, 29.6.

5′-O-Triphenylmethyl-3′-O-acetyl-2′-deoxyadenosine (*X*). 5′-*O*-Triphenylmethyl-2′-deoxyadenosine (I) (Anderson *et al.*, 1954) (9 g, 0.018 mole) was dissolved in 45 ml of hot AR pyridine (dried over $CaSO_4$) and cooled to 5°. Acetic anhydride (2.04 g, 0.02 mole) was added and the solution was allowed to stand at 15° for 15 hr. The yellow solution was poured into 250 ml of ice–water with vigorous stirring and the resulting emulsion was extracted with three 100-ml portions of ethyl acetate. The combined organic phase was washed with cold aqueous sodium bicarbonate and ice water to pH 7 and was dried over sodium sulfate. The drying agent was removed by filtration and the solvent was removed *in vacuo* (oil pump) at 60° (bath temperature) leaving a solid foam. This foam was dissolved in 20 ml of chloroform and applied to a column of neutral alumina (450 g). The product was eluted with chloroform and the solvent removed *in vacuo* to give X (9.3 g, 95%) as a colorless solid foam. Spectral data showed: λ_{max}^{MeOH} 259.5 mμ (ε 15,800), infrared band at 1750 cm^{-1} (OAc), pmr peak at δ 6.65 corresponding to two protons (NH_2).

Anal. Calcd for $C_{31}H_{29}N_5O_4$: C, 69.5; H, 5.42; N, 13.1. Found: C, 69.5; H, 5.52; N, 12.9.

3′-O-Acetyl-2′-deoxyadenosine (*XI*) (*Anderson et al., 1954; Hogenkamp and Oikawa, 1964*). Compound X (9 g, 0.017 mole) was dissolved in 80% acetic acid (100 ml) and the solution was heated at 95–96° (internal temperature) for 10 min. The acidic solution was cooled at 0° for several hours and the triphenylcarbinol (3.8 g) was collected by filtration. The solvent was removed *in vacuo* (oil pump) at 30° (bath temperature), and the resulting residue was triturated several times with boiling ether. The dried solid was powdered and placed in a Soxhlet extractor. Continuous extraction (10 days) with chloroform followed by solvent removal gave 3.26 g (66%) of crude product. Crystallization of this product from ethanol gave 2.16 g (48.8%) of XI, mp 220–223°. Spectral data showed: λ_{max}^{MeOH} 259 mμ (ε 16,500), strong infrared band at 1750 cm^{-1} (OAc).

Anal. Calcd for $C_{12}H_{15}N_5O_4$: C, 49.2; H, 5.12; N, 23.9. Found: C, 49.5; H, 5.34; N, 23.8.

5′-O-p-Toluenesulfonyl-2′-deoxyadenosine (*VII*). 2′-Deoxyadenosine (2.0 g, 0.012 mole) (previously dried at 100° (5 mm) for 12 hr) was dissolved in 75 ml of hot AR pyridine (dried over $CaSO_4$) and the solution was cooled to 0°. A solution of *p*-toluenesulfonyl chloride (2.3 g, 0.012 mole) in 30 ml of chloroform–pyridine (1:1) (both AR solvents dried over anhydrous calcium sulfate) was added dropwise with stirring at 0° over a period of 1 hr. The yellow solution was allowed to stir an additional 2 hr at 0° and then warmed slowly to room temperature (12 hr). The solution was cooled to 0° and treated with ice (300 g) and sodium bicarbonate (1.1 g, 0.013 mole). The chloroform layer was separated and the aqueous phase was extracted with three 20-ml portions of cold chloroform. The combined organic phase was washed with three 20-ml portions of ice–water and dried over sodium sulfate. The chloroform was re-

moved *in vacuo* (oil pump) at 20° (bath temperature). The residue was dissolved in 25 ml of ethanol without heating and the solvent was again similarly removed leaving a solid foam which was dissolved in 15 ml of ethyl acetate–ethanol (4:1) and applied to a column of neutral alumina (3 × 37 cm, 165 g of alumina). The column was washed with 450 ml of ethyl acetate–ethanol (4:1) and the wash was discarded. Elution with 600 ml of ethyl acetate–ethanol (1:1) followed by solvent removal *in vacuo* (oil pump) at 20° (bath temperature) gave a white foam. This solid was thoroughly triturated at room temperature with 60 ml of *n*-hexane and collected by filtration to give 2.06 g (42.6%) of VII. Spectral data showed: λ_{max}^{MeOH} 260 mμ (ε 14,400), strong infrared band at 1170 cm^{-1} (OTs).

Anal. Calcd for $C_{17}H_{19}N_5O_5S$: C, 50.4; H, 4.70; N, 17.3. Found: C, 50.2; H, 4.90; N, 17.3.

5′-S-Ethyl-5′-thio-2′,5′-dideoxyadenosine (VIII). METHOD A. 5′-*O*-*p*-Toluenesulfonyl-3′-*O*-acetyl-2′-deoxyadenosine (Anderson *et al.*, 1954) (2.77 g, 0.0062 mole) was dissolved in 10 ml of absolute methanol at room temperature and cooled to 0°. A cold solution of ethanethiol (1.92 g, 0.031 mole) in 20 ml of methanolic sodium methoxide (0.43 g of sodium, 0.018 g-atom) was added and the mixture was allowed to stir 24 hr, slowly warming to room temperature. The solution was then cooled to 0° and carefully neutralized to pH 7 with cold 1 N hydrochloric acid. The solution was evaporated to dryness *in vacuo* and the residue was extracted with three 30-ml portions of acetone. The combined extract was evaporated *in vacuo* to give a solid which was extracted with three 15-ml portions of boiling chloroform. The chloroform extract was concentrated to 15 ml, and 10 ml of *n*-pentane was added. An oil separated which crystallized upon standing at room temperature. The product (0.74 g, 41%) was collected by filtration and was recrystallized from water to give 0.22 g of 5′-*S*-ethyl-5′-thio-2′,5′-dideoxyadenosine monohydrate as fine colorless needles, mp 78–79°. Spectral data showed: λ_{max}^{MeOH} 259.5 mμ (ε 13,500), p.m.r. triplet centered at δ 1.20 ($-SCH_2CH_3$).

Anal. Calcd for $C_{12}H_{17}N_5O_2S \cdot H_2O$: C, 46.0; H, 6.1; N, 22.4. Found: C, 45.8; H, 6.3; N, 22.6.

To obtain the anhydrous product, 100 mg of the monohydrate was dissolved in 10 ml of boiling chloroform, and 15 ml of boiling *n*-pentane was added. The cooled solution deposited crystals which were again recrystallized from chloroform and *n*-pentane to give a product, mp 107–108°.

Anal. Calcd for $C_{12}H_{17}N_5O_2S$: C, 48.8; H, 5.7; N, 23.7. Found: C, 48.7; H, 5.8; N, 23.5.

METHOD B. 5′-*O*-*p*-Toluenesulfonyl-2′-deoxyadenosine (VII, 5.0 g, 0.012 mole) was dissolved in 15 ml of absolute methanol without heating. The solution was treated with ethanethiol (3.84 g, 0.062 mole) in 40 ml of methanolic sodium methoxide (0.85 g of sodium, 0.037 g-atom) as in method A. The solid foam resulting from evaporation of the chloroform extracts was dissolved directly in 60 ml of hot water (95°). The solution was treated with charcoal, cooled to 5°, seeded, and allowed to stand 15 hr at 5°. Crystalline 5′-*S*-ethyl-5′-thio-2′,5′-dideoxyadenosine monohydrate (2.34 g, 60%) which separated and collected by filtration. The product, mp 77–79°, was identical in every respect with the product obtained by method A as judged on the basis of infrared, ultraviolet, pmr spectroscopy, and paper chromatography in four solvent systems.

Anal. Calcd for $C_{12}H_{17}N_5O_2S \cdot H_2O$: C, 46.0; H, 6.06; N, 22.4. Found: C, 45.8; H, 5.92; N, 22.5.

6-Amino-9-(2′,5′-dideoxy-β-D-erythro-pentofuranosyl)-purine (2′,5′-Dideoxyadenosine) (IX). The monohydrate of VIII (5.80 g, 0.0185 mole) was dissolved in 50 ml of absolute ethanol and evaporated to dryness *in vacuo*. This procedure was repeated, the remaining white solid was dissolved in 250 ml of Methyl Cellosolve, and enough ethanol was added to give a 93° (inside) reflux temperature. The solution was heated at reflux with 87 g of Raney nickel (W-7) for 2 hr, an additional 58 g of Raney nickel was added, and the solution refluxed for another 3 hr. The catalyst was then removed by filtration and washed with 1 l. of boiling 15% aqueous ammonia. The filtrate was evaporated to dryness *in vacuo* at 30° (bath temperature). The green solid obtained was extracted with four 100-ml portions of boiling methanol. Evaporation of the combined methanol extracts gave a light yellow solid, which was extracted with four 100-ml portions of boiling ethyl acetate. The combined ethyl acetate extracts were concentrated to 200 ml and cooled to room temperature. A white crystalline solid (1.48 g) was removed by filtration. An additional 0.57 g of white crystals were obtained from the filtrate to give a total of 2.05 g (47%) of 6-amino-9-(2′,5′-dideoxy-β-D-*erythro*-pentofuranosyl)purine. Recrystallization from ethyl acetate gave 1.91 g (43.8%) of crystals, mp 186–188°. Spectral data showed: λ_{max}^{MeOH} 259.5 μ (ε 14,400), $[\alpha]^{22}D$ −38° (*c* 0.66, H_2O).

Anal. Calcd for $C_{10}H_{13}N_5O_2$: C, 51.1; H, 5.54; N, 29.8. Found: C, 51.2; H, 5.46; N, 29.6.

3′,5′-Di-O-p-Toluenesulfonyl-2′-deoxyadenosine (XIII). 2′-Deoxyadenosine (100 g, 0.4 mole) [previously dried for 12 hr at 100° (5 mm)] was dissolved in 1 l. of hot AR pyridine (dried over anhydrous $CaSO_4$) and the solution was cooled to 5°. *p*-Toluenesulfonyl chloride (232 g, 1.2 moles) was added, and the yellow solution was allowed to stand for 36 hr at 20°. The solution was cooled to 0° and neutralized with ice-cold aqueous sodium bicarbonate (135 g, 1.6 moles). The resulting solution was extracted with five 300-ml portions of cold chloroform, and the combined organic phase was washed with ice water and dried over sodium sulfate. The drying agent was removed by filtration and the solvent was removed *in vacuo* (oil pump) at 20° (bath temperature) leaving a solid tan foam. This was dissolved in 1.5 l. of chloroform and dripped into 12 l. of petroleum ether (bp 60–110°). Compound XIII (208 g, 93%) was collected by filtration. A small amount of this powder was reprecipitated as above to give an analytical sample. The ultraviolet absorption maximum of this amorphous product was 259 mμ in methanol.

Anal. Calcd for $C_{24}H_{25}N_5O_7S_2$: C, 51.5; H, 4.47; N, 12.5. Found: C, 51.3; H, 4.96; N, 12.5.

5'-S-Ethyl-3'-O-p-toluenesulfonyl-5'-thio-2',5'-dideoxyadenosine (XIV). Compound XIII (11.2 g, 0.02 mole) was added to a solution of ethanethiol (18.6 g, 0.3 mole) in 200 ml of ethanolic sodium ethoxide (4.6 g of sodium, 0.2 g-atom) at 0°. The mixture was allowed to stir for 15 hr during which time it slowly warmed to room temperature. The chalky suspension was concentrated *in vacuo* to 50 ml and then was poured into 1 l. of ice–water with vigorous stirring. The crude product (7 g, 78%) was collected by filtration. Recrystallization of the product from acetone or ethanol–water gave XIV (4.5 g, 50%), mp 183–184°. Spectral data showed: λ_{max}^{MeOH} 258 and 226 mμ (ε 16,000 and 16,400); strong infrared band at 1170 cm^{-1} (OTs); pmr, A_2X_2 system at 7.9 to 8.44 (OTs), triplet centered at 1.41 ($-SCH_2CH_3$) in dimethyl-d_6 sulfoxide (DMSO-d_6) with tetramethylsilane (TMS) as external reference.

Anal. Calcd for $C_{19}H_{23}N_5O_4S_2$: C, 50.8; H, 5.13; N, 15.6. Found: C, 50.9; H, 5.25; N, 15.4.

6-Amino-9-(3',5'-di-S-ethyl-3',5'-dithio-2',3',5'-trideoxy-β-D-threopentofuranosyl)purine (XV). The ethanolic sodium ethoxide solution of ethanethiol and XIV obtained as above was heated at reflux (internal temperature approximately 65°) for 5 hr in an 80° oil bath. Solution was effected after about 30 min and sodium *p*-toluenesulfonate began crystallizing from the boiling solution after approximately 2 hr. The yellow suspension was concentrated to 30 ml and poured slowly into 1 l. of ice water with vigorous stirring. The crude product (4 g, 59%) was recrystallized from acetone to yield 2 g (30%) of pure 6-amino-9-(3',5'-di-*S*-ethyl-3',5'-dithio-2',3',5'-trideoxy-β-D-*threo*-pentofuranosyl)purine (XV) as glistening needles, mp 182–183°. Spectral data showed: λ_{max}^{MeOH} 259.5 mμ (ε 15,700); pmr, multiplet at δ 0.7–0.9 corresponding to six protons (two $-SCH_2CH_3$) in DMSO-d_6 with TMS as external reference, A_2X_2 system at 7.9 to 8.5 (OTs) was absent. No infrared band was present at 1170 cm^{-1} (OTs).

Anal. Calcd for $C_{14}H_{21}N_5OS_2$: C, 49.6; H, 6.20; N, 20.6. Found: C, 49.8; H, 6.13; N, 20.5.

6-Amino-9-(2',3',5'-trideoxy-β-D-glycero-pentofuranosyl)purine (2',3',5'-Trideoxyadenosine) (XVI). Compound XV (5 g, 0.015 mole) was treated for 6 hr with 100 g of Raney nickel in 150 ml of Methyl Cellosolve with enough ethanol added to give a 100° (internal) reflux temperature. The catalyst was removed by filtration and washed with 300 ml of boiling ethanol. Evaporation of the combined filtrate gave a solid which was extracted with three 25-ml portions of boiling benzene. The combined benzene extract was concentrated to 20 ml and cooled at 10° for 2 days. Compound XVI (0.37 g, 11.5%) crystallized and was collected by filtration. Recrystallization of the product from 4 ml of acetone gave 0.32 g (9.9%) of crystals, mp 159–160°, $[\alpha]^{25.5}D$ −36.2° (*c* 1.11, MeOH). Spectral data showed: λ_{max}^{MeOH} 259.5 mμ (ε 15,400); pmr (Figure 3, a doublet centered at δ 1.42 (5' protons), multiplet at 1.67 to 2.73 corresponding to four protons (2' and 3' protons), multiplet at 4.02 (4' proton), and a triplet centered at 6.31 (1' proton).

Anal. Calcd for $C_{10}H_{13}N_5O$: C, 54.8; H, 5.95; N, 32.0. Found: C, 55.0; H, 5.92; N, 32.0.

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Influenza Virus-Induced Ribonucleic Acid Nucleotidyltransferase and the Effect of Actinomycin D on Its Formation*

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ABSTRACT: A ribonucleic acid (RNA) nucleotidyltransferase has been isolated from tissue infected with influenza virus PR-8. It shows a requirement for all four ribonucleoside triphosphates and Mg^{2+}. Guanidine and actinomycin D have no effect on the activity of this RNA nucleotidyltransferase preparation *in vitro*. However, actinomycin D prevents the appearance of viral RNA nucleotidyltransferase activity in tissues infected with influenza virus. The mode of action of actinomycin D is discussed.

Infection with RNA viruses causes the appearance of a new cytoplasmic RNA nucleotidyltransferase (nucleotide triphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) (Baltimore and Franklin, 1963; Baltimore *et al.*, 1963; Haruna *et al.*, 1963; Weissman *et al.*, 1963; August *et al.*, 1965a) and the conversion of the parental RNA into a double-stranded form which serves as template for the synthesis of progeny RNA (Ochoa *et al.*, 1964; Baltimore *et al.*, 1964; Burdon *et al.*, 1964; Fenwick *et al.*, 1964; Kaerner and Hoffmann, 1964; Kelly and Sinsheimer, 1964; Shipp and Haselkorn, 1964; Montagnier and Sanders, 1963; August *et al.*, 1965b). These processes are summarized by reactions 1 and 2, which might be catalyzed by viral RNA nucleotidyltransferase.

$$\text{viral RNA} \longrightarrow \text{RNA double-stranded} \quad (1)$$

$$\text{4 nucleoside triphosphate} \xrightarrow{\text{RNA double-stranded}} \text{4 pyrophosphate} + \text{viral RNA} \quad (2)$$

This report describes the isolation of virus-induced RNA nucleotidyltransferase from chorio-allantoic membrane (CAM)[1] infected with influenza virus, PR-8 strain, and the results obtained in the study of the mechanism of action of actinomycin D on influenza virus replication.

Influenza is one of the few RNA viruses inhibited by actinomycin D (Barry *et al.*, 1962). This antibiotic inhibits the formation of RNA by DNA-dependent RNA nucleotidyltransferase (Goodman and Rich, 1962; Reich *et al.*, 1962; Yankofsky and Spiegelman, 1963; Reich, 1964). Actinomycin-sensitive RNA synthesis has thus been suggested to be involved in the multiplication of influenza. However, it has been shown that actinomycin D inhibits only in the early part of the replication cycle (White *et al.*, 1965). It is suggested that influenza viral RNA replication is catalyzed by a viral RNA nucleotidyltransferase which is similar to that described by Baltimore *et al.* (1963), but is not synthesized in the presence of actinomycin D. We have found an RNA nucleotidyltransferase system in the cytoplasm of CAM infected with influenza and have carried out a study of the effects of actinomycin D on the appearance and activity of this enzyme system.

Experimental Procedures

Virus. The PR-8 strain of influenza A virus, adapted to egg, was used throughout these studies. The virus was prepared by inoculating 10-day-old chick embryos by the allantoic route with 0.2 ml of infected allantoic fluid diluted in broth to 10^{-5}. The infected eggs were incubated for 48 hr at 35°, and the allantoic fluid was collected. This virus solution was used immediately.

Hemagglutination Test. Serial twofold dilutions of the virus preparation were made in saline solution. To 0.5 ml of each dilution was added 0.5 ml of a 0.5% suspension of washed chicken red blood cells. The test mix-

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[1] Abbreviations used in this work: CAM, chorio-allantoic membrane; sucrose–Mg, 0.25 M sucrose containing 0.001 M $MgCl_2$; TCA, trichloroacetic acid; AMP, adenosine monophosphate; GMP and GTP, guanosine mono- and triphosphate.