boiling, then cooled immediately and filtered. The filtrate was acidified to *p*H 4–5 with 6 *N* hydrochloric acid and the resulting pale cream-colored precipitate was collected, washed with water, and dried *in vacuo* over phosphorus pentoxide at room temperature; λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 247–248 (15.0), 285 (17.5); *p*H 7, 267 (16.2); *p*H 13, 237–238 (18.4), 283–284 (13.0).

Anal. Calcd. for $C_9H_{13}N_5S \cdot H_2O$: C, 44.80; H, 6.27. Found: C, 44.84; H, 6.28.

2-Amino-6-(benzylthio)-purine.—A solution of 500 mg. (3.00 mmoles) of 2-amino-6-purinethiol[9] in 2.8 ml. of 1.07 *N* aqueous sodium hydroxide diluted to 30 ml. with water was treated, while stirred vigorously, with 0.35 ml. (3.0 mmoles) of α-chlorotoluene. A white solid precipitated within 5 minutes after the addition was completed, and stirring was continued for 2 hours to ensure complete reaction. The mixture then was chilled and the solid collected by filtration, washed with water and ethyl alcohol, and dried *in vacuo* over phosphorus pentoxide at 110°; yield 600 mg. (78%), m.p. 208°. The analytical and spectral data are recorded in Tables I and II, respectively.

In a later run in which dimethylformamide and potassium carbonate were used—according to a procedure similar to that described for Ib except that the mixture was heated between 50 and 60° for 30 minutes—a 94% yield of 2-amino-6-(benzylthio)-purine, m.p. 210°, was obtained; λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 277 (9.35), 320 (13.9); *p*H 7, 243 (11.4), 313 (11.5); *p*H 13, 316 (10.9).

BIRMINGHAM, ALA.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents.[1] XIX. Synthesis of 2′-Deoxyadenosine[2]

BY CHARLES D. ANDERSON, LEON GOODMAN AND B. R. BAKER

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The first synthesis of a naturally occurring purine 2′-deoxynucleoside has been achieved. The key reaction is an ethylthio migration from C.3′ to C.2′ based on the selectivity of nucleophilic attack on a nucleoside 2′,3′-episulfonium ion. This method should be adaptable to the synthesis of the 2′-deoxyribofuranosyl derivatives of both natural and unnatural bases and to the preparation of analogs of natural deoxynucleosides modified at C.3′. It therefore provides a route to two classes of potential deoxynucleoside antagonists that might exhibit anticancer activity.

The syntheses of the four nucleosides obtained on hydrolysis of ribonucleic acids, accomplished by Todd and his co-workers, were published in 1947 and 1948.[3] However, the first synthesis of a 2′-deoxyribofuranosylnucleoside, 2′-deoxyuridine, was announced only slightly more than a year ago.[4] Shortly afterward the synthesis of the first component of deoxynucleic acids, the pyrimidine 2′-deoxyribonucleoside thymidine, was reported by Shaw and Warrener.[5,6] In addition, a number of enzymatic syntheses of unnatural 2′-deoxynucleosides have appeared over the last few years.[7]

It was pointed out when this work was undertaken[8] that the then known chemical methods were inadequate for the preparation of 2′-deoxynucleosides. Davoll, Lythgoe and Trippett,[9] for instance, attempted to obtain 2′-deoxynucleosides from two 2′,3′-anhydrofuranosyl nucleosides by reaction with sodium ethyl mercaptide and subsequent desulfurization. Unfortunately, their method failed because the mercaptide ion attacked the anhydronucleosides almost exclusively at C.3′. This phenomenon of very predominant attack on 2,3-anhydro ribo- or lyxofuranosides at C.3 has been observed in all the additional cases that have been studied.[10] On the assumption that a 2,3-episulfonium ion, in its reactions with nucleophiles, would show a selectivity similar to that which is exhibited by 2,3-anhydrofuranosides, the suggestion was made that it should be possible to isomerize a 3′-alkylthio nucleoside having a *trans*-2′-hydroxyl group to a 2′-alkylthio nucleoside *via* the related 2′,3′-episulfonium ion.[8] That such an isomerization is indeed possible was first demonstrated in the methyl furanoside series.[11] Use of such an alkylthio migration has now made possible the first chemical synthesis of a naturally occurring purine 2′-deoxynucleoside, namely, 2′-deoxyadenosine (XI). The procedure employed is the first completely general method for the chemical synthesis of 2′-deoxynucleosides since the earlier deoxynucleoside syntheses[4,5] were dependent on activation of a C.2′ leaving group by a neighboring 2-oxo- or 2-thiopyrimidine moiety.

The starting material for the synthesis of 2′-deoxyadenosine (XI) was 1,2-di-*O*-acetyl-5-*O*-methoxycarbonyl-3-*O*-(*p*-tolylsulfonyl)-D-xylofuranose (I), an intermediate employed earlier in a synthesis of the methyl 2,3-anhydro-D-ribofuranosides[12]; the over-all yield of XI from I was 0.5%. The diacetate I was treated with ethereal hydrogen chlo-

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center of the National Cancer Institute, Contract No. SA-43-ph-1892. For the preceding paper of this series, *cf.* M. H. Gram, C. W. Mosher and B. R. Baker, THIS JOURNAL, **81**, 3103 (1959).

(2) For a preliminary announcement of this synthesis, see C. D. Anderson, L. Goodman and B. R. Baker, THIS JOURNAL, **80**, 6453 (1958).

(3) For reviews of this work, see G. W. Kenner, *Fortschr. Chem. org. Naturstoffe*, **8**, 96 (1951), and J. Baddiley in "Nucleic Acids," Vol. I, ed. by E. Chargaff and J. M. Davidson, Academic Press, Inc., New York, N. Y., 1955, p. 137.

(4) (a) D. M. Brown, D. B. Parihar, C. B. Reese and A. Todd, *Proc. Chem. Soc.*, 321 (1957); (b) *J. Chem. Soc.*, 3035 (1958); (c) D. M. Brown, D. B. Parihar and A. Todd, *ibid.*, 4242 (1958).

(5) G. Shaw and R. N. Warrener, *Proc. Chem. Soc.*, 81 (1958).

(6) An alternative synthesis of thymidine was reported by Brown, *et al.*, in the same paper in which they detailed the synthesis of 2′-deoxyuridine (see ref. 4b).

(7) *E.g.*, M. Friedkin, *Biochim. et Biophys. Acta*, **18**, 447 (1955); W. H. Prusoff, *J. Biol. Chem.*, **215**, 809 (1955); R. H. Hall and R. Hazelkorn, THIS JOURNAL, **80**, 1138 (1958); C. Heidelberger, L. Griesbach, O. Cruz, R. J. Schnitzer and E. Grunberg, *Proc. Soc. Exp. Biol. Med.*, **97**, 470 (1958).

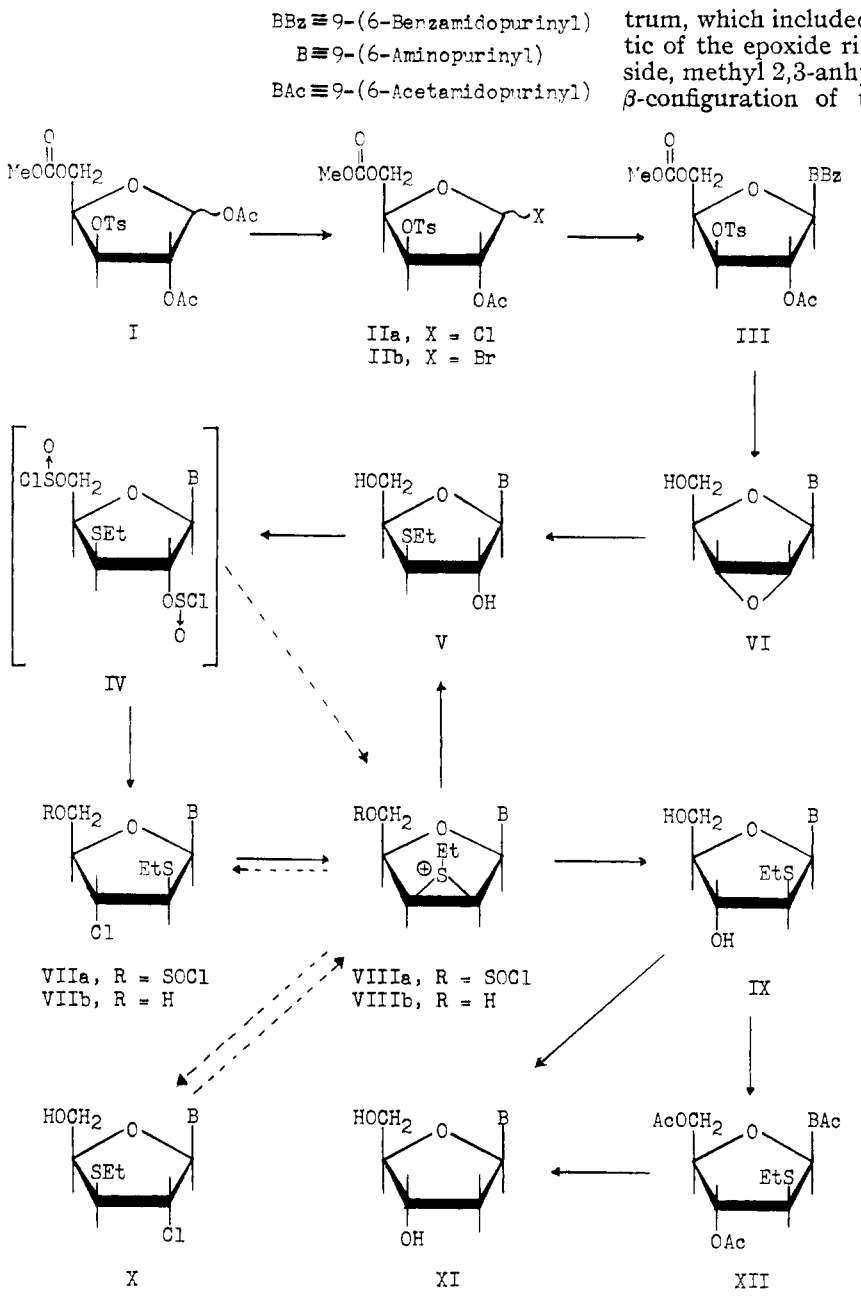
(8) L. Goodman, A. Benitez and B. R. Baker, Paper I of this series, THIS JOURNAL, **80**, 1680 (1958).

(9) J. Davoll, B. Lythgoe and S. Trippett, *J. Chem. Soc.*, 2230 (1951).

(10) For further discussion of this point, see ref. 11 and R. E. Schaub and M. J. Weiss, THIS JOURNAL, **80**, 4683 (1958).

(11) C. D. Anderson, L. Goodman and B. R. Baker, Paper XVI of this series, *ibid.*, **81**, 898 (1959).

(12) C. D. Anderson, L. Goodman and B. R. Baker, Paper VII of this series, *ibid.*, **80**, 5247 (1958).



ride to give 2-*O*-acetyl-5-*O*-methoxycarbonyl-3-*O*-(*p*-tolylsulfonyl)-D-xylofuranosyl chloride (IIa), an amorphous substance which was condensed directly with chloromercuri-6-benzamidopurine. The crude product, presumably containing the blocked nucleoside 9-[2′-*O*-acetyl-5′-*O*-methoxycarbonyl-3′-*O*-(*p*-tolylsulfonyl)-β-D-xylofuranosyl]-6-benzamidopurine (III), was treated with cold methanolic sodium methoxide, the three acyl functions being removed and an epoxide linkage introduced to give 6-amino-9-(2′,3′-anhydro-β-D-ribofuranosyl)-purine (VI) in crude form. After purification through the picrate, a low yield (8.9% over-all from I) of pure VI was obtained. That the crystalline nucleoside obtained was indeed VI was indicated by its elemental analysis; its ultraviolet spectrum, which was that of a 9-substituted adenine; and its infrared spectrum, which included a band at 11.67 μ characteristic of the epoxide ring of the corresponding glycoside, methyl 2,3-anhydro-β-D-ribofuranoside.[12] The β-configuration of this anhydronucleoside, while likely on the basis of mechanistic considerations,[13] was originally in some doubt because of its comparatively dextrorotatory optical rotation. The assignment was ultimately proved by the conversion of the anhydronucleoside VI to 2′-deoxyadenosine (XI), whose β-configuration has been demonstrated by its transformation to a 3,5′-cyclonucleoside.[14] The yield of anhydronucleoside VI obtained *via* the bromo sugar IIb was considerably lower in the one experiment performed than it was *via* the chloro sugar IIa.

The 9% yield of VI obtained is lower than the usual yield (20–50%) obtained in coupling reactions and could be partially attributed to possible side reactions involving the *trans*-2′-*O*-acetyl-3′-*O*-tosyl system. Nevertheless, this first example of nucleoside formation from a blocked sugar bearing a tosylate group has the advantage of having considerably fewer steps than alternative syntheses that were considered[15] and probably would not differ greatly in over-all yield from that of longer syntheses.

Reaction of the pure anhydronucleoside VI with sodium ethyl mercaptide in refluxing methanol afforded a 66% yield of a crystalline ethylthio nucleoside.[16] This product was presumed to be 6-amino-9-[3′-deoxy-3′-(ethylthio)-β-D-xylofuranosyl]-purine (V) by analogy with the known reactions of 2,3-anhydrofuranosides and, in particular, the reaction of methyl 2,3-anhydro-β-D-ribofuranoside with sodium ethyl mercaptide.[11] The optical rotation of this 3′-ethylthio nucleoside V was

(13) B. R. Baker, J. P. Joseph, R. E. Schaub and J. H. Williams, *J. Org. Chem.*, **19**, 1786 (1954); B. R. Baker, Ciba Foundation Symposium on "The Chemistry and Biology of Purines," J. and A. Churchill Ltd., London, 1957, pp. 120–130.

(14) W. Andersen, D. H. Hayes, A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, 1882 (1954).

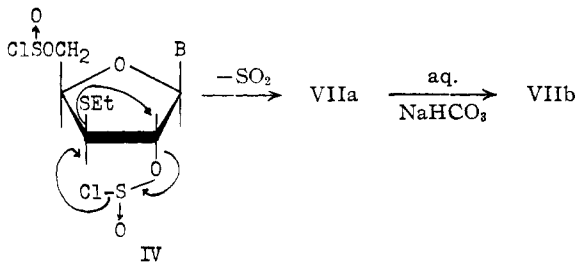
(15) One of these, which employs 9-β-D-xylofuranosyladenine (Baker and Hewson, ref. 36) as the starting material, is now being investigated; *cf.* R. E. Schaub, M. J. Weiss and B. R. Baker, THIS JOURNAL, **80**, 4692 (1958).

(16) In this experiment—the only one conducted using pure anhydronucleoside VI—the reaction mixture was acidified with Amberlite IRC-50(H). This resin presumably retained some of the ethylthio nucleoside, thus explaining, at least in part, the lower-than-expected yield.

considerably more levorotatory than that of the anhydronucleoside VI and did much to allay early suspicions concerning the anomeric configuration of the anhydronucleoside VI. The 3′-ethylthio nucleoside V, in contrast to the anhydronucleoside VI and most of the by-products formed during its preparation, could be isolated from aqueous solution by continuous chloroform extraction. This made it possible to avoid the tedium of isolating pure anhydronucleoside VI. The total product obtained on de-blocking the coupling product III was allowed to react with sodium ethyl mercaptide, the resultant crude product was dissolved in water, and the 3′-ethylthio nucleoside present was isolated by chloroform extraction. This procedure afforded a 6.8% over-all yield of pure 3′-ethylthio nucleoside V from the diacetate I. An additional amount of V representing approximately a 1% over-all yield could be obtained from the mother liquor residue by chromatography on seed test paper.[17]

The procedure employed for the isomerization of methyl 3-deoxy-3-(ethylthio)-β-D-xylofuranoside to methyl 2-deoxy-2-(ethylthio)-β-D-arabinofuranoside[11] involved treatment with excess *p*-toluenesulfonyl chloride in pyridine. This resulted in tosylation of the 5-hydroxyl group in addition to the essential reaction with the 2-hydroxyl group. Such a procedure is not applicable to a nucleoside such as V because a tosyloxy group at C.5′ could result in the undesirable formation of a 3,5′-cyclonucleoside during the subsequent "acetolysis" step.[14,18,19] The apparent solution was to block the 5′-hydroxyl group of V with a trityl group before reaction with *p*-toluenesulfonyl chloride, but this approach was found to be impractical (see below). Fortunately an alternative procedure was found that made it unnecessary to block the 5′-hydroxyl group.

Treatment of the 3′-ethylthio nucleoside V with thionyl chloride at room temperature afforded a monochloro derivative in high yield. The crystallinity of this product and its relatively narrow melting range suggested that it was isomerically pure. The conversion of simple β-hydroxy sulfides to β-chloro sulfides by this reagent is well known,[20]



IV

and therefore it was presumed that the product was either 6-amino-9-[3′-chloro-2′,3′-dideoxy-2′-(ethylthio)-β-D-arabinofuranosyl]-purine (VIIb) or the 2′,3′-isomer X. The 2′-ethylthio formulation VIIb is assumed to be the correct alternative. A concerted intramolecular reaction (see diagram) of the bis-chlorosulfinate IV of the 3′-ethylthio nucleoside V seems best able to explain the observed result: the apparently exclusive formation of one isomer.[21] An alternative mechanism consists of the two discrete steps: (a) elimination of the 2′-chlorosulfinate group of IV to yield the 2′,3′-episulfonium ion VIIIa and (b) reaction of VIIIa with chloride ion. The major product in this case also would very probably be the 3′-chloro nucleoside VIIb (formed on work-up by hydrolysis of its chlorosulfinate VIIa, as before), but one might have expected that a detectable amount of the other possible isomer (here, X) would have been formed also, as in the known methyl furanoside[11] and nucleoside (see below) cases. While the designation of the monochloro nucleoside obtained from the 3′-ethylthio nucleoside V as the 3′-chloro-2′-ethylthio isomer VIIb probably is correct, the possibility of its being the isomer X would have no effect on the later steps in this synthesis since either isomer would undergo nucleophilic reaction *via* the common 2′,3′-episulfonium ion VIIIb.

The reaction of the chloro nucleoside VIIb with sodium acetate in refluxing 95% aqueous methyl Cellosolve was expected to afford the 3′-*O*-acetyl derivative of 6-amino-9-[2′-deoxy-2′-(ethylthio)-β-D-arabinofuranosyl]-purine (IX) together with a smaller amount of the corresponding derivative of the isomeric 3′-ethylthio nucleoside V by attack of acetate ion on the intermediate episulfonium ion VIIIb. Instead, a mixture of the non-acetylated 2′-ethylthio IX and 3′-ethylthio V isomers in an approximately 6 to 1 ratio was obtained. Results obtained in the dityrityl series discussed below suggest that at least some acetolysis does occur but that the resulting *O*-acetyl nucleosides are subsequently hydrolyzed. Similar hydrolyses of sugar acetates and benzoates in refluxing 95% aqueous methyl Cellosolve containing sodium acetate have been reported recently by Jeanloz and Jeanloz.[22,23] Resolution of the mixture of ethylthio nucleosides obtained on "acetolysis" of the chloro nucleoside VIIb was accomplished by a combination of selective extraction from water by chloroform (the 2′-ethylthio isomer IX was less readily extracted than its isomer V) and chromatography on seed test paper.[17] In this way a 58% yield of pure 2′-ethylthio nucleoside IX and a 7% yield of recovered 3′-ethylthio nucleoside V were obtained.

To find conditions suitable for the desulfurization of the 2′-ethylthio nucleoside IX, a number of small-scale exploratory experiments were run using freshly prepared nickel catalyst, a 6-hour reaction period and methyl Cellosolve

(17) H. H. Brownell, J. G. Hamilton and A. A. Casselman, *Anal. Chem.*, **29**, 550 (1957).

(18) V. M. Clark, A. R. Todd and J. Zussman, *J. Chem. Soc.*, 2952 (1951).

(19) B. R. Baker and J. P. Joseph, THIS JOURNAL, **77**, 15 (1955).

(20) See, for example, V. Ettel and A. Kohlik, *Coll. Czech. Chem. Comm.*, **3**, 585 (1931); R. C. Fuson and J. H. Koehneke, *J. Org. Chem.*, **14**, 706 (1949); D. Klamann and H. Bertsch, *Ber.*, **88**, 201 (1955); and ref. 8.

(21) Such a cyclic (SNi′) process is analogous to that proposed for the reaction of allyl alcohols with thionyl chloride (J. D. Roberts, W. G. Young and S. Winstein, THIS JOURNAL, **64**, 2157 (1942)).

(22) R. W. Jeanloz and D. A. Jeanloz, *ibid.*, **80**, 5692 (1958).

(23) The thousand-fold greater nucleophilic activity of hydroxide ion relative to acetate ion toward bis-(2-chloroethyl) sulfide in water (W. E. Doering and R. P. Linstead, National Defense Research Committee Report, O.S.R.D. No. 1095, Dec. 9, 1942, p. 10), however, suggests that the observed result also is due in part to attack of the episulfonium ion VIIIb by hydroxide ion concurrent with attack by acetate ion.

as solvent.[24] Three forms of the starting material were investigated for use in this reaction: the free nucleoside IX, its 3′,5′-*O*-diacetyl derivative and its 3′,5′-*O*,6-*N*-triacetyl derivative XII. The two acetyl derivatives were studied in the hope that their use would reduce losses due to adsorption on the Raney nickel catalyst. By use of the Raney nickel "C" of Hurd and Rudner[25] in neutral methyl Cellosolve, all these forms of IX gave yields of 2′-deoxyadenosine (XI) and recovered starting material IX that varied randomly within the ranges of 7–15% and 25–50%, respectively. Under the same conditions, the nickel catalyst of Nishimura and Urushibara[26] gave similar yields of XI but only a very low recovery of IX (*ca.* 5%). In these experiments there was no clear indication that less adsorption loss was incurred by use of either of the acetyl derivatives of IX. Other variations in conditions that had no consistent effect on the course of the reaction were: (a) changes in reaction temperature from 100° to reflux temperature, (b) passing hydrogen through the reaction mixtures and (c) changing the weight ratio of catalyst to substrate (calculated as the free nucleoside IX) from 10:1 to 20:1.[27]

The most significant variant found consisted of the addition of approximately four mole-equivalents of 10% aqueous sodium hydroxide to the desulfurization mixture. Using either the free 2′-ethylthio nucleoside IX or its triacetyl derivative XII, this addition of base roughly doubled the yield of 2′-deoxyadenosine (XI). At the same time, the yield of recovered IX was substantially reduced. Use of the triacetyl derivative XII and catalyst "C"[25] in refluxing methyl Cellosolve containing aqueous base gave the most reproducible results of any set of conditions studied: average yields of 17 ± 2% of XI and 8 ± 3% of recovered IX in five experiments. Use of the same procedure on the free nucleoside IX gave the highest average yields (21 ± 6% of XI and 13 ± 4% of recovered IX in three experiments) although these results were not borne out by a later preparative run (see below).

During these desulfurizations in the presence of four mole-equivalents of sodium hydroxide, it was observed that the crude products obtained from the free 2′-ethylthio nucleoside were contaminated by a yellow decomposition product. Under these desulfurization conditions the triacetyl derivative XII was simultaneously deacetylated, and the crude products obtained from XII contained no detectable amount of the yellow decomposition product. It was demonstrated that the absence of the unknown decomposition product in the crude products from the triacetyl derivative XII was a result of the neutralization of three-fourths of the base originally present by hydrolysis of the acetyl groups, but the base concentration that would afford the maximum yield of 2′-deoxyadenosine (XI) in the face of the competing basic degradation was not determined.

In single preparative experiments, which were performed in the presence of four mole-equivalents of base, use of the triacetyl derivative XII gave a higher yield (13% based on unrecovered IX) of isolated synthetic 2′-deoxyadenosine (XI) than did the free nucleoside IX (7% on the same basis). This is a further example of the inexplicable results sometimes obtained in this reaction. That this desulfurization product was indeed 2′-deoxyadenosine (XI) was established by a rigorous comparison with natural 2′-deoxyadenosine,[28] both samples being characterized as their monohydrates (see Tables I and II). In addition to the data presented in these tables, the infrared spectra of the two samples were found to be identical.

TABLE I

COMPARISON OF PHYSICAL PROPERTIES OF SYNTHETIC AND NATURAL 2′-DEOXYADENOSINE MONOHYDRATE

Physical property	Synthetic XI	Natural XI	Literature or calcd. values
M.p., °C.	187–189[a]	186–189[a]	181,[b] 187–188,[c] 189–190[d]
Mixed m.p., °C.	186–189[a]		
$[\alpha]_D$	$-25 \pm 2^{\circ}$[e,f]	$-26 \pm 3^{\circ}$[e,g]	-26°,[b,g,h] -26.9°[c,i]
$\lambda_{max}^{pH\ 1}$	258	258	258
mμ, ε	14,400	14,600	14,100[j]
$\lambda_{max}^{H_2O}$, mμ,	260	260	
ε	14,800	14,900	
$\lambda_{max}^{pH\ 13}$, mμ,	261	260	
ε	14,900	14,900	
C, %	44.95	44.93	44.60
H, %	5.73	5.88	5.62
N, %	26.35	26.17	26.01
R_{ad} in solvent A[k]	0.73	0.73	
R_{ad} in solvent C	1.51	1.49	
R_{ad} in solvent D	1.23	1.24	
R_{ad} in solvent E	1.09	1.12	

[a] Corrected. [b] Ref. 29. [c] Ref. 30. [d] Ref. 31. [e] Temp. 23°. [f] c = 1.3% in water. [g] c = 1.0% in water. [h] Temp. 21°. [i] Temp. 20°, solvent water. [j] Ref. 32. [k] For solvent composition see Experimental.

Before the thionyl chloride-"acetolysis" isomerization sequence described above was available, the 6-*N*,5′-*O*-ditrityl derivative XIII of the 3′-ethylthio nucleoside V was prepared because of the anticipated need of blocking the 5′-hydroxyl group. This ditrityl nucleoside XIII was not obtained ana-

(24) In two desulfurization experiments employing toluene as solvent, no detectable amount of 2′-deoxyadenosine (XI) was formed. Under the same conditions but with methyl Cellosolve as solvent, approximately 12% yields of XI were obtained. The results of the exploratory experiments were determined by paper chromatography of the crude desulfurization products (deacetylated, if necessary) in water-saturated 1-butanol, elution of the spots due to XI and starting material IX, and measurement of the yields by ultraviolet spectroscopy.

(25) C. D. Hurd and B. Rudner, THIS JOURNAL, **73**, 5157 (1951).

(26) S. Nishimura and Y. Urushibara, *Bull. Chem. Soc. Japan*, **30**, 199 (1957).

(27) These preliminary experiments were performed in groups of five to seven so as to minimize the variations that occur in Raney nickel catalyst from one batch to another, even when prepared under carefully standardized conditions (*cf.* C. Djerassi, M. Shamma and T. Y. Kan, THIS JOURNAL, **80**, 4723 (1958), footnote 15). Nevertheless, the results were sufficiently capricious that only very powerful directing actors could be discerned

(28) The sample employed was obtained from the California Corporation for Biochemical Research, Los Angeles 63, Calif.

(29) W. K. Klein, *Z. physiol. Chem.*, **224**, 244 (1934).

(30) T. G. Brady, *Biochem. J.*, **35**, 855 (1941).

(31) W. Andersen, C. A. Dekker and A. R. Todd, *J. Chem. Soc.*, 2721 (1952).

(32) A. Deutsch, data published in the catalog of the California Corporation for Biochemical Research, Los Angeles 63, Calif.

TABLE II

X-RAY POWDER DIFFRACTION DATA FOR NATURAL AND SYNTHETIC 2'-DEOXYADENOSINE MONOHYDRATES[a,b]

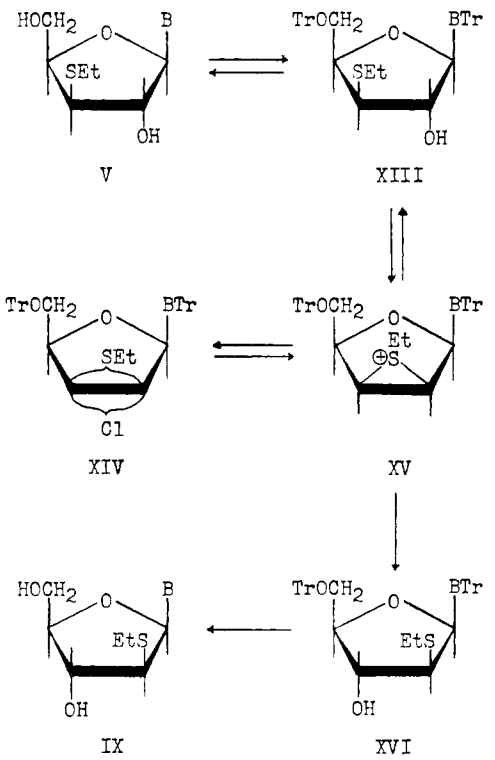
No.	Line intensity[c]	Natural d, Å.	Synthetic d, Å.	No.	Line intensity[c]	Natural d, Å.	Synthetic d, Å.
1	m	15.37	15.24	9	vs	3.87	3.88
2	m	8.01	8.04	10	w	3.73	3.74
3	s	7.05	7.08	11	s	3.37	3.36
4	s	5.63	5.64	12	s	3.09	3.09
5	w	4.68	4.67	13	m	2.72	2.72
6	m	4.43	4.44	14	m	2.15	2.15
7	vw	4.27	4.28	15	m	1.95	1.95
8	s	4.04	4.04	16	m	1.77	1.76

[a] Obtained using CuKα radiation. [b] All of the first 10 observed reflections are reported; thereafter only the stronger reflections are reported. [c] Estimated visually; s = strong, m = medium; w = weak, v = very.

lytically pure. This was at least partially explained by the fact that it occluded solvent on crystallization (it definitely occluded carbon tetrachloride and appeared to occlude heptane when recrystallized from ethyl acetate–heptane). Its structure and high purity were nevertheless reasonably certain since its ultraviolet spectrum is essentially identical (after allowing for occluded solvent) with that of the 6-*N*,5'-*O*-dityrityl derivative of adenosine,[33] which was measured for comparison.

B ≡ 9-(6-Aminopurinyl)

BTr ≡ 9-[6-(N-Tritylamino)-purinyl]



Treatment of the dityrityl nucleoside XIII with *p*-toluenesulfonyl chloride, using the conditions employed in the model sugar series,[11] introduced chlorine *via* the intermediate episulfonium ion XV, which reacts with chloride ion present in the reaction mixture. Unfortunately, the product, which presumably was largely the expected dityrityl derivative XIV of 6-amino-9-[3'(2')-chloro-2',3'-dideoxy-2'(3')-ethylthio-β-D-arabino(xylo)furanosyl]-purine, was difficult to purify; although originally crystalline, it could be recovered only in amorphous form on attempted recrystallization. In this respect, it was like the chloro nucleoside VIIb.[34] Treatment of the chloro dityrityl compound XIV with sodium acetate in refluxing 95% aqueous methyl Cellosolve for four hours gave a crude product that on analysis was free of chlorine but showed only very weak acetate carbonyl absorption in the infrared. Continued "acetolysis" of this product for an additional five hours gave a crude product completely devoid of acetate carbonyl absorption. Detritylation of this product, which must have been a mixture of the isomeric ethylthio dityrityl nucleosides XIII and XVI, in hot 80% aqueous acetic acid gave a mixture of the 2'-ethylthio nucleoside IX and its 3'-ethylthio isomer V. The ratio of IX to V was approximately 3 to 2. The lack of purity of the starting chloro dityrityl nucleoside XIV makes it impossible to say whether this unfavorable ratio of isomers was a result of low selectivity of nucleophilic attack on the episulfonium ion XV or of incomplete conversion of the 3'-ethylthio dityrityl nucleoside XIII to its chloro derivative XIV.

The successful completion of the synthesis of 2'-deoxyadenosine demonstrates the utility of the earlier predicted[8] alkylthio migration procedure for the synthesis of 2'-deoxy-β-D-ribonucleosides. Modifications of the present synthesis should yield two classes of fraudulent 2'-deoxyribonucleosides that may be of interest as antimetabolites. Substitution of unnatural bases for adenine can give one class. The second class, which would contain an unnatural 2'-deoxyribose moiety, could be obtained by reaction of the chloro ethylthio nucleoside VIIb (B ≡ a natural base) with a nucleophile other than hydroxide ion, *e.g.*, ammonia, an amine or an alkoxide ion.

Acknowledgments.—We wish to thank Dr. Peter Lim for interpretation of the infrared data; Mmes. B. Bonnell and J. Benz for the very extensive paper chromatographic work that was required; and Mr. O. P. Crews, Jr., and staff for large-scale preparation of the anhydronucleoside VI.

Experimental[35]

2-*O*-Acetyl-5-*O*-methoxycarbonyl-3-*O*-(*p*-tolylsulfonyl)-D-xylofuranosyl Chloride (IIa).—A solution of the diacetate I[12] (6.95 g., 15.6 mmoles) in 16 ml. of acetyl chloride was added to 230 ml. of ether saturated with hydrogen chloride at 0°, and the mixture was stored (stoppered) for 4 days at 0°. The infrared spectrum of the residue obtained on evaporation of a 0.5-ml. aliquot removed after 3 days showed

(33) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **121**, 131 (1937).

(34) A possible explanation is that on exposure to hot solvent, the initially formed 2',3'-isomer partially rearranges to the other possible 2',3'-isomer *via* episulfonium ion formation because of differences in thermodynamic stability.

(35) Melting points were taken on a Fisher–Johns apparatus and are uncorrected unless otherwise specified. Optical rotations were measured with a Standard Polarimeter model D attachment to the Beckman DU spectrophotometer calibrated with standard sucrose solutions For paper chromatographic solvent compositions, see last paragraph of the Experimental section.

that the 8.3-μ C–O–C band of the 1-*O*-acetate group of I had disappeared, suggesting complete conversion to IIa. Evaporation of the reaction mixture *in vacuo* while protected against moisture, and then evaporation of two 35-ml. portions of benzene from the residue, afforded IIa as a colorless oil. It was used immediately.

2-*O*-Acetyl-5-*O*-methoxycarbonyl-3-*O*-(*p*-tolylsulfonyl)-D-xylofuranosyl Bromide (IIb).—The bromo sugar IIb was prepared from 2.21 g. (5.0 mmoles) of diacetate I[12] by the method described by Baker and Hewson[36] for the preparation of 2,3,5-tri-*O*-benzoyl-D-xylofuranosyl bromide. The IIb was a yellow oil weighing 2.47 g. (107%) and was used immediately.

The 6-Amino-9-(2′,3′-anhydro-β-D-ribofuranosyl)-purine (VI). A. From Chloro Sugar IIa.—The crude IIa prepared as described above was dissolved in 50 ml. of dry xylene and added to an azeotropically dried suspension of chloromercuri-6-benzamidopurine[37] (14.8 g. (containing 50% Celite by weight), 15.7 mmoles) in 400 ml. of xylene. The mixture was refluxed with stirring for 2.5 hours, the then tan mixture was filtered through Celite, and the filtrate was evaporated to dryness *in vacuo*. The xylene-insoluble material was washed with two 165-ml. portions of chloroform, which then were combined and used to dissolve the residue from the xylene filtrate. This solution was washed with 30% aqueous potassium iodide (2 × 150 ml.) and saturated aqueous sodium bicarbonate (165 ml.) and dried over magnesium sulfate. Filtration and evaporation, finally at 30° and 0.5 mm., afforded 7.7 g. (79%) of crude blocked nucleoside III as a yellowish gum having λ_{max}^{film} 3.05 (amide NH), 5.72 (acetate and carbonate C=O), 5.89 (amide C=O), 6.25–6.34 (C=N, C=C), 7.29, 8.42 and 8.51 μ (—OSO_2—).

A solution of this crude III in 70 ml. of methanol was cooled to 0°, combined with 12.8 ml. (12.2 mmoles) of 0.95 *N* methanolic sodium methoxide, stoppered, and stored at 5° for 5 days. The still alkaline mixture was decanted from the insoluble salts, neutralized with glacial acetic acid, and evaporated to dryness *in vacuo*. The residue was partitioned between 140 ml. each of chloroform and water. Evaporation of the aqueous layer, finally at 35° and 0.5 mm., afforded 4.03 g. (104%) of unblocked nucleosidic material as a light brown solid. (This extremely crude product was normally treated directly with sodium ethyl mercaptide as described below.)

A solution of 3.93 g. of the above crude product in 40 ml. of hot methanol was combined with 53 ml. of 10% methanolic picric acid and stored at 5° for 1 hour. The resulting picrate was collected and washed with two 8-ml. portions each of cold methanol and water. The still damp picrate weighed 5.26 g. (73%). It was added portionwise with stirring to 100 ml. of water at room temperature together with 21 g. of Dowex 2 (CO_3).[38] When the picrate had dissolved, the mixture was filtered. The resin was washed with hot water (3 × 15 ml.), and the combined filtrate and washings were decolorized with Norit A, filtered through Celite, evaporated *in vacuo* to a volume of about 10 ml., and stored at 5°. The crystalline anhydronucleoside VI (0.47 g.) that separated had m.p. 160–190° dec. Recrystallization from absolute ethanol yielded 0.30 g. (8.9% over-all from I) of pure VI, m.p. 200–203° dec. A second recrystallization gave material of the same m.p.; $[\alpha]^{25}D$ −3° (0.6% in 20% aqueous pyridine); λ_{max}^{KBr} 3.02, 3.19 (OH, NH_2), 5.92, 6.08, 6.22 (NH_2, C=N, C=C), 11.67μ (epoxide); λ_{max}^{pH1} 257 mμ (ϵ 14,900); $\lambda_{max}^{H_2O}$ 260 mμ (ϵ 14,900); λ_{max}^{pH1} 260 mμ (ϵ 15,200). This product moved as a single spot in solvents A (R_{ad} 0.75) and B (R_{ad} 1.35).

Anal. Calcd. for $C_{10}H_{11}N_5O_3$: C, 48.2; H, 4.45; N, 28.1. Found: C, 48.2; H, 4.72; N, 28.0.

B. From Bromo Sugar IIb.—Similarly, reaction of the IIb prepared as described above with 4.41 g. (4.7 mmoles) of a 1:1 mixture of Celite and chloromercuri-6-benzamidopurine,[37] as described in section A, afforded 2.29 g. (78%) of crude blocked nucleoside III as a pale froth. Treatment with methanolic sodium methoxide gave 1.35 g. (110%) of crude unblocked nucleosidic material. Purification *via* the picrate (0.67 g., 28%) afforded 0.10 g. (8.1%) of poorly crystalline product, m.p. 160–170° dec., that on paper chromatography in solvent B showed two spots, R_{ad} 1.41 (anhydronucleoside VI) and 1.00 (adenine).

6-Amino-9-[3′-deoxy-3′-(ethylthio)-β-D-xylofuranosyl]-purine (V). A. From Pure Anhydronucleoside VI.—Analytically pure anhydronucleoside VI (142 mg., 0.57 mmole) was dissolved in 4.0 ml. of methanolic sodium ethyl mercaptide (prepared by dissolving 1.10 g. (20 mmoles) of sodium methoxide and 1.65 ml. (22 mmoles) of ethanethiol in 5.0 ml. of methanol with ice-bath cooling). This mixture was refluxed under nitrogen for 19 hours, cooled, and concentrated by evaporation *in vacuo* of the major portion of the methanol. The concentrate was dissolved in 20 ml. of water and stirred with 2.6 g. of Amberlite IRC-50(H)[39] until the mixture was neutral (about 4 hours). The resin was removed by filtration and washed with water (4 × 2 ml.). The combined filtrate and washings were evaporated to dryness *in vacuo*, and a portion of absolute ethanol was evaporated from the residue, which then weighed 228 mg. Crystallization from absolute ethanol, using Norit A, gave 116 mg. (66%) of crystalline 3′-ethylthio nucleoside V, m.p. 135–155° and 178–179.5° (cor.). Recrystallization from absolute ethanol of a 104-mg. portion afforded 94 mg. of crystals, m.p. 135–145° and 180.5–181.5° (cor.). A second recrystallization gave material having the same double m.p. (the first is ill-defined, but the second is very sharp); $[\alpha]^{27}D$ −76° (0.8% in 20% aqueous pyridine); λ_{max}^{KBr} 2.97, 3.13 (OH, NH_2), 6.10, 6.25 (NH_2, C=N, C=C), no absorption at 11.67 μ (epoxide); λ_{max}^{pH1} 258 mμ (ϵ 13,700); $\lambda_{max}^{H_2O}$ 260 mμ (ϵ 14,300); λ_{max}^{pH13} 260 mμ (ϵ 14,300). This product traveled as a single spot in solvents A (R_{ad} 1.48), B (R_{ad} 1.23) and C (R_{ad} 1.27).

Anal. Calcd. for $C_{12}H_{17}N_5O_3S$: C, 46.3; H, 5.50; N, 22.5; S, 10.3. Found: C, 46.2; H, 5.77; N, 22.2; S, 10.0.

B. From Crude Anhydronucleoside VI.—The crude VI that was obtained from a coupling reaction employing the chloro sugar IIa obtained from 56 g. (0.13 mole) of diacetate I was dissolved in methanolic sodium ethyl mercaptide (prepared from 34.5 g. (0.64 mole) of sodium methoxide, 160 ml. of methanol and 52 ml. (0.70 mole) of ethanethiol). The mixture was refluxed for 21 hours under nitrogen, cooled, and concentrated *in vacuo* to about 100-ml. volume. The concentrate was dissolved in 350 ml. of water, neutralized with glacial acetic acid (34.5 ml.), and extracted with 100 ml. of heptane (to remove unreacted ethanethiol). The aqueous phase was extracted continuously with chloroform for two 24-hour periods. On cooling, the first extract deposited 3.4 g. of crudely crystalline 3′-ethylthio nucleoside V, m.p. 130–145°. Evaporation of the filtrate *in vacuo* afforded 8.7 g. of gum. Evaporation of the second extract afforded 1.3 g. of gum. The combined 10 g. of gum on crystallization from absolute ethanol yielded 0.55 g. of crystalline V, m.p. 130–145° and 178–179°. Recrystallization of the combined 3.95 g. of crystalline product from absolute ethanol afforded 2.68 g. (6.8%) of pure V, m.p. *ca.* 130° and 180–181° (cor.), whose infrared spectrum and chromatographic behavior were identical with those of the analytical sample above. Chromatography of the residue from the combined mother liquors on seed test paper[17] using water as the developing solvent afforded an additional 0.35 g. (0.9%) of V, m.p. 179–181° (cor.).

6-Amino-9-[3′-chloro-2′,3′-dideoxy-2′-(ethylthio)-β-D-arabinofuranosyl]-purine (VIIb).—3′-Ethylthio nucleoside V (1.30 g., 4.2 mmoles) was dried for 1 hour at 56° and 0.2 mm. and then was cooled to 0° and dissolved in 8.0 ml. of cold thionyl chloride with stirring and ice cooling. The apparatus was continuously purged with dry nitrogen to protect against atmospheric moisture and sweep out the hydrogen chloride produced in the reaction. After the initial rapid reaction, the ice-bath was removed and the mixture was stirred at room temperature for 0.5 hour. The mixture then was poured slowly into an efficiently stirred mixture of sodium bicarbonate (40 g.), chloroform (70 ml.), and ice and water (equal parts, 320 ml. total volume). Chloroform (30 ml.) and saturated aqueous sodium bicarbonate (2 × 5 ml.) were used for rinsing. The mixture was stirred for 0.5 hour, the chloroform layer was separated, and the aqueous layer was extracted with chloroform (3 × 100 ml.). The combined extracts and initial chloroform layer were dried, fil-

(36) B. R. Baker and K. Hewson, *J. Org. Chem.*, **22**, 966 (1957).

(37) Prepared from 6-benzamidopurine and mercuric chloride as described for the preparation of chloromercuri-2,6-diacetamidopurine, see B. R. Baker and K. Hewson, *ibid.*, **22**, 959 (1957).

(38) An anion-exchange resin purchased from the Microchemical Specialties Company, Berkeley, Calif.

(39) A weak-acid cation-exchange resin manufactured by the Rohm and Haas Co., Philadelphia, Penna.

tered, and evaporated affording 1.18 g. (86%) of the chloronucleoside VIIb as white crystals having m.p. 188–192° dec.; $[\alpha]^{26}D$ −60° (1.0% in chloroform); λ_{max}^{KBr} 2.96 (OH, NH), 5.96 (shoulder), 6.11, 6.26 μ (NH_2, C=N, C=C); λ_{max}^{pH1} 259 mμ (ε 13,800); $\lambda_{max}^{H_2O}$ 261 mμ (ε 13,900); λ_{max}^{pH13} 261 mμ (ε 14,400). This nucleoside traveled as a single spot in solvent A (R_{ad} 1.86) but hydrolyzed in solvents B and C giving indefinite results.

Anal. Calcd. for $C_{12}H_{16}ClN_5O_2S$: C, 43.7; H, 4.89; Cl, 10.8; S, 9.72. Found: C, 43.2; H, 4.97; Cl, 10.9, 10.8; S, 9.74.

6-Amino-9-[2'-deoxy-2'-(ethylthio)-β-D-arabinofuranosyl]-purine (IX). A. From Chloro Nucleoside VIIb.—Chloronucleoside VIIb (0.96 g., 2.9 mmoles) and 1.80 g. of anhydrous sodium acetate were dissolved in 18 ml. of 95% aqueous methyl Cellosolve and refluxed under nitrogen for 3 hours The mixture was evaporated to dryness *in vacuo*, and the residue was dissolved in 120 ml. of water and extracted continuously with chloroform for these successive periods: (a) 4.5 hours, (b) 24 hours, (c) 39 hours and (d) 24 hours. Evaporation of the corresponding extracts *in vacuo*, finally at 35° and 0.5 mm. afforded these residues: (a) 0.26 g., partially crystalline; (b) 0.34 g., partially crystalline; (c) 0.25 g., m.p. 210–212° with preliminary softening; and (d) 0.06 g., partially crystalline. Recrystallization of residue c from acetone by the addition of hexane afforded 0.19 g. (21%) of IX, m.p. 211.5–213.5°. A second such recrystallization gave material of unchanged m.p.; $[\alpha]^{25}D$ −65° (0.9% in 20% aqueous pyridine); λ_{max}^{KBr} 2.99 (OH, NH), 6.12, 6.29, 6.36 μ (NH_2, C=C, C=N); λ_{max}^{pH1} 260 mμ (ε 14,800); $\lambda_{max}^{H_2O}$ 262 mμ (ε 15,200); λ_{max}^{pH13} 262 mμ (ε 15,400). This material traveled as a single spot on paper chromatography in solvents A (R_{ad} 1.51), C (R_{ad} 1.63) and D (R_{ad} 1.33).

Anal. Calcd. for $C_{12}H_{17}N_5O_3S$: C, 46.3; H, 5.50; N, 22.5; S, 10.3. Found: C, 46.3; H, 5.12; N, 22.4; S, 10.6.

The residues a, b and d were resolved by chromatography on Whatman seed test paper[17] using water as the developing solvent. Elution of the corresponding spots with methanol in a Soxhlet apparatus and recrystallization of the crude extracted material afforded 0.33 g. (37%) of additional IX in two crops, (a) m.p. 209–212° with preliminary softening and (b) m.p. 208–210° (both of which were chromatographically pure), and 66 mg. (7%) of the 3'-ethylthio isomer V, m.p. 125–155° and 178–179° (cor.), whose identity was confirmed by mixed m.p. determination and infrared spectroscopy.

B. From the 2'-Ethylthio Dityril Nucleoside XVI.—A suspension of 20 mg. of the crude 2'-ethylthio dityril nucleoside XVI (see below) in 0.60 ml. of 80% aqueous acetic acid was heated on the steam-bath for 20 minutes with periodic stirring, then diluted with 3.6 ml. of hot water and extracted with hot heptane (3 × 4 ml.). The aqueous phase was evaporated to dryness *in vacuo*, and the resultant residue was twice dissolved in absolute ethanol and recovered by evaporation, finally at 35° and 0.5 mm. The colorless, residual gum weighed 5.5 mg. (70%). On paper chromatography in solvent B, spots were observed at R_{ad} 1.00 (trace, adenine), 1.20 (3'-ethylthio nucleoside V) and 1.61 (2'-ethylthio nucleoside IX). In solvent C, the corresponding spots appeared at R_{ad} 1.02, 1.36 and 1.70. Ultraviolet spectroscopic measurements made on these chromatograms indicated that the ratio of IX to V was approximately 3:2.

6-Amino-9-[3',5'-di-*O*-acetyl-2'-deoxy-2'-(ethylthio)-β-D-arabinofuranosyl]-purine.—A solution of 2'-ethylthio nucleoside IX (71 mg., 0.23 mmole) and 0.25 ml. of acetic anhydride in 3 ml. of pyridine was allowed to stand (stoppered) 20 hours at room temperature and then evaporated to dryness *in vacuo*. The residue was twice dissolved in toluene and recovered by evaporation, finally at 35° and 0.5 mm. The residue was a colorless gum weighing 111 mg. (111%) that had λ_{max}^{film} 3.03 (NH_2), 5.74 (acetate C=O), 6.10, 6.26 μ (C=N), C=C), λ_{max}^{EtOH} 262 mμ, showing no 6-*N*-acetylation. In solvent A this product moved as a single spot (R_{ad} 1.86), but in solvent C there was a trace spot (R_{ad} 1.99), due to the presence of some triacetyl derivative XII (see below), besides the major spot (R_{ad} 1.63).

6-Acetamido-9-[3',5'-di-*O*-acetyl-2'-deoxy-2'-(ethylthio)-β-D-arabinofuranosyl]-purine (XII).—2'-Ethylthio nucleoside IX (22 mg., 0.07 mmole) and 0.04 g. of anhydrous sodium acetate were refluxed in 2.0 ml. of acetic anhydride for 10 minutes (protected from moisture), and the mixture was evaporated to dryness *in vacuo*. The solid residue was extracted with two 4–5-ml. portions of hot acetone, which were filtered, combined and evaporated, finally at 35° and 0.5 mm., yielding 33 mg. (106%) of non-crystalline triacetyl derivative XII. This material moved as a single spot in solvents A and C (R_{ad} 1.93 in both systems) and had λ_{max}^{film} 3.09 (amide NH), 5.7–5.8 (acetate C=O) with shoulder at 5.88 (amide C=O), 6.20, 6.30 (C=C, C=N), 6.66 μ (amide NH); λ_{max}^{EtOH} 272 mμ (ε 16,700), showing 6-*N*-acetylation.

2'-Deoxyadenosine (XI). A. From the Triacetyl Derivative XII.—Crude triacetyl derivative XII, prepared from 404 mg. (1.30 mmoles) of 2'-ethylthio nucleoside IX by the method described above, was dissolved in 40 ml. of chloroform, and 5.0-ml. aliquots were placed in each of eight flasks and evaporated to dryness, finally at 35° and 0.5 mm. Freshly prepared Raney nickel "C"[25] (1.00 g., moist with ethanol), 10 ml. of methyl Cellosolve and 0.30 ml. of 10% aqueous sodium hydroxide (0.75 mmole) were added to each flask, and the mixtures were refluxed for 6 hours with stirring. The hot mixtures were each diluted with 5 ml. of absolute ethanol and filtered through Celite. The catalyst was washed with hot absolute ethanol (4 × 10 ml.). The colorless filtrate and washings from each of the eight separate mixtures were combined and evaporated to dryness *in vacuo*. Absolute ethanol (20 ml.) was evaporated from the residue (finally at 35° and 0.5 mm.), which then weighed 813 mg. The infrared spectrum of this residue showed essentially no *O*- or *N*-acetyl carbonyl absorption. The remaining 799 mg. of crude product were resolved by chromatography on Whatman seed test paper (previously washed as described by Brownell, Hamilton and Casselman[17] except that after the recommended 4 days with 0.01 *N* hydrochloric acid the paper was washed for 1 day with 0.01 *N* aqueous ammonia and then for 2 days with water to assure its non-acidity) using water-saturated 1-butanol as the developing solvent. The resulting chromatograms had strongly ultraviolet-absorbing bands at R_{ad} 0.83 (2'-deoxyadenosine (XI)) and 1.55 (IX) and a less intense band of unknown origin at R_{ad} 1.09. Chromatographic elution[17] of the 2'-deoxyadenosine bands with water afforded on evaporation of the combined eluates *in vacuo* (finally at 35° and 0.5 mm.) 44 mg. of waxy solid. Ultraviolet spectroscopy indicated that this crude product contained 93% XI monohydrate. Recrystallization from water gave 35 mg. of an almost white crystalline solid, m.p. 168–174° (cor.) with preliminary softening. For further purification, this material was combined with 21 mg. of once recrystallized synthetic XI monohydrate from earlier desulfurization experiments. Recrystallization from water then afforded 53 mg. of essentially white crystals, m.p. 179–184° (cor.). A final recrystallization gave 49 mg. of pure XI monohydrate which had m.p. 187–189° (cor.) and λ_{max}^{KBr} 2.98, 3.1–3.2 (NH_2, OH, H_2O), 5.89, 6.07–6.09, 6.19, 6.33 μ (NH_2, H_2O, C=N, C=C). The infrared spectrum of a sample of natural XI monohydrate[28] (recrystallized from water to a constant m.p. of 186–189° (cor.)) was identical. A comparison of the other physical properties of these two samples appears in Tables I and II. In the case of the synthetic XI monohydrate, elemental analyses were obtained on the material recovered after measurement of the optical rotation, which was dried overnight at room temperature and about 0.5 atmosphere and then had m.p. 189–191° (cor.).

Chromatographic elution[17] with water of the bands at R_{ad} 1.55 on the seed test paper chromatograms used to purify the crude desulfurization product gave on evaporation of the combined eluates, finally at 35° and 0.5 mm., 175 mg. of crude IX as a yellowish gum (ultraviolet measurements indicated it was 74% pure). Crystallization from acetone–hexane gave 114 mg. (28% recovery) of starting material IX, m.p. 207–210°. The yield of pure XI monohydrate based on unrecovered IX (and corrected for the material from earlier experiments) was therefore 13%.

B. From the Free 2'-Ethylthio Nucleoside IX.—A solution of 2'-ethylthio nucleoside IX (50 mg., 0.16 mmole) and 0.30 ml. of 10% aqueous sodium hydroxide in 10 ml. of methyl Cellosolve containing 1.00 g. (moist with ethanol) of freshly prepared Raney nickel "C"[25] was refluxed for 6 hours with stirring. The deeply yellow reaction mixture then was worked up as described in section A to afford a crude product weighing 130 mg. Chromatography on seed test paper[17] and subsequent recrystallization as described in section A gave 3.2 mg. (7%) of pure XI monohydrate, m.p. 185–188° (cor.). The yield of recovered IX was 1.4%

by spectroscopic measurement; no attempt at isolation was made.

In an exactly parallel experiment, except for the substitution of 0.10 ml. of 10% aqueous sodium hydroxide and 0.20 ml. of 2.5 *N* aqueous sodium acetate for the 0.30 ml. of the 10% (2.5 *N*) aqueous sodium hydroxide employed above, work-up of the completely colorless reaction mixture afforded 5.8 mg. (13%) of XI monohydrate, m.p. 185–188° (cor.), and a 7.4% yield (by spectroscopic measurement) of recovered IX.

9-[3′-Deoxy-3′-(ethylthio)-5′-*O*-trityl-β-D-xylofuranosyl]-6-(*N*-tritylamino)-purine (XIII).—A solution of 3′-ethylthio nucleoside V (1.00 g., 3.22 mmoles) and trityl chloride (3.06 g., 11.0 mmoles) (both previously dried at 56° and 0.1 mm. for 1 hour) in 26 ml. of pyridine was heated at 56° for 116 hours, protected from moisture. The cooled mixture was dissolved in 45 ml. of chloroform and poured into a mixture of 90 ml. of 1 *N* aqueous sodium bicarbonate and 90 g. of ice with mechanical stirring. After 1.5 hours, the layers were separated. The aqueous layer was washed with 25 ml. of chloroform. The combined organic layers were washed with 35 ml. of 1 *N* aqueous sodium bicarbonate, dried over magnesium sulfate, filtered, and evaporated *in vacuo*. The residue was dissolved in toluene and re-evaporated, finally at 35° and 0.5 mm., affording 3.88 g. of residual gum. This was dissolved in *ca.* 10 ml. of carbon tetrachloride; on standing, 1.25 g. (41%) of XIII was deposited as solvated, white crystals, m.p. 139–145° with gas evolution. The mother liquor was evaporated to dryness *in vacuo*, dissolved in ethyl acetate, and re-evaporated. The residue was dissolved in 5 ml. of ethyl acetate and diluted to turbidity with heptane. On standing, a second crop of XIII (0.51 g., 17%) was deposited as a tan solid, m.p. 138–158° with gas evolution. Recrystallization of the first crop from carbon tetrachloride gave 0.93 g. of white crystals which had m.p. 138–156° with gas evolution and λ_{max}^{KBr} 2.91 (OH, NH), 6.23 (C=N plus phenyl C=C), no absorption at 9.82 and 13.13 μ (tritanol).

Anal. Calcd. for $C_{50}H_{45}N_5O_3S \cdot CCl_4$: C, 64.5; H, 4.78; Cl, 14.9; S, 3.38. Found: C, 66.0; H, 4.99; Cl, 12.5; S, 2.44, 2.68.

A second analytical sample, m.p. 153–158° with gas evolution, was prepared by recrystallization of XIII from ethyl acetate–heptane. Its infrared spectrum showed no acetate carbonyl absorption; $\lambda_{max}^{CHCl_3}$ 278 mμ (ε 21,000) with shoulders at 272 (ε 19,400) and 286 mμ (ε 14,900).

Anal. Calcd. for $C_{50}H_{45}N_5O_3S \cdot C_7H_{16}$: C, 76.4; H, 6.86; S, 3.58. Found: C, 75.5, 75.5; H, 6.55, 6.49; S, 3.54.

9-(5′-*O*-Trityl-β-D-ribofuranosyl)-6-(*N*-tritylamino)-purine.[40]—By proper modification of the procedure of Levene and Tipson,[33] the yield was increased several-fold and the work-up considerably simplified. A mixture of 310 ml. of reagent pyridine, 20 g. of adenosine and 63 g. of trityl chloride was stirred (protected from moisture) in a bath at 50–60° until solution was complete (about 4.5 hours). After being heated in the bath an additional 48 hours, the solution was poured in a thin stream into a rapidly stirred solution of 25 g. of sodium bicarbonate in 3 l. of ice-water. The precipitate was collected on a filter, washed with cold water, and dried overnight in a desiccator over phosphorus pentoxide. The dry product was stirred for 30 minutes with 600 ml. of acetone, then filtered from 5′-*O*-trityladenosine. The combined filtrate and washings were evaporated to dryness *in vacuo*. Recrystallization of the residue from 1 l. of ethyl acetate gave 31.8 g. (57%) of product in two crops, m.p. 213–215°; λ_{max}^{KBr} 2.95 (OH, NH), 6.25 μ (C=N plus phenyl C=C); $\lambda_{max}^{CHCl_3}$ 277 mμ (ε 23,200) with shoulders at 271 (ε 21,700) and 286 mμ (ε 16,400).

9-[3′(2′)-Chloro-2′,3′-dideoxy-2′(3′)-(ethylthio)-5′-*O*-trityl-β-D-arabino(xylo)furanosyl]-6-(*N*-tritylamino)-purine (XIV).—A solution of 282 mg. (0.35 mmole) of ditrityl nucleoside XIII and 204 mg. (1.1 mmoles) of *p*-toluenesulfonyl chloride in 2.0 ml. of pyridine was allowed to stand (stoppered) at room temperature for 64 hours. Water (0.10 ml.) was added, and 2 hours later the mixture was poured into 20 ml. of ice and water. The crystalline product that separated (presumably XIV) was collected, washed with ice-water (3 × 3 ml.), and dried *in vacuo*. It then weighed 274 mg. (94%) and had m.p. 109–115° and λ_{max}^{KBr} 2.96 (NH), 6.24 (phenyl C=C), no 7.3 or 8.4 tosylate absorption, and decreased C—O absorption in the region of 9.2–9.5 μ. It could not be recrystallized from the non-hydroxylic solvents tried and on recovery was an amorphous solid.

Anal. Calcd. for $C_{50}H_{44}ClN_5O_2S$: Cl, 4.35. Found: Cl, 5.05.[41]

9-[2′-Deoxy-2′-(ethylthio)-5′-*O*-trityl-β-D-arabinofuranosyl]-6-(*N*-tritylamino)-purine (XVI).—A solution of 427 mg. (0.52 mmole) of XIV in 10.4 ml. of 95% aqueous methyl Cellosolve containing 1.01 g. of anhydrous sodium acetate was refluxed (under nitrogen) for 4 hours. The mixture was cooled, diluted with 35 ml. of chloroform, washed with water (2 × 14 ml.) and with saturated aqueous sodium chloride (14 ml.), then dried over magnesium sulfate. Filtration and evaporation to dryness *in vacuo* gave a residue that was dissolved in toluene and re-evaporated, finally at 40° and 0.5 mm. The residual oil weighed 494 mg. (118%) and had λ_{max}^{film} 2.95 (shoulder), 3.00 (OH, NH), 6.25 (phenyl C=C), 5.72 μ (very weak, acetate C=O). For analysis, a portion was dried 1 hour at 56° and 0.1 mm.

Anal. Calcd. for $C_{50}H_{45}N_5O_3S$: C, 75.4; H, 5.70. Found: C, 76.7; H, 6.44; Cl, <0.1%.

Retreatment of the above crude XVI with sodium acetate in refluxing 95% aqueous methyl Cellosolve for an additional 5 hours gave a 115% yield of a pale yellow oil whose infrared spectrum was identical with that above, except that the acetate carbonyl band had disappeared entirely.

The detritylation of this material was described in an earlier section.

Paper Chromatography.—The paper chromatograms were run by the descending technique on Whatman No. 1 paper (except where indicated) in the following solvent systems: A, water-saturated 1-butanol[42]; B, 5% disodium hydrogen phosphate[43] (without the usual organic phase); C, water; D, ammonium sulfate–2-propanol–water (2:28:70)[44]; and E, ethyl acetate–pyridine–water (2:1:2).[45] Adenine was used as a standard (spot locations are expressed as R_{ad} units with adenine at 1.00), and spots were detected by visual examination under ultraviolet light.

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(40) This experiment was performed by R. R. Spencer.

(41) The chloronucleoside VIIb later was found to give high chlorine analyses by the combustion method used for this analysis; if the Carius method, which worked well on VIIb, had been used here, the result might have been better.

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