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Puromycin. Synthetic Studies. XV. 3′-Amino-3′-deoxyadenosine

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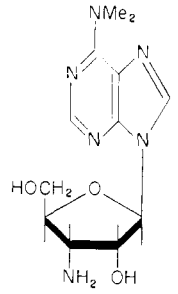
The title compound VI, the N-demethylated analog of the biologically active aminonucleoside I from puromycin, has been synthesized by condensation of chloromercuri-6-benzamidopurine with 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-acetamido-3-deoxy-D-ribofuranose (III), in the presence of titanium tetrachloride, followed by deacylation. Both α- and β-nucleosides were obtained, the β-nucleoside IV being anomerized to the α-nucleoside V by the titanium tetrachloride. Condensation of chloromercuri-6-benzamidopurine with 2,5-di-*O*-benzoyl-3-phthalimido-3-deoxy-β-D-ribofuranosyl chloride followed by deacylation gave a much higher over-all yield of the title compound VI since no anomerization to α-nucleoside took place. It was shown that "basic" nucleosides could be separated from "neutral" nucleosides by absorption on a carboxylic acid ion-exchange resin.

The active moiety of the puromycin molecule against *Trypanosoma equiperdum* and the transplanted mammary adenocarcinoma of the C_3H mouse (J-tumor) has been shown to be the aminonucleoside, 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine (I).[1] The activity



I

against *Trypanosoma equiperdum* can be reversed *in vivo* by a number of purines, including adenine and, strikingly, even the purine moiety of puromycin, namely, 6-dimethylaminopurine.[2] The activity against the J-tumor *in vivo* is not reversed by these purines,[3] indicating that in these two test systems the mechanisms of action are not necessarily related. Microbiologically, it has been observed that large amounts of 6-dimethylaminopurine can replace the adenine necessary for growth of *E. coli*.[4] The fact that 6-diethylaminopurine can replace adenine in these two biological systems can be rationalized by enzymatic demethylation of the 6-dimethylaminopurine to adenine. If such is the case, then it is equally possible that the aminonucleoside I from puromycin is also enzymatically demethylated to 3′-amino-3′-deoxyadenosine (VI). The synthesis of the latter compound is the subject of this paper.

Biological Activity.—3′-Amino-3′-deoxyadenosine (VI) has only $^1/_2$ the activity of the aminonucleoside I against *Trypanosoma equiperdum*,[5] thus indicating that enzymatic demethylation is not a factor in the activity. Against the J-tumor grown in tissue culture, VI is about twenty times as active as the aminonucleoside I, but neither I nor VI show a selective *in vitro* action against tumor cells over normal cells.[6] In the C_3H mouse, 3′-amino-3′-deoxyadenosine (VI) is much more toxic than the aminonucleoside I agreeing with the tissue culture data. When the activity of VI against the J-tumor in the C_3H mouse was tested at the maximum tolerated dose ($^1/_{50}$ of I), the growth of the tumors was reduced to 48% of the tumor controls, compared to a 6% growth of tumors when the mice were treated with aminonucleoside I.[7] This combination of *in vivo* and *in vitro* data shows that the selective *in vivo* action of I against the J-tumor[3] cannot be rationalized by enzymatic demethylation of I to 3′-amino-3′-deoxyadenosine (VI), otherwise I should have shown a selective action against tumor cells *in vitro*. It is probable that the aminonucleoside I is metabolized to some unknown active compound which has a selective action on tumor cells. Further synthesis and testing of suitable additional analogs of I may lead to this unknown active metabolic product.

Synthesis.—Condensation of 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-acetamido-3-deoxy-D-ribofuranose (III anomeric mixture)[8] with chloromercuri-6-benzamidopurine[9] in ethylene dichloride in the presence of titanium tetrachloride[10] gave a 57% yield of crude nucleoside IV.

During debenzoylation of IV in methanolic sodium methoxide a pure, highly insoluble, crystalline acetamido nucleoside, m.p. 271° dec., $[\alpha]D$ +64°, separated from the boiling methanol solution in 10% yield (from III). Concentration of the mother liquor gave 33% of another crystalline acetamino nucleoside which, when purified, had a m.p. 247° dec. and $[\alpha]D$ +10°. Both compounds had the combustion analyses, ultraviolet and infrared spectra expected for 3′-acetamino-3′-deoxyadenosine. Thus the higher melting isomer, by reason of its higher positive rotation, appeared to be the α-nu-

(1) B. R. Baker, J. P. Joseph and J. H. Williams, THIS JOURNAL, **77**, 1 (1955), paper VII of this series.

(2) R. I. Hewitt, A. R. Gumble, W. S. Wallace and J. H. Williams, *Antibiotics and Chemotherapy*, **4**, 1222 (1954).

(3) J. J. Oleson, P. L. Bennett, S. L. Halliday and J. H. Williams, 6th International Cancer Congress, Sao Paulo, Brazil, July, 1954.

(4) G. B. Elion, G. H. Hitchings and H. Vanderwerff, *J. Biol. Chem.*, **192**, 505 (1951).

(5) Private communication from Dr. R. I. Hewitt of these laboratories.

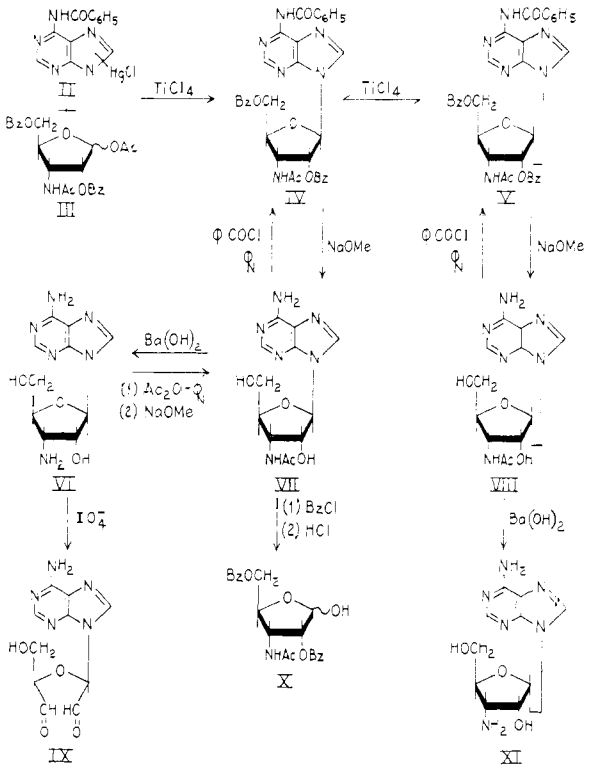
(6) Private communication from Dr. P. A. Eichorn of these laboratories.

(7) Private communication from Dr. J. J. Oleson of these laboratories.

(8) B. R. Baker, R. E. Schaub, J. P. Joseph and J. H. Williams, THIS JOURNAL, **77**, 12 (1955), paper IX of this series.

(9) J. Davoll and B. A. Lowy, *ibid.*, **73**, 1650 (1951).

(10) This procedure was found superior to the method[8] of preformation of the chloro sugar–titanium chloride complex for the synthesis of 6-dimethylamino-9-(3′-acetamino-3′-deoxy-α-D-arabinofuranosyl)-purine; *cf.* B. R. Baker and R. E. Schaub, THIS JOURNAL, **77**, 2396 (1955), paper XII of this series.



cleoside VIII and the lower melting isomer the expected β-nucleoside VII. That VII was the desired β-D-ribofuranosyl nucleoside VII was unequivocally demonstrated by (a) rebenzoylation back to IV and hydrolysis with the refluxing two phase system 2 N hydrochloric acid–ethylene dichloride[8] to 2,5-di-*O*-benzoyl-3-acetamido-3-deoxy-D-ribose (X); (b) removal of the N-acetyl group to VI and periodate oxidation to IX, discussed in detail later. Although VIII could be rebenzoylated back to V, hydrolysis with the two-phase hydrolysis system did not give any X (or any other crystalline product). However, hydrolysis of VIII with 2% hydrochloric acid gave authentic 3-amino-3-amino-3-deoxy-D-ribose,[11] showing that in VIII no rearrangement of the sugar configuration had taken place. It is reasonable to assume that the ring size of the sugar in the nucleoside VIII was still furanose since ring expansion in a nucleoside condensation in the presence of titanium tetrachloride has not been observed previously.[8,10] Thus the higher melting acetamino nucleoside most probably, though not unequivocally, has structure VIII.

Hydrolysis of the N-acetyl β-nucleoside VII to VI with 0.5 N barium hydroxide at 100° appeared to be complete in 1 hour since the amorphous product no longer had amide carbonyl absorption at 5.90 μ (characteristic of VII). However, the product could not be crystallized by conventional methods, but was crystallized after development of a new technique for the purification of nucleosides bearing an amino group in the sugar moiety.

Since the purine moiety of a nucleoside is a very weak organic base, it would not be expected to be absorbed from neutral solution on a carboxylic acid type ion-exchange resin. However, any nucleoside bearing the much stronger aliphatic amine in the sugar moiety would be expected to be absorbed.[12] Experimentally, it has now been shown that the carboxylic acid resin, Amberlite IRC-50 (H-form) was not strong enough, as anticipated, to hold a "neutral" nucleoside such as 6-dimethylamino-9-β-D-ribofuranosylpurine. However, the basic nucleoside, 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine (I), was completely absorbed from an aqueous solution and washing with large quantities of water failed to remove it. The basic nucleoside I then could readily be eluted from the resin with 2 N ammonium hydroxide.[13] In these experiments the concentration of nucleosides in the effluent fractions was conveniently followed by ultraviolet inspection. These data showed that it should be possible to separate quantitatively a "neutral" nucleoside from a "basic" nucleoside.

The aqueous solution of VI obtained from alkaline hydrolysis of VII was freed from barium hydroxide with carbon dioxide, then passed through a column of Amberlite IRC-50 (H). The column was then washed with 50% methanol until the effluent contained no appreciable purine observable at 260 mμ in the ultraviolet. The column was then washed with 2 N ammonium hydroxide in 50% methanol until no more of the basic nucleoside was eluted. The desired 3′-amino-3′-deoxyadenosine (VI) crystallized directly from the richest fraction. A total of 26% of crystalline VI could be isolated by this method. Although VI is not appreciably water soluble, direct seeding of the hydrolysis solution still failed to give any crystals of VI. Apparently this compound readily supersaturated in water in the presence of certain impurities, a property also characteristic of adenosine. That VI had the β furanose configuration was checked by periodate oxidation in pH 4.5 buffer. The resultant solution of dialdehyde IX had M_D −6400°, in reasonable agreement with the same dialdehyde obtained from adenosine[14] (M_D −5600° to −7700° depending on the pH) and in marked contrast to the dialdehyde of α-configuration, M_D +4000°, obtained by periodate oxidation of 9-α-D-arabinofuranosyladenine.[15]

In order to prove that no change in the basic structure of VII had taken place during hydrolysis, pure 3′-amino-3′-deoxyadenosine (VI) was fully acetylated with acetic anhydride and pyridine, then *O*-deacetylated to give pure VII, m.p. 247° dec., $[\alpha]_D$ +7 ± 2°.

The formation of the α-nucleoside V as a by-product during nucleoside synthesis contradicts the

(11) B. R. Baker and R. E. Schaub, *J. Org. Chem.*, **19**, 646 (1954), paper III of this series.

(12) J. C. Winters and R. Kunin, *Ind. Eng. Chem.*, **41**, 461 (1949), have used Amberlite IRC-50 for separation of basic amino acids from neutral amino acids. They have also recorded that the weekly basic purine, adenine, is not held by this resin.

(13) Basic amino acids have been eluted from Amberlite IRC-50 with hydrochloric acid.[12] Acid elution was not considered compatible with the acid lability of the sugar–purine linkage. This difficulty was avoided by elution with ammonium hydroxide, a procedure also used by H. Tauber, *Proc. Soc. Exp. Biol. Med.*, **86**, 838 (1954), for elution of amino acids from the sulfonic acid type ion-exchange resin Amberlite IR-105.

(14) J. Davoll, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 833 (1946).

(15) N. W. Bristow and B. Lythgoe, *ibid.*, 1465 (1949).

previously postulated rule[16] that when a halo acylated sugar is coupled with a heavy metal salt of a purine, the resultant nucleoside will have a C_1-C_2 *trans*-configuration. The formation of the α-nucleoside V with a C_1-C_2 *cis*-configuration can be reasonably explained by assuming that the β-nucleoside IV with a C_1-C_2 *trans*-configuration is formed initially, then IV is anomerized by the titanium chloride present to the α-nucleoside V in the same fashion that acylated methyl glycosides are anomerically equilibrated by titanium tetrachloride.[17] Attempts to prove that this mechanism was possible by rebenzoylation of the pure α- or β-nucleoside back to the tribenzoate IV (or V), treatment with titanium tetrachloride in ethylene dichloride, then debenzoylation back to IV and/or V did not give conclusive results.

It is interesting to note that this anomerization has so far only been observed with nucleosides of 6-benzamidopurine; an example of the same anomerization with a different sugar as 6-benzamidopurine has been found and will be published in a future paper. Nucleosides from 2-methyl-mercapto-6-dimethylaminopurine with either the α- or β-configuration do not anomerize in the presence of titanium chloride.[8,10] A number of other purines have been used in this method and also do not anomerize. In spite of the fact that 6-benzamidopurine and others may be discovered is an exception to the rule of formation of C_1-C_2 *trans*-nucleosides, this rule has been valuable for predicting the route to stereochemically controlled syntheses of nucleosides.[10,18] This difficulty can be avoided by the use of other substituted purines which do not anomerize and which by further transformation of the nucleosides lead to adenine nucleosides (to be published).

Since the anomerization of the β-nucleoside IV to the α-nucleoside V by titanium chloride causes considerable loss of the desired β-nucleoside, a synthesis was sought which would avoid the anomerization. One of the answers found was the use of the N-phthalyl blocking group[19] for the amino sugar moiety. Condensation of crystalline 2,5-di-*O*-benzoyl-3-phthalimido-3-deoxy-β-D-ribofuranosyl chloride[19] with chloromercuri-6-benzamidopurine followed by debenzoylation gave a 58% yield of crude non-crystalline 3′-phthalimido-3′-deoxyadenosine. Removal of the N-phthalyl group with hydrazine[19] and isolation of the product by the ion-exchange column technique gave crystalline 3′-amino-3′-deoxyadenosine (VI) in 41% yield or an over-all yield of 24% from the original chloro sugar. Reaction of pure VI with phthalic anhydride in boiling dimethylformamide gave crystalline 3′-phthalimido-3′-deoxyadenosine which could be recrystallized from water. Recrystallization of the crude synthetic 3′-phthalimido-3′-deoxyadenosine from water afforded a 61% recovery of pure material. Dephthalation of this pure phthalimido nucleoside gave VI in 60% yield. Thus it can be calculated that the crude synthetic 3′-phthalimido-3′-deoxyadenosine had a purity of about 68%. Slightly higher over-all yields of VI can be obtained if the intermediate N-phthalyl derivative is not purified.

The over-all yield of VI from 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-phthalimido-3-deoxy-D-ribofuranose is almost five times that obtained from 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-acetamido-3-deoxy-D-ribofuranose (III). With the N-phthalyl group, no formation of α-nucleoside was noted, although a relatively small yield could have escaped isolation.

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Experimental

α- and β-Anomers of 3′-Acetamido-3′-deoxyadenosine (VII and VIII). (A).—A mixture of 8.5 g. of chloromercuri-6-benzamidopurine (II),[9] 10 g. of Celite (diatomaceous earth), 6.36 g. of III (anomeric mixture)[8] and 570 cc. of ethylene chloride was freed from traces of moisture by distillation of 50 cc. of solvent. Then a solution of 1.95 cc. of titanium tetrachloride in 15 cc. of ethylene dichloride was added portionwise with stirring. After being refluxed and stirred for 19 hours, the mixture was cooled to room temperature, treated with 275 cc. of 1 *N* hydrochloric acid and stirred for 1 hour. The mixture was filtered and the Celite cake washed with 100 cc. of hot chloroform. The Celite cake was stirred vigorously with 125 cc. of chloroform and 125 cc. of 1 *N* hydrochloric acid to break down the remainder of the titanium complex. The mixture was filtered and the solids washed with two 100-cc. portions of boiling chloroform. The combined organic solutions and washings, separated from water, were evaporated to dryness *in vacuo*. The residue was dissolved in 100 cc. of chloroform and washed with 100 cc. of 30% aqueous potassium iodide, then water. Dried with magnesium sulfate, the chloroform solution was evaporated to dryness *in vacuo* leaving 5.49 g. (57%) of a crude mixture of IV and V, λ_{max}^{alc} 230 mμ (ε 37,700), 278 mμ (ε 14,100).

A solution of 5.48 g. of this benzoate in 75 cc. of hot methanol was clarified with Norit and filtered through Celite. After the addition of 2.7 cc. of 1 *N* methanolic sodium methoxide the solution was refluxed for 30 minutes, the *p*H remaining < 10 when spotted on moist indicator paper. After 15 minutes white crystals had begun to separate. The solution was filtered hot and the crystals of α-anomer VIII were washed with hot methanol; yield 440 mg. (9.9% from III), m.p. 271° dec., $[\alpha]^{25}D$ +64 ± 1° (2% in 0.1 *N* HCl), $\lambda_{max}^{H_2O}$ 259 mμ (ε 14,800); λ_{max}^{KBr} 2.98 μ (broad) (OH, NH), 5.88 μ (C=O), 6.02, 6.13 μ (C=N).

Anal. Calcd. for $C_{12}H_{16}N_6O_4$: C, 46.8; H, 5.23; N, 27.2. Found: C, 46.8; H, 5.63; N, 26.8.

This compound was analytically, optically and spectrographically (ultraviolet) pure. When twice recrystallized by extraction with methanol in a Soxhlet apparatus the above properties did not change from the original in each recrystallization. The α-anomer then had $[\alpha]^{25}D$ +63 ± 1° (2% in 0.1 *N* HCl).

The filtrate from the 440 mg. was concentrated to about 1/3 *in vacuo*, chilled in an ice-bath, the gelatinous crystals of crude β-anomer VII were collected and washed with cold methanol, then with benzene to remove methyl benzoate; yield 1.49 g. (33%), m.p. 241° dec., $\lambda_{max}^{H_2O}$ 259 mμ (ε 12,200). Thus, ultraviolet analysis showed a purity of 82% based on the mol. wt. of VII (or VIII). It can also be estimated from recrystallization data that this crude VII contains about 10% VIII. Recrystallization of 500 mg. from 6 cc. of water with the aid of Norit gave 310 mg. (62% recovery) of white needles of VIII, m.p. 247° dec., $[\alpha]^{25}D$ +10 ± 2° (1.5% in 0.1 *N* HCl), $\lambda_{max}^{H_2O}$ 259 mμ (ε 14,700); λ_{max}^{KBr} 2.97 μ (broad) (OH, NH), 5.90 μ (C=O), 6.03, 6.14 μ (C=N), which were analytically, spectrographically (ultraviolet) and optically pure. The compound forms a hydrate which is obtained

(16) B. R. Baker, J. P. Joseph, R. E. Schaub and J. H. Williams, *J. Org. Chem.*, **19**, 1786 (1954), paper V of this series.

(17) E. Pacsu, THIS JOURNAL, **52**, 2563, 2568, 2571 (1930); E. Piel and C. B. Purves, *ibid.*, **61**, 2978 (1939).

(18) B. R. Baker and R. E. Schaub, *ibid.*, **77**, 5900 (1955), paper XIII of this series.

(19) B. R. Baker, J. P. Joseph and R. E. Schaub, *ibid.*, **77**, 5905 (1955), paper XIV of this series.

anhydrous after drying at 110° for 18 hours, but not 3 hours, in high vacuum.

Anal. Calcd. for $C_{12}H_{16}N_6O_4$: C, 46.8; H, 5.23; N, 27.2. Found: C, 46.8; H, 5.04; N, 27.3.

Further recrystallizations from water did not change the optical rotation. The β-anomer VII is readily separated from α-anomer VIII since the latter, when dissolved in hot water, does not crystallize out from solution after standing for several days. The rotation of neither VII nor VIII changed when the solutions in 0.1 *N* hydrochloric acid were allowed to stand for 48 hours.

In a pilot run the yield of combined VII and VIII was 50% (173 mg.). Replacing III with 2,5-*O*-benzoyl-3-acetamido-3-deoxy-D-ribofuranose (X) gave a 29% yield of combined VII and VIII. When the 2,5-di-*O*-benzoyl-3-acetamido-3-deoxy-D-ribofuranosyl chloride titanium chloride complex was preformed,[8] then condensed with chloromercuri-6-benzamidopurine the yield of combined VII and VIII was 47%. All three runs were the same size.

(B).—A mixture of 50 mg. of pure VI, 1 cc. of reagent pyridine and 0.2 cc. of acetic anhydride, protected from moisture, was heated on the steam-bath under a condenser for 2 hours when solution was complete. After standing for 2 days the solution was diluted with 5 cc. of water and extracted with chloroform (3 × 5 cc.). The combined extracts, dried with magnesium sulfate, were evaporated to dryness *in vacuo*. The residue was twice evaporated with toluene *in vacuo* leaving 65 mg. of glassy solid. To a solution of this 65 mg. in 2 cc. of hot absolute methanol was added 0.02 cc. of 1 *N* methanolic sodium methoxide. The solution was refluxed for 30 minutes, crystals separating after 10 minutes. The cooled solution was filtered and the product washed with methanol; yield 25 mg. of white crystals, m.p. 249° dec., $[\alpha]^{25}D$ +7 ± 3° (0.6% in 0.1 *N* HCl). This compound was identical with VII of preparation A. From the mother liquor was isolated an additional 10 mg. (total 60%), m.p. 247° dec.

N-Acetylation of VI with acetic anhydride in water, as described for I,[1] was unsuccessful.

Regeneration of 2,5-Di-*O*-benzoyl-3-acetamido-3-deoxy-D-ribose from 3'-Acetamido-3'-deoxyadenosine (VI).—A mixture of 100 mg. of VI, 2 cc. of reagent pyridine and 0.13 cc. of benzoyl chloride, protected from moisture, was heated on the steam-bath under a condenser for 90 minutes, solution being complete in 10 minutes. The cooled mixture was diluted with 10 cc. of water and extracted with ethylene dichloride (3 × 5 cc.). The combined extracts, washed with aqueous sodium bicarbonate and dried with magnesium sulfate, were evaporated to dryness leaving 160 mg. (80%) of benzoylated nucleoside IV as a glass.

The 160 mg. of glass was hydrolyzed with 5 cc. of 2 *N* hydrochloric acid and 4 cc. of ethylene dichloride as described for the hydrolysis of 6-dimethylamino-9-(2',5'-di-*O*-benzoyl-3'-acetamido-3'-deoxy-β-D-ribofuranosyl)-purine[5]; yield 20 mg. (20%) of 2,5-di-*O*-benzoyl-3-acetamido-3-deoxy-D-ribose, m.p. 153–155°, which gave a positive Benedict test and was identical with an authentic sample.[8]

Benzoylation and attempted hydrolysis of VIII in a similar fashion gave no reducing sugar.

Hydrolysis of 9-(3'-Acetamido-3'-deoxy-α-D-ribofuranosyl)-adenine (VIII).—A solution of 200 mg. of VIII in 4 cc. of 2% hydrochloric acid and 0.054 cc. of 12 *N* hydrochloric acid was refluxed for 22 hours. The solution, clarified with Norit, was warmed to about 60° and treated with a hot solution of 182 mg. of picric acid in 2 cc. of absolute alcohol. Yellow crystals of adenine picrate immediately separated, which were collected and washed with water and alcohol; yield 120 mg. (51%), m.p. 283° dec. (lit. m.p. 280° dec.).

The aqueous solution was washed with chloroform (6 × 10 cc.) to remove picric acid, then decolorized with Norit and evaporated to a sirup *in vacuo* (bath 40–45°). The residue was dissolved in acetic acid by addition of a few drops of water. The solution soon deposited white crystals of 3-amino-3-deoxy-D-ribose hydrochloride; yield 65 mg. (54%), m.p. 159° dec., which gave a positive Benedict test. Recrystallization by solution in 0.3 cc. of water and addition of 6 cc. of acetic acid gave 49 mg. of white crystals, m.p. 161° dec., $[\alpha]^{25}D$ −23° (2% in H_2O), which had an infrared spectrum identical with that of an authentic sample,[11] $[\alpha]^{25}D$ −25° (H_2O).

3'-Phthalimido-3'-deoxyadenosine. (A).—A mixture of 2.47 g. of chloromercuri-6-benzamidopurine,[9] 2.86 g. of Celite and 250 cc. of xylene was distilled until moisture was removed (about 75 cc. distilled). After the addition of a warm solution of 2,5-di-*O*-benzoyl-3-phthalimido-3-deoxy-β-D-ribofuranosyl chloride[18] in 50 cc. of xylene, the mixture was refluxed with stirring for 3 hours. The hot solution was filtered and the filter cake was washed with 50 cc. of hot toluene. The combined filtrate and washing was evaporated to dryness *in vacuo*. Meanwhile the filter cake was washed with four 25-cc. portions of boiling chloroform. The residue from the xylene solution was dissolved in the chloroform solution. Washed with 100 cc. of 30% aqueous potassium iodide, then water, the chloroform solution was dried with magnesium sulfate. Evaporation to dryness *in vacuo* left 3.3 g. (99%) of crude 6-benzamido-9-(2',5'-di-*O*-benzoyl-3'-phthalimido-3'-deoxy-β-D-ribofuranosyl)-purine as a cream colored glass.

To the 3.3 g. of blocked nucleoside was added 66 cc. of reagent methanol and 5.15 cc. of 1 *N* methanolic sodium methoxide. The mixture was refluxed for 40 minutes, solution being complete after 2 minutes at the b.p.; the *p*H remained <10 throughout the reaction when spotted on moist indicator paper. Evaporation to dryness *in vacuo* left an amorphous solid mixed with methyl benzoate. The latter was removed by trituration with ether and decantation. The ether-insoluble residue was warmed *in vacuo* to remove traces of ether, then dissolved in 20 cc. of dimethylformamide containing 0.54 cc. of acetic acid and refluxed for 30 minutes. The dark solution was evaporated to dryness *in vacuo*. Addition of 33 cc. of water gave a gum which gradually hardened. The crude solid was collected on a filter and washed with water; wt., 1.08 g. (58%). The crude phthalimido nucleoside (0.88 g.) was heated to boiling with 90 cc. of water. The solution was decanted from some insoluble tar. The cooled solution deposited 0.54 g. (61% recovery) of crystalline phthalimido nucleoside, m.p. and mixed with preparation B, 228–229°.

(B).—A mixture of 50 mg. of pure VI, 31 mg. of phthalic anhydride and 2 cc. of dimethylformamide was refluxed for 45 minutes, solution being complete at the b.p. The slightly darkened solution was evaporated to dryness *in vacuo*. The residue was heated on the steam-bath with 3 cc. of water which caused crystallization of the gum. The cooled mixture was filtered and the product washed with water; yield 51 mg. (69%), m.p. 222–224°. Recrystallization from water afforded nearly white crystals, m.p. 228–230°, $[\alpha]^{24}D$ −175° (0.6% in EtOH); λ_{max}^{KBr} 2.86, 2.94, 3.06 μ (OH), 5.62, 5.81 μ (phthalyl C=O), 6.05, 6.23 μ (C=N).

Anal. Calcd. for $C_{18}H_{16}N_6O_5$: C, 54.5; H, 4.07; N, 21.2. Found: C, 54.2; H, 4.01; N, 21.0.

Chromatographic Behavior of Nucleosides with the Carboxylic Acid Exchange Resin IRC-50. (A).—A chromatographic column (2 cm. diam.) was packed with 10 g. of wet Amberlite IRC-50 (H-form) resin (column height 9 cm.). To the top of the column was added a solution of 29.5 mg. of 6-dimethylamino-9-β-D-ribofuranosylpurine[20] in 3 cc. of water. The column was washed with water until ultraviolet inspection of the elution fractions no longer showed purine content (540 cc.). The combined eluates were evaporated to dryness *in vacuo*. Crystallization from methanol–acetone gave 19.6 mg. of starting material, m.p. and mixed m.p. 168–172°. This data showed that a "neutral" nucleoside could be recovered unchanged.

(B).—A solution of 29.5 mg. of 6-dimethylamino-9-(3'-amino-3'-deoxy-β-D-ribofuranosyl)-purine[1] (I) in 3 cc. of water was added to the top of the column described under (A). The column was washed with 300 cc. of water; ultraviolet inspection of the eluate fractions showed that no purine containing material has been eluted. The column was then eluted with 2 *N* ammonium hydroxide. Ultraviolet analysis showed that the first two 15-cc. fractions contained 15.3 mg. of purine derivative. An additional five 15-cc. fractions contained the remainder of the nucleoside in diminishing amounts. The combined eluates were evaporated to dryness *in vacuo*. Recrystallization of the residue from absolute alcohol with the aid of Darco gave 21.9 mg. of starting material, m.p. and mixed m.p. 215–218°. The column, when washed with 2 *N* hydrochloric acid, then water until neutral was again ready for another chromatogram.

3'-Amino-3'-deoxyadenosine (VI). (A).—A mixture of 1.68 g. of VII (about 80% pure) and 84 cc. of 0.5 *N* barium hydroxide was heated on the steam-bath for 1 hour, solution

(20) H. M. Kissman, C. Pidacks and B. R. Baker, THIS JOURNAL, **77**, 18 (1955), paper XI of this series.

being complete in a few minutes. The excess barium hydroxide was precipitated with solid carbon dioxide. After being filtered through Celite, the solution was added at the top of a chromatographic column containing about 10 g. of wet Amberlite IRC-50 (H-form). The column was washed with a total of 1150 cc. of 50% aqueous methanol when ultraviolet inspection showed that only a negligible amount of purine was in a 50-cc. cut. The pooled fractions showed a content of 472 mg. of nucleosides calculated for the mol. wt. of starting material.

The column was eluted with 400 cc. of 2 *N* ammonia in 50% aqueous methanol. The last 100-cc. cut showed by ultraviolet inspection only 4.3 mg. of nucleoside. The four 100-cc. aliquots were allowed to stand overnight. The first 100-cc. aliquot was the only one to deposit crystals; yield 245 mg. (17%), m.p. 263° dec. This filtrate was combined with the other three cuts and evaporated to dryness *in vacuo*. Crystallization of the residue from 20 cc. of water gave, after 2 days at 3° to complete crystallization, 366 mg. (total yield 26%) of product, m.p. 260–261° dec.

In a pilot run the yield was 31% (22 mg.), m.p. 259–260° dec., $[\alpha]^{25}D$ −40° (0.4% in dimethylformamide); $\lambda_{max}^{H_2O}$ 259 mμ (ε 15,000); λ_{max}^{KBr} 2.97, 3.12 μ (OH, NH), 5.98, 6.22 μ (C=N), 9.13, 9.24, 9.66 μ (OH and C–O–C).

Anal. Calcd. for $C_{10}H_{14}N_6O_3$: C, 45.1; H, 5.30; N, 31.5. Found: C, 44.8; H, 5.50; N, 31.1.

The β-configuration was established by periodate oxidation in *p*H 4.5 acetic acid–sodium acetate buffer. The resultant solution then had $[\alpha]^{24}D$ −24°, M_D −6400.[14,15] The oxidation was complete in a few minutes and did not change further after an additional 16 hours.

A similar hydrolysis and work-up of pure VIII gave a 46% yield of 9-(3′-amino-3-deoxy-α-D-ribofuranosyl)-adenine (XI) as an amorphous solid which could not be crystallized.

(B).—A mixture of 200 mg. of pure 3′-phthalimido-3′-deoxyadenosine and 1.35 cc. of methyl Cellosolve containing 0.027 cc. of 100% hydrazine hydrate was heated on the steam-bath for 1.5 hours. After 10 minutes solution was complete and after 15 minutes another solid began to separate. The mixture was treated with 0.28 cc. of acetic acid and 1.4 cc. of methyl Cellosolve and heated 10 minutes more. The mixture was evaporated to dryness *in vacuo*. Addition of 5 cc. of water left 73 mg. (89%) of insoluble phthalhydrazide. The solution was chromatographed as in procedure A to give 81 mg. (60%) of product, m.p. and mixed m.p. with preparation A, 264° dec. Both compounds had identical infrared spectra.

Similarly, hydrazinolysis of 200 mg. of crude 3′-phthalimido-3′-deoxyadenosine gave 55 mg. (41%) of product, m.p. 264° dec., identical with preparation A.

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[CONTRIBUTION FROM THE STAMFORD LABORATORIES, RESEARCH DIVISION, AMERICAN CYANAMID CO.]

Preparation of Ethylenimine and Triethylenemelamine

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An improved process for preparing triethylenemelamine from ethylenimine and cyanuric chloride has been developed. It features the use of an aqueous reaction medium and the generation of ethylenimine in solution, which obviates the isolation and handling of anhydrous ethylenimine. The rates of formation of ethylenimine from 2-aminoethyl hydrogen sulfate, 2-chloroethylamine hydrochloride, and 2-bromoethylamine hydrobromide have been compared. An improved laboratory preparation of anhydrous ethylenimine is described. Some solubility and stability data on triethylenemelamine are presented.

Triethylenemelamine, 2,4,6-tris-(1-aziridinyl)-*s*-triazine (I) was first prepared during World War II in Germany.[2,3] Called Persistol Hö 1/193, it was found to be very effective as a cross-linking agent for wool and as a hydrophobizing agent for regenerated cellulose. More recently it has shown promise as a chemotherapeutic agent against certain types of cancer.[4]

In the German method for preparing I,[2,3,5] ethylenimine is condensed with cyanuric chloride in benzene solution using triethylamine as hydrogen chloride acceptor. This process has several disadvantages. The use of benzene is objectionable on a large scale because of its inflammability. Also, its relatively low solvent power for the product I results in small yields per unit volume. Triethylamine is expensive to use as well as to recover. Finally, anhydrous ethylenimine is costly to prepare, is highly toxic, and can polymerize explosively.[6]

We have been successful in using water as the reaction medium for the preparation of I and have circumvented the use of anhydrous ethylenimine. In so doing, the disadvantages of the German process have been overcome.[7]

The use of an aqueous reaction medium has been possible because of the remarkable speed of the reaction at low temperatures and the unusual solubility properties of I (Table I).

TABLE I

APPROXIMATE SOLUBILITY OF TRIETHYLENEMELAMINE IN VARIOUS SOLVENTS AT 26°

Solvent	Solubility % by wt.	Solvent	Solubility % by wt.
Water	40.0	Ethanol	7.7
Chloroform	28.1	Benzene	5.6
Methylene chloride	19.7	Dimethyl cellosolve	4.8
Methanol	12.5	Methyl ethyl ketone	4.7
Nitromethane	(11.5)	Ethyl acetate	4.5
Acetone	10.6	Carbon tetrachloride	3.6
Dioxane	9.6		

(1) Olin Mathieson Chemical Corp., New Haven, Conn.

(2) R. A. Pingree and M. A. Dahlen, "Textile Finishing Treatments," PB-1576, Office of Technical Sales, Department of Commerce, Washington, D. C. Also published in Great Britain as B.I.O.S. Miscellaneous Report No. 18.

(3) H. Bestian, *Ann.*, **566**, 210 (1950), has published the chemistry of ethylenimine as investigated in the laboratories of I. G. Farbenindustrie A.G.

(4) F. C. Schaefer, J. T. Geoghegan and D. W. Kaiser, THIS JOURNAL, **77**, 5918 (1955). References to the literature pertaining to the use of triethylenemelamine in cancer therapy are cited.

(5) J. Heyna and W. Weibezahn, German Patent 859,025 (July 8, 1949).

(6) The hazards of handling ethylenimine are discussed by Pingree and Dahlen, ref. 2, Appendix XVI, p. 3.

(7) *Cf.* (a) V. P. Wystrach and D. W. Kaiser, U. S. Patent 2,520,619 (August 29, 1950); (b) D. W. Kaiser and F. C. Schaefer, U. S. Patent 2,653,934 (Sept. 29, 1953).