

crystd. Drying at 56° (0.05 mm) for 24 hr gave a white solid foam; $[\alpha]_{385} -82.6^\circ$, $[\alpha]_{436} -188.4^\circ$ (*c* 0.14, CHCl₃); uv max (0.1 N HCl) 270 m μ ($\log \epsilon$ 4.308); (H₂O) 277 (4.317); (0.1 N NaOH) 277 (4.317); ir (KBr) 3375 broad (OH, NH₂, NH), 1650 (amide C=O), 1590 cm⁻¹ (arom, NH₂); ir (CHCl₃) 1650, 1597; mass spectrum (probe temp ca. 260°), *m/e* above 80 (relative intensity) 439 (1.0), 422 (1.6), 421 (1.5), 420 (1.8), 419 (3.7), 418 (2.7), 405 (1.0), 404 (1.8), 403 (1.1), 319 (6.5), 318 (34.2), 301 (22.6), 300 (100), 289 (6.5), 271 (8.8), 228 (11.8), 190 (17.5), 165 (7.7), 164 (76.8), 163 (17.5), 150 (13.2), 148 (12.3), 134 (23.2), 122 (6.7), 121 (39.0), 120 (5.4), 109 (4.1), 82 (9.8), metastable transitions same as those of 19. Anal. (C₂₂H₂₉N₇O₅) C, H, N.

Hydrogenolysis of a mixt of 2a and 19a prepd by method B gave a 91% yield of 2 and 19 which, after sepn by chromatog, had $[\alpha]$ within experimental error of those of samples prepd by method A.

Acknowledgments. The excellent technical assistance of Maxine Palm is acknowledged. The authors also are indebted to Dr. Herb Nagasawa for providing the data on nephrotoxicity studies.

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Identification and Synthesis of the Major Nucleoside Metabolite in Rabbit Urine after Administration of Puromycin Aminonucleoside^{1†}

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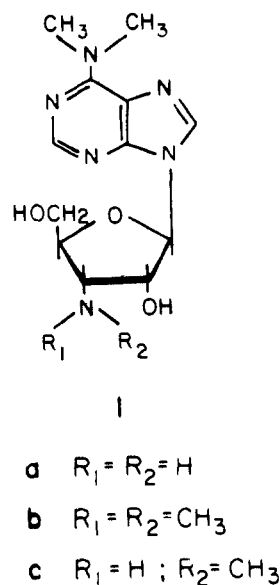
9-(3'-Amino-3'-deoxy- β -D-ribofuranosyl)-6-dimethylamino-9H-purine (1a), the aminonucleoside of puromycin, when administered to rabbits is monodemethylated at the 6-N position to give 9-(3'-amino-3'-deoxy- β -D-ribofuranosyl)-6-methylamino-9H-purine (9), the latter constituting the major nucleoside metabolite of 1a in the urine. The 3'-N-acetylated derivative of the metabolite, 9, *i.e.*, 9-(3'-acetamido-3'-deoxy- β -D-ribofuranosyl)-6-methylamino-9H-purine (8) was identical in all respects (tlc patterns, degradation products, mass spectral fragments) to 8 synthesized chemically by methylation of 9-(3'-acetamido-3'-deoxy- β -D-ribofuranosyl)-6-amino-9H-purine (6) on the 1 position with MeI, followed by rearrangement in dil NH₄OH. Contrary to earlier speculations, rabbits do not metabolize 1a by methylation on the 3'-amino group of the amino ribose moiety, as shown by comparison of the urinary metabolites of 1a with chemically synthesized 3'-N-methylated derivatives of 1a, *viz.*, 9-(3'-methylamino-3'-deoxy- β -D-ribofuranosyl)-6-dimethylamino-9H-purine (1c), and 9-(3'-dimethylamino-3'-deoxy- β -D-ribofuranosyl)-6-dimethylamino-9H-purine (1b).

The aminonucleoside 1a produced by the Edman degradation of the antibiotic puromycin and independently synthesized, by Baker, *et al.*,^{2,3} exhibits trypanocidal as well as antitumor properties.^{4,5} The appearance of massive, though reversible, proteinuria frustrated clinical trials of 1a as a tumor chemotherapeutic agent in man.⁶ When ad-

ministered to rats by oral, sc, or ip routes, 1a elicits a nephrotic syndrome characterized by hypoproteinemia, hyperlipidemia, hypercholesterolemia, proteinuria, edema, and ascites—a syndrome that is clinically indistinguishable from the kidney disease frequently observed in children.⁷ 1a has since been utilized extensively for the experimental induction of this disease in rats.⁸

The striking species susceptibility to toxicity by 1a manifested by its lack of nephrotoxicity in mice, guinea pigs, or rabbits, does not appear to be reflected in differential

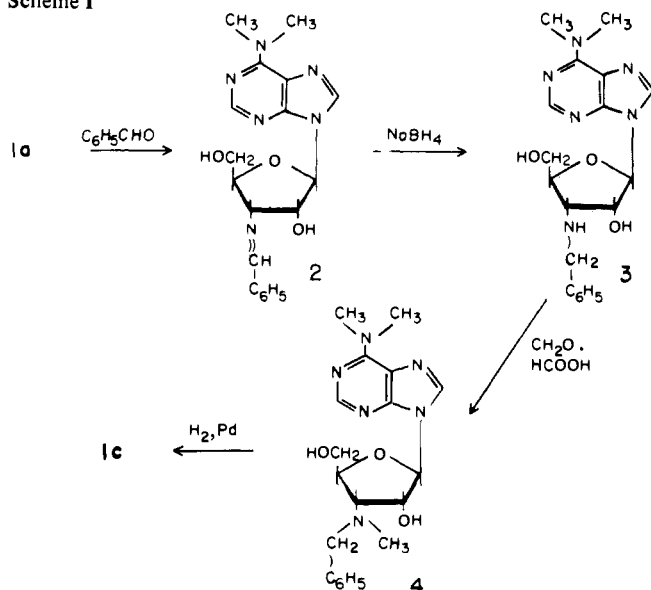
†This work was supported in part by Grant HE-04983, United States Public Health Service, and Grant 65G078, American Heart Association, and was presented in part at the 52nd Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N. J., April 18, 1968.



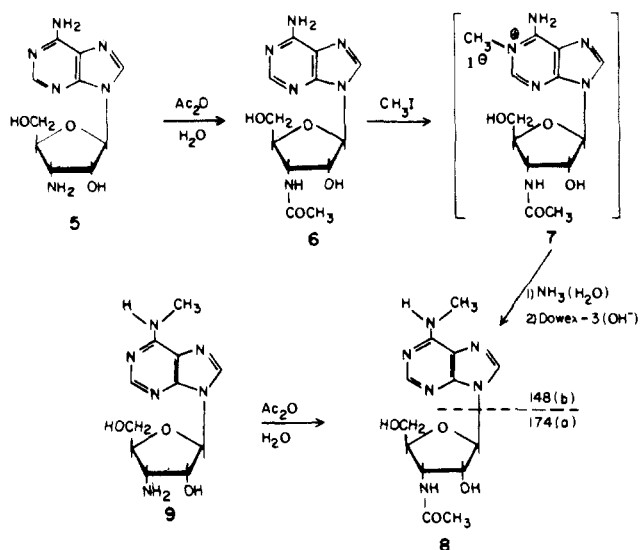
metabolic handling of the drug by the different species. Thus, the metabolic fate of 1a follows similar courses, qualitatively and quantitatively, in both susceptible (rat) and nonsusceptible species (guinea pigs, mice).^{9,10} Wilson, *et al.*,¹¹ have suggested that rabbits, which are refractory to toxicity by 1a, metabolize this compound differently from rats, and speculated that perhaps rabbits detoxified 1a by methylation of the 3'-amino group on the aminoribose moiety, although direct chemical evidence was not presented. This possibility is not without merit as rabbits are known to possess an enzyme system uniquely capable of N-methylating a variety of structurally unrelated amines quite nonspecifically, and this N-methyl transferase, which is found predominantly in lung tissue, is absent in the lung fractions of the rat, mouse, or guinea pig.¹² In the present study we have attempted to clarify this point by administration of 1a to rabbits and examining their urines for the 3'-N-methylated products, 1b and 1c.

Chemistry. Synthetic 3'-N-substituted dimethyl (1b) and monomethyl (1c) derivatives of 1a were required for comparison with the metabolites of 1a isolated from rabbit urine. The preparation of 1b by reductive methylation

Scheme I



Scheme II



of 1a with formaldehyde under catalytic hydrogenation conditions¹³ led to mixtures of the 3'-N-dimethyl (1b) and 3'-N-monomethyl (1c) derivatives as revealed by tlc, unless the catalyst was prereduced prior to addition of the Schiff base. Eschweiler-Clarke methylation of 1a also gave 1b in good yield and free from 1c.

An unambiguous synthesis of the 3'-N-monomethylated compd 1c is depicted in Scheme I. The benzilidene derivative 2 of the aminonucleoside 1a was reduced to the benzyl analog 3 with NaBH₄ in MeOH, and 3 methylated with CH₂O-HCO₂H to the benzyl methyl derivative 4. Catalytic hydrogenolysis of the benzyl group gave 1c.‡

9-(3'-Amino-3'-deoxy-β-D-ribofuranosyl)-6-methylamino-9H-purine (9), the major nucleoside metabolite of 1a in the rat, guinea pig, the mouse⁹⁻¹¹ has previously been prepared by solvolysis of the halogen in the corresponding blocked 6-chloropurineaminonucleoside with MeNH₂ followed by deblocking¹⁵⁻¹⁷ and by enzymatic demethylation of 1a.¹⁸ We have prepared 9 acetylated on the 3'-amino N, *viz.*, 8, by an alternate method (Scheme II) starting from 3'-amino-3'-deoxyadenosine (5), the antitumor principle isolated from the broth of *Helminthosporium* sp. No. 215.¹⁹ The latter has been synthesized previously by Baker, *et al.*²⁰ Treatment of 5 in aq soln with Ac₂O selectively acetylated the 3'-amino group to give 6. Methylation of 6 with MeI in DMA gave the 1-methiodide (7) which rearranged[§] in dil NH₄OH to 8.

Metabolism and Degradation Studies. After administration of 1a to rabbits, the nucleoside fraction isolated from urine by ion-exchange chromatography contained 3 aminonucleoside components separable and detectable by tlc (Figure 1A). One of these was unchanged 1a, but none of the metabolites corresponded in R_f to the 3'-N-methylated derivatives 1b and 1c prepared above. Although one of the two metabolites appeared to have low mobility such as would be expected for 3'-amino-3'-deoxyinosine, this product was present only in small amounts and our inter-

‡Lee, *et al.*,¹⁴ have recently reported the synthesis of 1b and 1c by somewhat different procedures.

§This rearrangement very likely proceeds by opening of the pyrimidine ring followed by recyclization in a manner similar to the rearrangement of 1-methyladenosine and 1-methyl-2'-deoxyadenosine to 6-N-methyladenosine and 6-N-methyl-2'-deoxyadenosine, respectively.²¹

Table I. Diagnostic Ions in the Mass Spectra of Puromycin Aminonucleoside Metabolite and Derivatives

Compound	Ion source temp, °C	M ⁺	Ion fragment ^a (<i>m/e</i> relative intensity)														
			M - 30	b + 60	b + 44	b + 30	b + 14	b + 2H	b + H	b	b + 2H - 29	b + H - 29	a	Other			
Ib	300	322/1.3	292/-	222/1.5	206/3.8	192/7.8	176/9.3	164/49.0	163/5.7	162/3.8	135/3.7	134/18.9	160/7.5	248/3.6 ^b	148/7.6 ^c	142/51.5 ^d	101/100 ^e
Ic	240	308/3.9	278/1.0	222/33.3	206/22.1	192/32.7	176/17.9	164/100	163/23.4	162/8.8	135/12.1	134/77.2	144/7.5	248/3.6 ^b	148/29.0 ^c	128/32.0 ^d	
Acetylated metabolite (8)	410	322/8.1	292/1.4	208/2.1	192/26.7	178/69.0	162/10.3	150/100	149/62.9	148/12.7	121/35.8	120/43.1	174/70.7	305/3.2 ^f	291/1.6 ^g	263/0.68 ^h	
Synthetic 8	360	322/12.8	292/1.8	208/2.3	192/29.7	178/79.7	162/24.4	150/100	149/65.6	148/12.2	121/29.7	120/37.5	174/75.6	305/3.9 ^f	291/1.6 ^g	263/0.46 ^h	
Metabolite (9)	400	280/2.5	250/0.1	208/11.1	192/14.6	178/36.5	162/10.4	150/100	149/34.7	148/10.7	121/26.7	120/34.1	132/0.2				

^ab = purine base fragment, a = sugar fragment. ^b(M + 86). ^c(b + H - 15). ^d(a - 18). ^e(C₅H₁₁NO). ^f(M - 17). ^g(M - 31). ^h(M - 59).

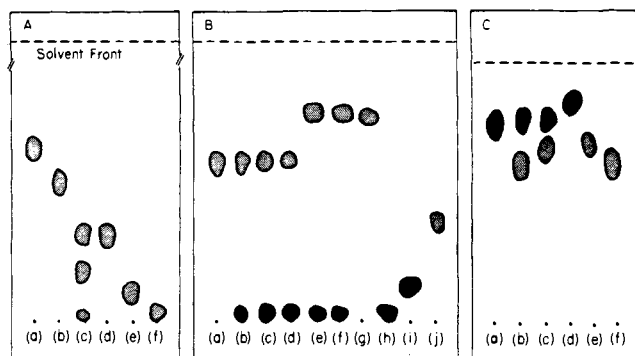


Figure 1. Tlc patterns of 1a metabolites and their degradation products. ○ = Fluorescence-quenching under 2537-Å lamp; ● = red after spraying with aniline phthalate reagent and heating at 110° for 0.5 hr. A. Silica gel HF₂₅₄, CMA-II. (a) 1b; (b) 1c; (c) crude nucleoside fraction from rabbit urine after administration of 1a; (d) 1a; (e) 5; (f) 3'-amino-3'-deoxyinosine. The patterns were essentially reversed on Silicar tlc 7-GF with 88% HCO₂H-abs EtOH-H₂O (3:16:3). B. Silicar tlc 7-GF, CMA-I. (a) 6-Methylaminopurine; (b) major nucleoside metabolite (hydrolyzed = hyd); (c) acetylated metabolite (hyd); (d) synth 8 (hyd), (e) 1a (hyd); (f) acetylated 1a (hyd); (g) 6-dimethylaminopurine; (h) synth 3-amino-3-deoxy-D-ribose; (i) D-ribose; (j) adenine. C. Silicar tlc 7-GF, 88% HCO₂H-abs EtOH-H₂O (3:32:6). (a) Synth 3-amino-3-deoxy-D-ribose; (b) 1a (hyd); (c) major nucleoside metabolite (hyd); (d) D-ribose; (e) 6-methylamino purine; (f) 6-dimethylaminopurine.

est was focused on the other metabolite which predominated. This major nucleoside metabolite, which was isolated by prep tlc, exhibited a uv absorption maximum at 266 nm (minimum, 236 nm), and gave a positive test with ninhydrin under alk conditions. Acetylation gave a product no longer chromogenic with ninhydrin reagent. Hydrolysis of either the metabolite or its acetylated derivative gave a sugar moiety which tested positive with ninhydrin, aniline phthalate, and alkaline AgNO₃ reagents, and a purine base whose uv absorption spectra in acid and alkali resembled that for 6-methylaminopurine. The tlc patterns of the hydrolysate compared against 6-methylaminopurine and synthetic 3-amino-3-deoxy-D-ribose in several solvent systems (Figures 1A and 1B) confirmed their identity, leading to the conclusion that this metabolite was 9-(3'-amino-3'-deoxy-β-D-ribofuranosyl)-6-methylamino-9H-purine, *i.e.*, 9.

Further evidence was adduced from analysis of the mass spectra of the metabolite and its acetylated derivative (Table I). The aminonucleoside metabolite exhibited a molecular ion at *m/e* 280, while the acetylated product showed the expected molecular ion at *m/e* 322. The presence of unsubstituted 5'-OH groups in both compounds was indicated by the (M - 30) peaks (loss of CH₂O) at *m/e* 250 and *m/e* 292. The (M - 59) peak (loss of CH₃CONH₂)^{22,23} at *m/e* 263 in the acetylated derivative verifies the chemical evidence that the acetylation occurred on the 3'-amino N. The series of ions (Table I) which include the purine base fragment b, the base plus various portions of the sugar skeleton, and the ions *m/e* 121 (b + 2H - 29) and *m/e* 120 (b + H - 29), clearly establish the purine base moiety of the molecule as 6-methylaminopurine.²⁴ Synthetic 8 had essentially identical mass spectral fragmentation patterns (Table I) as well as identical R_f values on tlc in several solvent systems as this acetylated metabolite, thereby fully identifying the latter as 8. It follows that the metabolite of 1a isolated from rabbit urine is, in fact, 9.

Thus, the early metabolic transformations of 1a in the rabbit appear to be similar to that observed in the other

species examined,^{9,10} the 6-N-monomethylated product of 1a, viz. 9, predominating, and no evidence for the presence of 3'-N-methylated metabolites of 1a could be found in the urine of rabbits dosed with 1a. Both New Zealand white and Shingler strain rabbits gave the same results, and no qualitative differences in the metabolic handling of 1a were observed in young or mature rabbits. Furthermore, incubation of 1a with S-adenosylmethionine and the soluble fraction from rabbit lung containing the N-methyl transferase described by Axelrod¹² failed to give 1b or 1c as determined by tlc.

Toxicity Studies. The nephrotoxicity of some of the intermediates and products synthesized in the course of this work was evaluated in rats by measuring their daily urinary protein excretion after administration of the compounds according to previously established protocols.^{25,#} The benzylidine derivative 2 (dose: 51 μ moles/kg per day for 24 days, sc) to male albino rats weighing 45–50 g caused the appearance of mild proteinuria commencing at day 14, which is 7–10 days later than the usual onset of massive proteinuria following treatment with equimolar doses of 1a. Ascites and edema were absent in these animals even at day 25; 2 is therefore comparable in nephrotoxicity to puromycin itself.²⁶ No evidence of nephrotoxicity to rats was observed with 1b (dose: 51 μ moles/kg per day for 23 days); however, rats given 1c (dose: 51 μ moles/kg per day for 27 days) developed slight proteinuria commencing on day 23 which persisted until the drug was withdrawn on day 28. Proteinuria gradually subsided thereafter and disappeared by day 41. Rats sacrificed on days 29 and 30 had 1–2 g of ascitic fluid in the peritoneal cavity.

Experimental Section**

Isolation of Nucleoside Metabolites of 1a from Rabbit Urine. 1a as a 1% aq soln was administered sc to male New Zealand white rabbits housed in metabolism cages (dose: 30 mg/kg per day in divided doses to 2 different sites) for 6 days. The daily urine collections were adjusted to pH 8.0 \pm 0.5, pooled, and stored refrigerated under toluene. Control urine samples collected for 6 days prior to administration of 1a were separately pooled and treated similarly in the following description. After centrifugation of the urine at 2500 rpm at +5° for 20 min, the clear amber supernatant was dild five- to tenfold with distd H₂O, adjusted to pH 1 with concd HCl, and charged on a 3.6 \times 14.5 cm column of Bio-Rad AG 50-X4 cation-exchange resin (100–200 mesh, NH₄⁺). The column was washed with 500 ml of 0.01 N HCl and then approx 60 ml of the discolored resin at the topmost portion of the column was withdrawn and transferred to a smaller column contg about 20 ml of fresh resin. The new column was washed with 3 l. of 0.05 M ammonium formate buffer (pH 5.9) to remove the free purine bases. The desired nucleoside fraction was obtained by elution with 3 l. of 0.2 M ammonium formate buffer (pH 7.9). This latter fraction was adjusted to pH 1 and desalted by (a) charging onto a fresh column of AG 50-X4 (NH₄⁺), (b) washing the column with distd H₂O, and (c) eluting the nucleoside metabolites with 1.0 N NH₄OH. Eluates having uv absorbance of >0.1 were pooled and lyophilized. This crude isolate contd 3 uv-absorbing compds as detd by tlc, one of which was unchanged 1a (Figure 1A). None of these spots corresponded in R_f to synthetic 1b and 1c, and control

urine samples did not show the presence of any of these components.

Purification and Acetylation of the Major Nucleoside Metabolite. The metabolite with R_f ~0.15 was sep'd from 1a and from the small amount of unknown product near the origin by preparative tlc twice on silica gel with CMA-II and eluted with 0.1 N NH₃. The eluate was conc'd to dryness, and the residue was recrystd from MeOH to give colorless crystals, mp 238–241°, uv max (H₂O) 266 nm, min 236 nm. This product on tlc or paper chromatog gave a blue-violet color with ninhydrin reagent at a level of 5 μ g, as did 1, 5, and 3'-amino-3'-deoxyinosine; but 1b, 1c, or acetylated 1a did not. This metabolite was therefore unsubstituted on the 3'-amino group of the amino sugar moiety. Treatment of this metabolite in H₂O with Ac₂O gave a product, mp 236–237° after recrystn from MeOH-Et₂O, which was no longer chromogenic with ninhydrin indicating that the 3'-amino group was now acetylated. The mass spectra of these products are recorded in Table I.

Hydrolytic Degradation Studies. The metabolite was hydrolyzed in 0.5 ml of 2 N HCl for 3 hr at 100°. For comparative purposes, the following compds were also subjected to the conds of this hydrolysis, viz., 1a, acetylated 1a, acetylated metabolite, 1b, 1c, and 8 prep'd synthetically (*vide infra*). 1b was not completely hydrolyzed under these conds, and some unchanged nucleoside was still detectable by tlc after the prescribed period (Silicar tlc 7-GF; 5% (NH₄)₂CO₃-95% EtOH, 90:7 or 90:3). The purines liberated from this hydrolysis were compared with 6-dimethylamino-purine, 6-methylaminopurine, and adenine, and the results (Figure 1B) showed the product from the metabolite to be 6-methylaminopurine, confirmed by comparison of its uv spectrum in 0.01 N HCl and 0.02 N NaOH with an authentic sample. The 3-dimethylamino-3-deoxy-D-ribose and the 3-methylamino-3-deoxy-D-ribose resulting from the hydrolysis of 1b and 1c, respectively, were not chromogenic with ninhydrin but gave the characteristic red color for pentoses with aniline phthalate reagent. The sugars liberated from all of the above nucleosides gave positive alk AgNO₃ tests, but only the sugar resulting from 1a, acetylated 1a, the metabolite, acetylated metabolite, and synthetic 8 had identical R_f values in two solvent systems as synthetic 3-amino-3-deoxy-D-ribose²⁷ (Figures 1B and 1C).

9-(3'-Dimethylamino-3'-deoxy- β -D-ribofuranosyl)-6-dimethylamino-9H-purine (1b). A. By Reductive Methylation. PtO₂ (500 mg) was prerduced in 50 ml of 95% EtOH-HOAc (1:1) at 1.05 kg/cm² for 30 min. The mixt was carefully equilibrated to the atm (caution: fire hazard) and 594 mg (2.02 mmoles) of 1a in 5 ml of 37% aq CH₂O was added followed by an addnl 50 ml of 95% EtOH-HOAc. The mixt was hydrogenated at room temp until H₂ uptake ceased (5 hr), the catalyst then was removed by filtration (Celite), the filtrate was conc'd to dryness, the residue was dissolved in abs EtOH, and the solvent was evap'd *in vacuo*. This was repeated until the odor of HOAc and CH₂O was no longer detectable. Recrystn of the crude product from EtOAc gave 519 mg (80.6%) of 1b, mp 184–185° (lit.¹⁴ mp 184.5–186°). A second crop, 106 mg, mp 183–184°, was obtained by concn of the mother liquor; total yield, 625 mg (97%); uv max (MeOH) 274 nm (ϵ 19,700); [α]_D²⁰ -60.1° (c 1.60, Py). *Anal.* (C₁₄H₂₂N₆O₃) C, H, N. If the catalyst was not activated by prerduction, or if the catalyst was Pd black, mixts of the mono (1c) and dimethyl (1b) derivatives were formed requiring extensive chromatog manipulation for sepn.

B. By Eschweiler-Clarke Methylation. 1a (147 mg, 0.50 mmole) in 2.0 ml of 88% HCO₂H and 0.5 ml of 37% aq CH₂O was heated under reflux for 2 hr. Tlc indicated that 1a had completely reacted by this time. The reaction mixt was dild with 2 vols of abs EtOH and conc'd to incipient dryness *in vacuo*, and the excess HCO₂H and CH₂O were removed as azeotropes by successive addn of PhMe and EtOAc followed by evapn. The glassy residue which was contaminated with the 5'-formyl derivative of 1b [ir (KBr) 1730 cm⁻¹ (ester); *m/e* 350 (M⁺)] was dissolved in MeOH contg 1.0 ml of Et₂NH and heated under reflux for 1 hr. Evapn of the solvent and recrystn of the residue from CH₃CN gave 122 mg (76% yield) of 1b, mp 184–185° (lit.¹⁴ 184.5–186°). An analytical sample recryst from CH₃CN gave the same mp. *Anal.* (C₁₄H₂₂N₆O₃) C, H, N.

9-(3'-Benzalimino-3'-deoxy- β -D-ribofuranosyl)-6-dimethylamino-9H-purine (2). A mixt of 1.00 g (3.40 mmoles) of 1a and 1.3 ml of freshly distd PhCHO in 30 ml of abs EtOH was heated on the steam bath under reflux for 2 hr. The solvent was evap'd to incipient dryness *in vacuo* and the glassy residue was crystd from EtOAc-petr ether to give 1.20 g (92% yield) of 2, colorless crystals, mp 162.5–164.5°; uv max (MeOH) 270 nm (ϵ 23,800). *Anal.*

#Doses are based on the weights of the animals on day 1.

**Melting points were taken on a Fisher-Johns mp apparatus and are corrected; optical rotations were detd in a Perkin-Elmer Model 141 polarimeter. Spectrophotometers used were: uv, Beckman DK-2A; ir, Beckman IR-10; ms, Hitachi-Perkin Elmer RMU-6 (ionization energy, 70 eV; ion source temps as indicated). Microanalyses by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y., or Galbraith Laboratories, Knoxville, Tenn. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within \pm 0.4% of the theoretical values. Tlc solvents: CHCl₃-MeOH-2.5 N NH₃ (50:20:3) (CMA-I); CHCl₃-MeOH-1 N NH₃ (5:2:1, lower phase) (CMA-II).

(C₁₅H₂₂N₆O₃) C, H, N. A second crop, 75 mg, obtained from the mother liquor melted at 158–161°.

9-(3'-Benzylamino-3'-deoxy-β-D-ribofuranosyl)-6-dimethylamino-9H-purine (3). To a stirred soln of 1.23 g (3.22 mmoles) of 2 in 50 ml of MeOH was added slowly at room temp, 600 mg of cryst NaBH₄. The reaction mixt was stirred at room temp for 15 min, then heated under reflux on a steam bath for 2.5 hr and filtered. The filtrate was concd to incipient dryness, and the solid residue was dissolved in 25 ml of H₂O. After multiple extns with EtOAc, the combined EtOAc exts were washed (H₂O), dried (Na₂SO₄), and reduced in vol when 3 crystd spontaneously, 1.06 g (86% yield), mp 165–167° (lit.¹³ 162–164°; uv max (MeOH) 274 nm (ε 19,400); [α]_D²⁵ -49.2° (c 1.60, Py) (lit.¹³ [α]_D²⁵ -44°, Py). *Anal.* (C₁₉H₂₄N₆O₃) C, H, N. In some runs recrystn of the crude product from EtOAc-petr ether or MeOH-Et₂O gave crystals melting at 122–125° or slightly lower. That this was a dimorphic cryst form of the higher melting product was indicated by (a) the identity of their R_f values on tlc (CMA-II); (b) the identity of their ir spectra in CHCl₃, and (c) the elemental analysis. *Anal.* (C₁₉H₂₄N₆O₃) C, H, N.

9-[3'-(N-Methyl,N-benzyl)amino-3'-deoxy-β-D-ribofuranosyl]-6-dimethylamino-9H-purine (4). 3 (384 mg, 1.0 mmole) in a mixt of 0.6 ml of 88% HCOOH, 0.3 ml of 37% aq CH₂O, and 3.0 ml of 95% EtOH was heated under reflux until tlc showed no trace of remaining 3 (10 hr). The solvent was evapd, and the residual glass was subjected to the action of a mechanical vac pump for 18 hr. Abs EtOH was added to dissolve the residue, and the EtOH was evapd *in vacuo*. This process was repeated until the odor of HCOOH was no longer detectable. The fluffy white solids were recrystd from EtOAc-petr ether, 345 mg (86.6%), mp 137–139°; uv max (MeOH) 274 nm (ε 19,800); [α]_D²⁵ -32.1° (c 0.93, Py). *Anal.* (C₂₀H₂₆N₆O₃) C, H, N.

9-(3'-Methylamino-3'-deoxy-β-D-ribofuranosyl)-6-dimethylamino-9H-purine (1c). A soln of 154 mg (0.462 mmole) of 4 in 6 ml of 95% EtOH-HOAc (1:1) was heated on the steam bath for a few min with a spatulaful of Pd black, and the catalyst removed by filtration. This procedure was necessary to remove traces of impurities which poisoned the catalyst. Fresh Pd black catalyst (78 mg) was added to the filtrate, and the mixt was hydrogenated at room temp in an all-glass micro hydrogenator until tlc (CMA-II) indicated complete removal of the benzyl group (3 hr). After removal of the catalyst, the solvent was evapd to dryness *in vacuo*, and the residue was triturated with ether to give 127 mg of crude product, mp 208–212° dec. Recrystn from abs EtOH gave 117 mg (82%) of 1c, mp 212–214° (lit.¹⁴ 216.5–218°); uv max (MeOH) 247 nm (ε 19,600); ir (KBr) 3275 (OH), 3125 (NH), 1595 cm⁻¹ (C=N). *Anal.* (C₁₃H₂₀N₆O₃) C, H, N.

9-(3'-Acetamido-3'-deoxy-β-D-ribofuranosyl)-6-amino-9H-purine (6). A 100-mg (0.37 mmole) sample of 5¹⁹ in 1.0 ml of H₂O was acetylated in the cold by addn of 200 μl of freshly distd Ac₂O. The reaction, monitored by tlc (CMA-I), was found to be complete within 1 hr. The mixt was evapd to dryness *in vacuo* and the residue recrystd from abs EtOH to give 57 mg (50% yield) of 6, mp 250–252°; ir (KBr) 1690 (amide I), 1660 (C=N), 1565 cm⁻¹ (amide II). *Anal.* (C₁₂H₁₆N₆O₄) C, H, N. The highest melting sample obtd was 258–259° dec (lit.^{22,20} 263–265°; 247° dec), but the mp (dec pt) appears to be a poor criterion for assessing purity, and tlc was relied upon. Crop 2 (33 mg) was obtd by diln of the mother liquor with Et₂O, homogeneous by tlc.

9-(3'-Acetamido-3'-deoxy-β-D-ribofuranosyl)-6-methylamino-9H-purine (8). To 74 mg (0.24 mmole) of 6 dissolved in 5 ml of dimethylacetamide was added 500 μl of MeI, and the reaction mixt was stirred at room temp until 6 disappeared (19 hr) as revealed by tlc (CMA-II). The reaction mixt was poured into 250 ml of petr ether, and the yellow oil that settled out was taken up in Me₂CO. The white hygroscopic product which pptd on addn of Et₂O was collected, washed (petr ether), and dried to give 11 mg of crude 7. This product was dissolved in 1 N NH₃ and passed through a column containing 10 g of 50–100 mesh Dowex-3 (OH) resin at a rate of 12–15 drops/min collecting 10-ml fractions. The tubes having uv max of 265 nm and min below 235 nm were combined and lyophilized. The residue was dissolved in abs EtOH and the EtOH evapd to remove traces of H₂O. The residue was

then extd several times with hot EtOAc and the combined EtOAc extracts concd to give 58.2 mg (73%) of crude 8, mp 219–223° dec. Recrystd from MeOH-Et₂O, mp 235–237° (lit.^{16,17} 229–230° and 233° for compd with 0.25 mole of H₂O). *Anal.* (C₁₃H₁₈N₆O₄·H₂O) C, H, N.

Acknowledgments. We are indebted to Dr. Nancy N. Gerber for an inoculum of *Helminthosporium* sp No. 215 as well as a sample of 5 isolated from this organism and to Dr. Elmer Reist for experimental quantities of chemically synthesized 5. Both samples behaved similarly in the tlc system described in the Experimental Section. We also thank K. F. Swingle and M. Coleman for technical assistance in the toxicity screening and J. McMahon for the mass spectra. Lederle Laboratories Division, American Cyanamide Co., provided generous samples of puromycin aminonucleoside.

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