NaOH—310 (19.6); $\tilde{\nu}$ (in cm^{-1}) 3400 (OH), 3140 (broad) (CH), 1590, 1545, 1475 (C=C, C=N), 1075, 1015 (POC); M_{Tlc} 0.67.

Anal. Calcd for $C_{10}H_{14}N_5O_6PS \cdot C_2H_5OH$: C, 35.21; H, 4.92; N, 17.11; P, 7.57. Found: C, 35.40; H, 4.95; N, 17.00; P, 7.50.

Bis[*cis*-3-(1,6-dihydro-6-thioxopurin-9-yl)cyclopentanemethyl] Phosphate (13).—To a cold solution of 221 mg (0.885 mmole) of *cis*-3-[6(1*H*)-thio-9*H*-purine]cyclopentanemethanol[12] in 20 ml of dry pyridine was added 113 mg (0.442 mmole) of *p*-nitrophenylphosphorodichloridate.[2] The resulting solution was stirred in the cold for 15 min and then left at room temperature for 20 hr. It was then poured into 25 ml of ice water and the resulting solution was evaporated to dryness *in vacuo*. The residue was dissolved in 10 ml of 0.3 *N* NaOH and the solution was left at room temperature for 2 hr. Upon neutralization (HCl), the solution deposited a gelatinous precipitate that was collected by filtration and purified by dissolving it in 50% $NaHCO_3$ followed by precipitation as a gel with AcOH; yield 72 mg (25%). The analytical sample was obtained in another run in which the crude material was purified by solution in 0.3 *N* NaOH and a precipitation by addition of concentrated HCl. It was dried at 100° (0.07 mm, P_2O_5, 20 hr); λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 0.1 *N* HCl—325 (35.9), pH 7—321 (38.7), 0.1 *N* NaOH—310 (40.8); $\tilde{\nu}$ (in cm^{-1}) 3420 (OH), 1585, 1525, 1465 (C=C, C=N), 1000 (POC); M_{Tlc} 0.58.

Anal. Calcd for $C_{22}H_{27}N_5O_4PS_2 \cdot 0.75H_2O$: C, 45.87; H, 4.99; N, 19.49; P, 5.36. Found: C, 46.02; H, 5.06; N, 19.60; P, 4.80.

Acknowledgments.—The authors are indebted to Dr. W. J. Barrett and members of the Analytical and Physical Chemistry Division of Southern Research Institute who performed most of the microanalytical and spectral determinations reported and to Dr. G. J. Dixon and Miss E. A. Dulmadge for the biological results reported.

Analogs of 6-Methyl-9-β-D-ribofuranosylpurine[1]

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Received July 18, 1967

6-Methyl-9-β-D-ribofuranosylpurine, 9-(2-deoxy-β-D-ribofuranosyl)-6-methylpurine, 6-methyl-9-β-D-xylofuranosylpurine, 2-fluoro-6-methyl-9-β-D-ribofuranosylpurine, and 6-ethyl-9-β-D-ribofuranosylpurine were prepared by fusion of the appropriate O-acetyl sugars and purines. The assignment of the anomeric configuration to the nucleosides thus obtained was based on an analysis of their pmr spectra. Evidence is presented that the cytotoxicity of these nucleosides, determined using human epidermoid carcinoma cells no. 2 in culture, may correlate with the efficiency with which they are converted to nucleotides by adenosine kinase.

The toxicity[2] and antitumor activity[3] of 6-methylpurine caused Gordon, *et al.*,[4] to synthesize its ribonucleoside, a compound that is more than 200-fold as cytotoxic to HEp-2 cells as 6-methylpurine itself.[5] In addition, a HEp-2 cell line that has lost AMP pyrophosphorylase and is resistant to 2-fluoroadenine (HEp-2/FA) and cross-resistant to 6-methylpurine is sensitive to 6-methylpurine ribonucleoside.[5] This activity can be explained by the fact that the ribonucleoside is an excellent substrate for adenosine kinase[6] and therefore can be converted to its cytotoxic form, the ribonucleotide, in cells lacking the pyrophosphorylase that normally converts the purine base to its ribonucleotide.

In pursuit of compounds with greater cytotoxic specificity for cancer cells than 6-methylpurine ribonucleoside, it seemed logical to select other nucleosides that should be substrates for adenosine kinase. Consequently, we selected the deoxyribonucleoside and the xylonucleoside of 6-methylpurine and the ribonucleosides of 2-fluoro-6-methylpurine[7] and 6-ethylpurine.[8]

These nucleosides were all prepared by the fusion method of Sato, *et al.*[11] Although an excellent preparative procedure, the fusion method is known to give rise to anomeric mixtures[12-18] even with sugars that exert steric control by orthoester ion formation in the halosugar–heavy metal purine derivative condensation.[19]

In all of the fusion reactions of 6-methylpurine (**1**), we observed formation of the *cis*(α anomer) as well as the *trans* (β anomer) nucleoside. However, in contrast to the results of Lee, *et al.*,[12] who obtained approximately equal amounts of α and β anomers from the fusion of tetra-*O*-acetyl-D-xylofuranose and *N*-nonanoyladenine, we obtained roughly 10β to 1α in the

(1) This work was supported by funds from the C. F. Kettering Foundation and the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. PH43-64-51.

(2) F. S. Phillips, S. S. Sternberg, L. Hamilton, and D. A. Clark, *Ann. N. Y. Acad. Sci.*, **60**, 283 (1954).

(3) D. A. Clark, F. S. Phillips, S. S. Sternberg, and C. C. Stock, *ibid.*, **60**, 235 (1954).

(4) M. P. Gordon, V. S. Weliky, and G. B. Brown, *J. Am. Chem. Soc.*, **79**, 3245 (1957).

(5) L. L. Bennett, Jr., M. H. Vail, S. Chumley, and J. A. Montgomery, *Biochem. Pharmacol.*, **15**, 1719 (1966).

(6) H. P. Schnebli, D. L. Hill, and L. L. Bennett, Jr., *J. Biol. Chem.*, **242**, 1997 (1967).

(7) J. A. Montgomery and K. Hewson, *J. Am. Chem. Soc.*, **82**, 463 (1960).

(8) Although these compounds might be poorer substrates for adenosine kinase than 6-methyl-9-β-D-ribofuranosylpurine, they would be expected, by analogy with known substrates,[6,9] to be phosphorylated to some extent to the cytotoxic form. One factor in the cytotoxicity of purine nucleosides appears to be the ease of phosphorylation,[10] and selective action could result from differences in the kinase from normal and neoplastic cells. Such a difference might be more evident with poorer substrates.

(9) B. Lindberg, H. Klenow, and K. Hansen, *J. Biol. Chem.*, **242**, 350 (1967).

(10) L. L. Bennett, Jr., and J. A. Montgomery, unpublished observations.

(11) T. Sato, T. Simadate, and Y. Ishido, *Nippon Kagaku Zasshi*, **81**, 1140 (1960).

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(13) L. Pichat, P. DuFay, and Y. Lamorre, *Compt. Rend.*, **259**, 2453 (1964).

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(16) K. Imai, A. Nohara, and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **14**, 1377 (1966).

(17) K. Onodera, S. Hirano, H. Fukumi, and F. Masuda, *Carbohydrate Res.*, **1**, 254 (1965).

(18) K. Onodera and H. Fukumi, *Agr. Biol. Chem.* (Tokyo), **27**, 864 (1963).

(19) B. R. Baker, *Ciba Found. Symp. Chem. Biol. Purines*, 120 (1957).

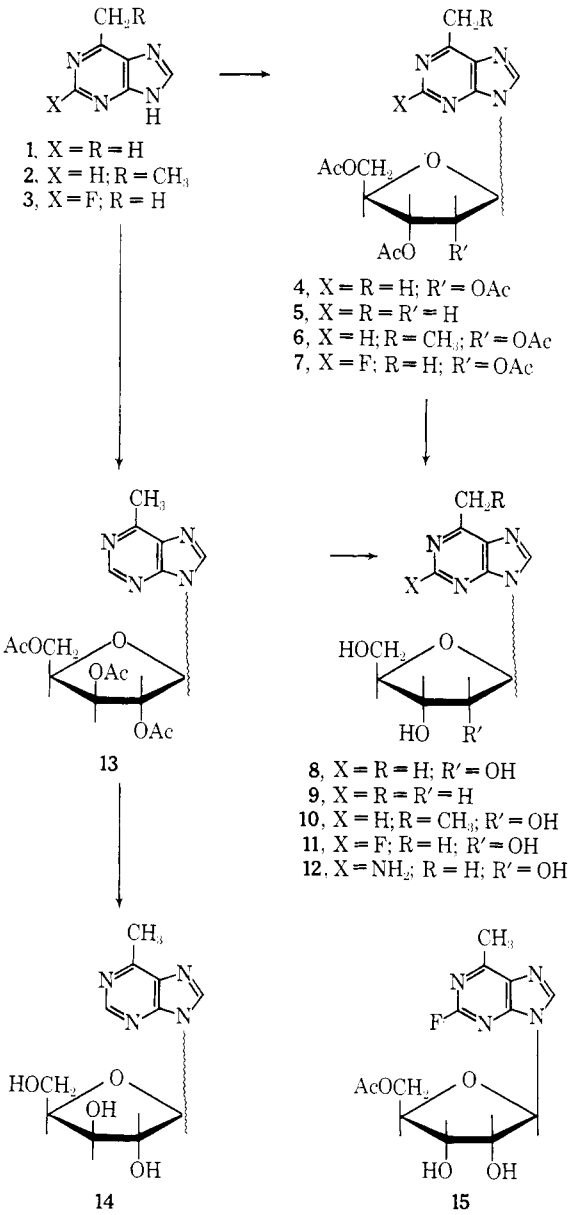
fusion of 6-methylpurine with this sugar[20] and with tetra-*O*-acetyl-β-D-ribofuranose. On the other hand Pichat, DuFay, and Lamorre[13] have found that the fusion of N-acetyladenine and tetra-*O*-acetyl-β-D-ribofuranose gives almost equal quantities of 9-α- and -β-D-ribofuranosyladenines. Thus it would appear that the anomer ratio formed, or at least isolated, from the fusion reaction may depend more on the nature of the purine base than on the sugar,[22] provided that the sugar contains a 2-acyloxy group, since proton magnetic resonance spectral analysis of the reaction mixture from the fusion of tri-*O*-acetyl-2-deoxy-D-ribose with 6-methylpurine showed that approximately equal amounts of α and β anomers were formed in this case in which the 2-acyloxy group is not present.[23] The present work coupled with previous observations on the fusion of tetra-*O*-acetyl-D-ribofuranose, however, tend to support the conclusion that, in general, the 2-acyloxy group does exert steric control in the fusion reaction, presumably through the orthoester ion intermediate postulated for the halosugar–heavy metal purine derivative coupling.[19,25]

The crude nucleosides (**5–7**, **13**) prepared by the fusion reaction were freed from unchanged sugar and other contaminants by chromatography on silica gel (see Experimental Section) before removal of the acetyl group by treatment with ethanolic ammonia (Chart I). The pure β anomers of the desired nucleosides (**8–11**, **14**) were obtained by recrystallization. Small amounts of the α anomers of **8** and **14** were also obtained pure. In the case of the 2-deoxyribonucleoside **9**, more α than β anomer was isolated pure.

9-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-2-fluoro-6-methylpurine (**7**) was obtained as a pure, crystalline solid, which gave, on treatment with liquid ammonia, a 1:2 mixture of the desired 2-fluoro-6-methyl-9-β-D-ribofuranosylpurine (**11**) and the partially deacylated compound, 9-(5-*O*-acetyl-β-D-ribofuranosyl)-2-fluoro-6-methylpurine (**15**). Treatment of this mixture, which could not be easily resolved, with ethanolic ammonia at 5° gave a mixture of **11** and 2-amino-6-methyl-9-β-D-ribofuranosylpurine (**12**). Finally **11** was obtained, along with some **15**, by the use of diisopropylamine in ethanol. In this case **11** could be freed of **15** by recrystallization. The identity of **12** was established by means of its ultraviolet and infrared spectra. Compound **15** was identified by its ultraviolet and infrared spectra and by the fact that it gave a positive metaperiodate–Schiff test for *cis* vicinal hydroxyl groups.

The anomeric configuration of these nucleosides was established by their pmr spectra (Table I). It has been fairly well established by now that the C-1′ proton of a *cis*-furanosylpurine occurs downfield from the C-1′ proton of the corresponding *trans*-furanosylpurine.[16,27,28]

CHART I



Thus the β (*trans*) configuration was established for the major product of the reaction of **1** and **2** with 1,2,3,5-tetra-*O*-acetylribofuranose and with 1,2,3,5-tetra-*O*-acetylxylofuranose.[29] The identity of β-**7** was established by a comparison of its pmr spectrum with those of α- and β-**4**. Finally the pmr spectrum, optical rotation, and melting point of 6-methyl-9-(2′-deoxy-α-D-ribofuranosyl)purine (**9**) was in agreement with literature values,[24] and pmr spectrum of the β anomer (**9**) showed the pseudo-triplet with an apparent coupling constant of 6.6 cps typical of β-2-deoxyribonucleosides.

Biological Data.—The cytotoxicity of the nucleosides described in this paper to human epidermoid carcinoma cells no. 2 (HEp-2) in culture is compared with that of

(20) About the same isomer ratio was obtained with 2,6-dichloropurine and tetra-*O*-acetylxylofuranose.[21]

(21) J. A. Montgomery and K. Hewson, unpublished observations.

(22) However, the acyloxy group at C-1 of a sugar apparently does affect the yield of anomers.[17,18]

(23) Robins isolated only the α anomer from the fusion of 6-methyl purine with tri-*O*-acetyl-2-deoxy-D-ribofuranose, but gave no indication of anomer ratio formed in the reaction.[24]

(24) M. J. Robins and R. K. Robins, *J. Am. Chem. Soc.*, **87**, 4934 (1965).

(25) It should be remembered that in certain cases small amounts of *cis* nucleosides have been isolated from the chlorosugar–heavy metal purine derivative coupling.[26]

(26) See references in J. A. Montgomery and H. J. Thomas, *Advan. Carbohydrate Chem.*, **17**, 30 (1962).

(27) J. A. Montgomery and H. J. Thomas, *J. Am. Chem. Soc.*, **87**, 5402 (1965).

(28) T. Nishimura and B. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **13**, 803 (1965).

(29) The anomeric configuration of the 6-methyl-9-D-ribofuranosylpurine previously reported was not established nor was its optical rotation reported.[4] On the basis of melting points it would appear that this material is the β anomer that one would expect from Baker's *trans* rule.[19]

TABLE I

PROTON MAGNETIC RESONANCE DATA

No.	Compd	Anomer	Solvent	Chemical shifts (ppm) and coupling constants (cps) $H_{1'}$	$J_{H_1'}$	H_2	H_8
4	9-(2,3,5-Tri-*O*-acetyl-D-ribofuranosyl)-6-methylpurine	β	$CDCl_3$	6.23 (d)	5.0	8.82	8.45
		α	$CDCl_3$	6.73 (d)	*Ca.* 5.5	8.79	8.30
5	9-(3,5-Di-*O*-acetyl-2′-deoxy-D-ribofuranosyl)-6-methylpurine	β	$CDCl_3$	6.47 (q)		8.82	8.18
		α	$CDCl_3$	6.57 (q)		8.82	8.28
6	9-(2,3,5-Tri-*O*-acetyl-D-ribofuranosyl)-6-ethylpurine	β	$CDCl_3$	6.23 (d)	5	8.89	8.17
		α	$CDCl_3$	6.77 (d)	*Ca.* 5	8.89	8.32
7	9-(2,3,5-Tri-*O*-acetyl-D-ribofuranosyl)-2-fluoro-6-methylpurine	β	$CDCl_3$	6.15 (d)	5.5	...	8.13
13	9-(2,3,5-Tri-*O*-acetyl-D-xylofuranosyl)-6-methylpurine	β	$CDCl_3$	6.28 (d)	2.5	8.82	8.28
8	6-Methyl-9-D-ribofuranosylpurine	β	DMSO-d_6	6.00 (d)	6	8.75	8.68
		α	DMSO-d_6	6.40 (d)	*Ca.* 5	8.70	8.58
9	9-(2-Deoxy-D-ribofuranosyl)-6-methylpurine	β	DMSO-d_6	6.45 (t)	6.6	8.77	8.68
		α	DMSO-d_6	6.45 (q)	3 and 7	8.76	8.70
10	6-Ethyl-9-D-ribofuranosylpurine	β	DMSO-d_6	6.08 (d)	5	8.83	8.75
11	2-Fluoro-6-methyl-9-D-ribofuranosylpurine	β	DMSO-d_6	5.93 (d)	5.4	...	8.75
14	6-Methyl-9-D-xylofuranosylpurine	β	DMSO-d_6	6.02 (d)	1.8	8.77	8.58
		α	DMSO-d_6	6.47 (d)	*Ca.* 3.6	8.73	8.43

TABLE II

CELL CULTURE CYTOTOXICITY DATA[a]

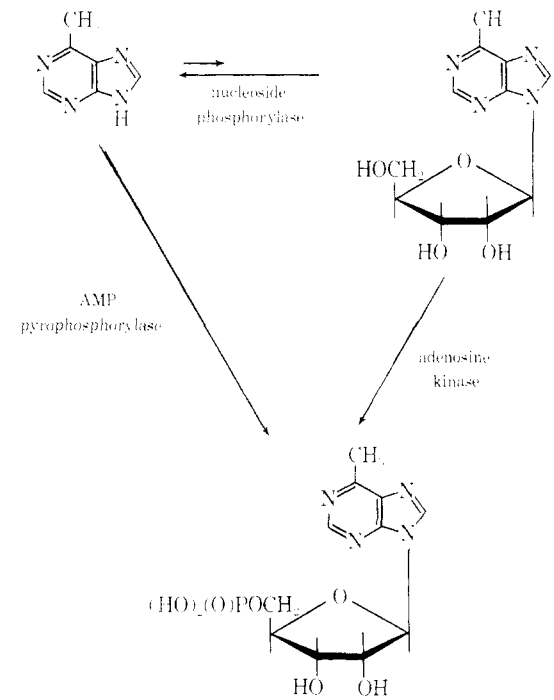
No.	ED_{50}, μmole/l.[b] /S	/FA	/MeMPR	/FA/FAR
1	0.75	>5	0.75	>150
8	0.004	<0.4	8	40–80
9	21.6			
10	0.43			
11	>3.5			
14	11.3		76	

[a] Human epidermoid carcinoma cells no. 2. [b] The concentration of compound required to inhibit the growth of treated cells to 50% of that of untreated controls as measured by colony counts.[5] Cell lines: /S = sensitive line, /FA = line resistant to 2-fluoroadenine, /MeMPR = line resistant to 6-methylthiopurine ribonucleoside, /FA/FAR = line resistant to 2-fluoroadenine and to 2-fluoroadenosine.

6-methylpurine (**1**) itself in Table II. 6-Methyl-9-β-D-ribofuranosylpurine (**8**) is about 140 times as toxic to the sensitive (or normal) HEp-2 cells as **1**, presumably because **8** is a better substrate for adenosine kinase than **1** is for adenylic pyrophosphorylase. The HEp-2/MeMPR cell line, which has lost adenosine kinase,[30] is 2000-fold resistant to **8** supporting the proposition that the great degree of cytotoxicity of **8** is due to phosphorylation to the nucleotide. The fact that **8** is moderately cytotoxic (8 μmoles/l.) to this cell line suggests that **8** itself may serve as an anitmetabolite or that it is cleaved by a phosphorylase to 6-methylpurine, which in this cell line could then be converted by adenylic pyrophosphorylase to the cytotoxic nucleotide. The fact that the HEp-2/FA/FAR cell line, which has lost both the adenosine kinase and adenylic pyrophosphorylase,[30] is five- to tenfold more resistant to **8** than the kinase-deficient line (HEp-2/MeMPR) argues that some cleavage to 6-methylpurine (**1**) must occur and that the nucleoside itself (**8**) is only weakly cytotoxic. These interconversions are pictured in Chart II. 6-Ethyl-9-β-D-ribofuranosylpurine (**10**) is only one-hundredth as toxic as **8** but still twice as toxic as **1**. Insertion of a fluoro group at C-2 produced

(30) L. L. Bennett, Jr., H. P. Schnebli, M. H. Vail, P. W. Allan, and J. A. Montgomery, *Mol. Pharmacol.*, **2**, 369 (1966).

CHART II



a compound (**11**) that was not toxic at the highest level tested. Changes in the sugar moiety gave moderate to slightly toxic compounds (**9** and **14**). Substrate specificity of adensoine kinase could explain these relative cytotoxicity values, since it is known, for example, that the xylosyl compound **14** is not a good substrate for the kinase.[6]

Experimental Section

The melting points reported were determined on a Kofler Heizbank and are corrected unless specified otherwise. The optical rotations were determined in the solvents specified with a Rudolph Model 80 polarimeter. Silica gel H (Brinkmann) was used for thin layer chromatographic analyses. Spots were detected with ultraviolet light after spraying the plates with Ultraphor WT highly concentrated (BASF Colors & Chemicals, Inc., Charlotte, N. C.). The pmr spectra were determined in DMSO-d_6 or $CDCl_3$ with a Varian A-60a spectrometer.

TABLE III
FUSION REACTION

Purine	Poly-O-acetyl-furanose	Molar ratio sugar/purine	% p-TSA[a]	Temp, °C	Fusion time, min	Crude yield, %[b]	Anomer ratio β/α	Purifn method	Solvent system	Yield of β anomer, %
6-Methyl-	2-Deoxyribo	3	2	160	15	75	*Ca.* 1	B	C_6H_6–Me_2CO (1:1)	46
6-Methyl-	Xylo	3	10	190	15	>59	*Ca.* 10	B	EtOAc	59
6-Methyl-	Ribo	3	10	180	45	78	*Ca.* 10	A	EtOAc	63
6-Ethyl-	Ribo	4	10	180	15	77	>10	A	EtOAc	77
2-Fluoro-6-methyl-	Ribo	2	2	170	10	80	>10	A	EtOAc–$CHCl_3$ (1:1)	80

[a] % by weight of purine. [b] No anomer separation.

Fusion Procedure.—A small round-bottom flask containing a dry mixture of the two reactants was evacuated with a water aspirator and heated with continuous stirring in an oil bath until vigorous gas evolution had ceased or until an essentially clear melt was obtained (10–20 min). The flask was removed from the heating bath, the catalyst was added, and the flask containing the reaction mixture was reevacuated and heated with continuous stirring for the desired period of time. After reaction was complete, the reaction melt was cooled to room temperature and dissolved in benzene (20 ml/mmole of purine) and the solution was washed (aqueous $NaHCO_3$ and then H_2O) before it was dried ($MgSO_4$) and concentrated to a small volume *in vacuo*. This crude product was purified by either column or preparative thin layer chromatography using silica gel (the method of purification was chosen on the basis of convenience for the size of the reaction). See Table III.

A. Purification by Column Chromatography.—A slurry of silica gel (50 g of SilicAR TLC-7, Mallinckrodt) in benzene was packed to give a 2.0 × 30 cm long column. The crude product (2–5 g in 10 ml of benzene) was absorbed on the column which was eluted with a total of 100 ml of benzene, and then the eluting solvent was changed to $CHCl_3$. Essentially all of the unreacted sugar was eluted before the $CHCl_3$ front. The desired nucleoside began to elute with the $CHCl_3$ front and the elution was continued until all of the desired β anomer had been eluted as evidenced by thin layer chromatography. Evaporation of the fractions containing the nucleoside to dryness *in vacuo* gave the purified product as an oil suitable for deacetylation.

9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-2-fluoro-6-methylpurine (7).—The column fractions of **7** solidified on evaporation to dryness. A sample (2 g) of this solid was dissolved in benzene (30 ml) and the solution was filtered through dry Celite before it was diluted with ligroin (1 vol.). The crystals that formed were collected by filtration, washed, and dried *in vacuo* to give the pure material: yield 1.3 g, mp 137°, $[\alpha]^{25}D$ −18.8 ± 0.2° (*c* 2.0 g/100 ml of MeOH).

Anal. Calcd for $C_{17}H_{19}FN_4O_7$: C, 49.75; H, 4.67; N, 13.66. Found: C, 49.81; H, 4.96; N, 13.74.

B. Purification by Thin Layer Chromatography.—Preparative thin layers 200 × 200 × 1 mm prepared from a methanol slurry of SilicAR TLC-7 (Mallinckrodt) were activated at 110° for 1 hr and were stored in a desiccator prior to use. The plates were developed a distance of 17 cm in unlined tanks. The bands were located by their ultraviolet light absorption at 256 mμ.

9-(3,5-Di-O-acetyl-2-deoxy-β-D-ribofuranosyl)-6-methylpurine (5).—The anomeric mixture of **5** was obtained by streaking the crude product (550 mg) dissolved in benzene (1 ml) on a preparative thin layer plate and developing the plate with EtOAc. The nucleoside band was eluted from the silica gel with EtOAc. Evaporation of this solution to dryness gave the pure mixture (260 mg) which was dissolved in benzene (1 ml) and rechromatographed on a preparative thin layer using C_6H_6–Me_2CO (1:1) as the eluent. Although separation of the anomers was not complete, bands separated that were of sufficient purity for use as intermediates. The band of the β anomer, which traveled ahead of the α anomer, was eluted from the silica gel with acetone. Evaporation of the Me_2CO solution to dryness gave the product as an oil.

9-(2,3,5-Tri-O-acetyl-β-D-xylofuranosyl)-6-methylpurine (13).—Crude **13** (700 mg) was dissolved in benzene (1 ml) and streaked on a preparative thin layer plate. The plate was developed with $CHCl_3$–EtOAc (1:1) for a distance of 6 cm, removed from the solvent, air dried, and returned to the solvent for complete development. The nucleoside band was eluted from the silica gel with MeOH. The MeOH solution was evaporated to dryness and the residue was dissolved in benzene and filtered to remove traces of silica gel, and the filtrate was evaporated to dryness to give the purified product as an oil.

Deacetylation Procedure.—A solution (2 mmoles/100 ml) of the acetylated nucleoside in EtOH saturated with NH_3 at 5° was refrigerated for 3 days before it was evaporated to dryness and the residue was dissolved in water. After extraction with $CHCl_3$ and filtration through dry Celite, the water solution was evaporated to dryness to give the crude product which was purified as follows.

6-Methyl-9-β-D-ribofuranosylpurine (8).[4]—Crude **8** (679 mg) was triturated with boiling EtOH (10 ml) and the mixture was cooled to room temperature before the insoluble solid was collected by filtration and recrystallized from EtOH (20 ml). Thin layer chromatography using $CHCl_3$–MeOH (4:1) as the eluent indicated the recrystallized product was anomerically homogeneous: yield 247 mg (45%), mp 209°, $[\alpha]^{23}D$ −52.1 ± 0.4° (*c* 1.0 g/100 ml of MeOH).

6-Methyl-9-(2′-deoxy-β-D-ribofuranosyl)purine (9).—Crude **9** (211 mg) was triturated with boiling EtOAc (5 ml) and the less soluble α anomer was removed by filtration. The purified β anomer (97 mg) that crystallized on chilling the filtrate was collected by filtration and recrystallized from acetone (4 ml) to give the anomerically pure product: yield 29 mg (13%), mp 153° (Mel-Temp). Thin layer chromatography using $CHCl_3$–MeOH (4:1) showed a single spot: $[\alpha]^{25}D$ −12.3 ± 0.2° (*c* 0.93 g/100 ml of MeOH).

Anal. Calcd for $C_{11}H_{14}N_4O_3$: C, 52.80; H, 5.64; N, 22.39. Found: C, 52.59; H, 5.80; N, 22.50.

A sample of chromatographically homogeneous α anomer was obtained by two recrystallizations of the EtOAc-insoluble solid from EtOH; mp 190–191° (Mel-Temp), $[\alpha]^{25}D$ +70.5 ± 0.2° (*c* 0.99 g/100 ml of MeOH).

6-Ethyl-9-β-D-ribofuranosylpurine (10).—Crude **10** (675 mg) was triturated with anhydrous ether and the insoluble solid that formed was collected by filtration and recrystallized. After two recrystallizations from acetone, the pure material was isolated as a monohydrate: yield 150 mg (23%), mp 105°. Thin layer chromatography using $CHCl_3$–MeOH (4:1) as the eluent indicated the product was anomerically homogeneous; $[\alpha]^{22}D$ −50.0 ± 0.4° (*c* 1.02 g/100 ml of MeOH).

Anal. Calcd for $C_{12}H_{16}N_4O_4 \cdot H_2O$: C, 48.36; H, 6.09; N, 18.80. Found: C, 48.28; H, 6.04; N, 18.66.

2-Fluoro-6-methyl-9-β-D-ribofuranosylpurine (11).—A solution of crude **11** (680 mg) in Me_2CO (50 ml) was filtered to remove insoluble pigments and the filtrate was concentrated *in vacuo* to 15 ml. The solid that formed in the concentrate after overnight refrigeration was removed by filtration and set aside and the filtrate was further concentrated. The concentrate was streaked on a preparative thin layer plate which was developed with $CHCl_3$–MeOH (9:1), air dried, and redeveloped with fresh solvent before the bands were eluted with warm EtOH. Evaporation of the faster moving major band to dryness gave 200 mg (37%) of impure 2-fluoro-6-methyl-9-β-D-ribofuranosylpurine (**11**) as identified by its uv spectrum. Elution of the slower moving band with EtOH gave additional acetone-insoluble solid which was pooled with the original acetone-insoluble solid to give a total of 189 mg (35%) of 2-amino-6-methyl-9-β-D-ribofuranosylpurine (**12**) as identified by its uv spectrum.

The analytically pure sample of **11** was obtained by deblocking with diisopropylamine. A solution of 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-fluoro-6-methylpurine (**7**, 410 mg, 1 mmole) and diisopropylamine (2 ml) in absolute EtOH (20 ml) was allowed to stand at room temperature for 3 days before it was evaporated to dryness. The residue was chromatographed on

a preparative thin layer plate using $CHCl_3$–MeOH (9:1) as the eluent. The major band was eluted from the silica gel with MeOH and the solution was evaporated to dryness *in vacuo*. The residue (137 mg) was dissolved in EtOAc (3 ml) and filtered through dry Celite, and the filtrate was refrigerated until crystallization was complete. The crystals were collected by filtration and recrystallized from EtOAc; yield 59 mg (20%), mp 174–176°, $[\alpha]^{25}D$ −40.9 ± 0.1° (*c* 0.86 g/100 ml of MeOH). Thin layer chromatography using $CHCl_3$–MeOH (4:1) as the eluent showed a single spot.

Anal. Calcd for $C_{11}H_{13}FN_4O_4$: C, 46.49; H, 4.61; N, 19.70. Found: C, 46.55; H, 4.74; N, 19.36.

6-Methyl-9-β-D-xylofuranosylpurine (14).—Crude **14** (646 mg) was triturated with anhydrous ether. The insoluble gum that formed was dissolved in EtOH (1 ml), crystallization was initiated by scratching, and the solution was diluted with additional EtOH and refrigerated overnight. The crystals (180 mg) that formed were collected by filtration, washed (EtOH, Et_2O), and recrystallized from EtOH (5 ml) to give the pure β anomer: yield 117 mg (24%), mp 162–163° (Mel-Temp), $[\alpha]^{25}D$ −53.6 ± 0.1° (*c* 1.03 g/100 ml of MeOH). Thin layer chromatography using $CHCl_3$–MeOH (4:1) as the eluent showed a single spot.

Anal. Calcd for $C_{11}H_{14}N_4O_4$: C, 49.62; H, 5.31; N, 21.05. Found: C, 49.91; H, 5.32; N, 21.04.

A sample of chromatographically homogeneous α anomer was isolated as an oil by repeated preparative thin layer chromatography using $CHCl_3$–MeOH (3:1) as the eluent; $[\alpha]^{25}D$ −10.4 ± 0.3° (*c* 1.0 g/100 ml of MeOH).

Acknowledgment.—The authors are indebted to Dr. W. J. Barrett and members of the Analytical and Physical Chemistry Division of Southern Research Institute for the spectral and microanalytical data reported, to Mrs. Martha Thorpe for her help in the interpretation of the pmr spectra, and to Mrs. Margaret H. Vail for the cytotoxicity data reported.

The Synthesis and Properties of 2,6-Dihydroxylaminopurine and Its 9-β-D-Ribofuranosyl Derivative[1]

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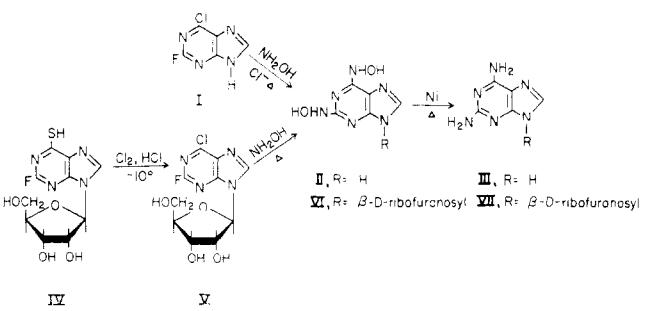
Received August 12, 1967

Syntheses of 2,6-dihydroxylaminopurine and its 9-β-D-ribofuranosyl derivative from the corresponding 6-chloro-2-fluoro compounds and hydroxylamine are described. The dihydroxylaminopurines were converted into the corresponding diamines by reduction with Raney nickel. Several mouse leukemias and Ridgeway osteogenic sarcoma were inhibited by 2,6-dihydroxylaminopurine, and the pattern of activity in various resistant lines of mouse leukemia suggested that this compound may exert its antileukemic effect by a mechanism similar to that of 6-mercaptopurine.

The biological activity shown by the adenine analog, 6-hydroxylaminopurine,[2] and the marked inhibitory effect of its 9-β-D-ribosyl derivative on mouse leukemias[3] stimulated our interest in the synthesis of the corresponding 2,6-dihydroxylamino compounds. These derivatives can be considered as analogs of 2,6-diaminopurine, the first purine reported to exert an inhibitory effect on mouse leukemia.[4] A bishydroxylamino derivative, dihydroxyurea, has recently been found to be a powerful inhibitor of DNA biosynthesis in HeLa cells[5] and to be active against several experimental tumors.[6] Related hydroxylamine derivatives induce chromosomal aberrations in cultured mammalian cells and exert a direct degradative action on DNA.[7]

The bishydroxylamino derivatives were obtained by interaction of 6-chloro-2-fluoropurine or its 9-ribosyl derivative with ethanolic hydroxylamine. Reaction of 6-chloro-2-fluoropurine[8] (I) (Scheme I) with ethanolic

SCHEME I



hydroxylamine in the presence of chloride ions afforded 2,6-dihydroxylaminopurine (II) in 70% yield, which

(1) This investigation was supported by funds from the National Cancer Institute (Grant No. CA 08748), The Atomic Energy Commission (Contract No. AT[30-1], 910), and aided by Grant No. T-128F from the American Cancer Society, Grant T45, an Ethel A. Shaffer Memorial Grant for Cancer Research from the American Cancer Society, and U. S. Public Health Service Fellowship No. 1-F3-CA-32,812.

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