bath for 2 hr with protection from atmospheric moisture. The NaCl was removed by filtration and H_2O was added to the filtrate until turbitidity appeared. An amber oil formed within 20 min, which, on scratching, crystallized. The crystals were washed (H_2O) and recrystallized from 50% EtOH; mp 87–89°, yield 11.4 g (83%). *Anal.* ($C_{28}H_{38}O_{18}S \cdot H_2O$) C, H.

2,3,4-Tri-*O*-acetyl-α-D-ribopyranosyl Chloride (XXIX).—1,2,3,4-Tetra-*O*-acetyl-α-D-ribopyranose[31] was converted to XXIX by an adaptation of the Pacsu[20] procedure, yield 61%, mp 93–95°, lit.[31] mp 95°. *Anal.* ($C_{11}H_{15}O_7Cl$) C, H.

1-Chloro-2,5-di-*O*-acetyl-α-D-glucofururonolactone (XXXII).—1,2,5-Tri-*O*-acetyl-α-D-glucofururonolactone[30c] (XXXI) (85 g, 0.28 mole) was dissolved in 500 ml of $CHCl_3$ (U.S.P.) and 44 g (0.23 mole) of $TiCl_4$ was added slowly with stirring. After stirring for 3.5 hr at 25°, the solution was poured into 2 l. of ice water. The $CHCl_3$ layer was washed (5% $NaHCO_3$, H_2O), dried (Na_2SO_4), decolorized with charcoal, and concentrated to a syrup *in vacuo* at 50°. The syrup was dissolved in anhydrous ether and a fine white crystalline product precipitated, yield 20 g (26%), mp 152–157°, $[\alpha]^{25}D$ +240° (*c* 2.0, $CHCl_3$). *Anal.* ($C_{10}H_{11}O_7Cl$) C, H.

Goebel and Babers[30b] prepared XXXII by a different method and reported mp 107.5–108.5° and $[\alpha]^{20}D$ 95.5° (*c* 1.257, $CHCl_3$). It is possible that this large discrepancy in melting point and optical rotation represents two stereoisomers, although the optical rotation of XXXII reported here is close to that of the analogous 1-bromo-2,4-di-*O*-acetyl-α-D-glucofururonolactone, $[\alpha]^{25}D$ +236°, mp 138–130°.

Hydrolysis of 6-Purinyl Thioglycosides.[32] **(a) Acid Catalysis (Figure 1).**—Stock solutions were prepared to contain 0.23 μmole of thioglycosides/ml in H_2O. One milliliter of stock solution was diluted to 10.0 ml with appropriate buffers. Periodic readings of uv absorption were recorded at 280 mμ and 325 mμ. Decreased absorbance at 280 mμ with simultaneous increase at 325 mμ demonstrated hydrolysis of the thioglycoside with liberation of 6-MP in solutions with pH values below 5. Products of hydrolysis were further identified by paper chromatography. The thioglycosides here described were stable at neutral and alkaline pH values, but were readily hydrolyzed in dilute acid.

(b) Enzyme Catalysis.—Hydrolysis of purine thioglycosides is catalyzed by a wide variety of mammalian thioglycosidase.[2a] The action of hog liver thioglycosidase on MPG is illustrated in Figure 2.

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(32) Details concerning the kinetics of hydrolysis, the preparation, and the properties of mammalian and other thioglycosidases will appear in a subsequent publication.

The Synthesis and Biological Properties of Hydroxylaminopurines and Related Derivatives[1]

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Syntheses are described for the preparation of substituted hydroxylaminopurines, the related methoxyamino, methylhydroxylamino, methylhydrazino, and methylmercapto derivatives, and some ribonucleosides thereof. These compounds were tested against L1210 mouse leukemia. Two compounds, 6-methoxyaminopurine and 2-hydroxylamino-6-methylmercaptopurine, were active against the parent L1210 line but not against a subline resistant to 6-mercaptopurine, suggesting that they may be converted to active nucleotides by a mechanism similar to that of 6-mercaptopurine.

The marked inhibition of several mouse leukemias by 6-hydroxylamino-9-β-D-ribofuranosylpurine,[2] its 2-amino derivative,[3] and 2,6-dihydroxylaminopurine and its ribosyl derivative[4] indicates that hydroxylamino derivatives of purines or their nucleosides are worthy of further investigation as potential chemotherapeutic agents. We now report the synthesis and biological activity of other substituted hydroxylaminopurines as well as related methoxyamino, methylhydroxylamino, and methylhydrazino derivatives and their nucleosides.

Reaction of 8-methylthiopurine[5] (I) with ethanolic hydroxylamine in the presence of a catalytic amount of chloride ions[3] led to 8-hydroxylaminopurine (II) (Table I). When 2-fluoro-6-mercaptopurine[6] (III) was treated with the hydroxylamine solution, substitution of the 2-fluoro was accompanied by hydrolysis of the mercapto group, leading to the known[7] 2-hydroxylamino-6-hydroxypurine (IV). When 2-fluoro-6-methylthiopurine (V) was similarly treated, 2-hydroxylamino-6-methylthiopurine (VI) was obtained, even in the presence of a catalytic amount of chloride ions. This behavior contrasts with the ease of replacement of a 6-thiomethyl group by hydroxylamino when the C_2 is substituted by NH_2.[3]

Upon reaction with hydroxylamine in the presence of chloride ions, 2,6-dichloropurine[8] (VII) afforded 2-chloro-6-hydroxylaminopurine (VIII). This is analogous to the reported conversion of VII to 2-chloro-6-aminopurine upon aminolysis.[9]

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TABLE I: REACTIONS OF HYDROXYLAMINOPURINES AND RELATED DERIVATIVES

Starting material	R_1	R_2	R_3	R_4	Amount, g	Reagent[a]	Mol ratio (reagent: purine)	Reaction time, hr (reflux)	Reaction product	R_5	R_6	R_7	Yield, %	Mp, °C	Formula	Analyses
I[b]	H	H	SCH_3	H	4	A	15	6	II	H	H	$NHOH$	76	315	$C_5H_5N_5O$	C, H, N
III	F	SH	H	H	5	A	30	18	IV	$NHOH$	OH	H	54	260	$C_5H_5N_5O_4 \cdot 0.67H_2O$	C, H, N
V[b]	F	SCH_3	H	H	4.6	A	30	18	VI	$NHOH$	SCH_3	H	60	250	$C_6H_7N_5OS$	C, H, N, S
VII	Cl	Cl	H	H	3	A	20	1	VIII	Cl	$NHOH$	H	80	320	$C_5H_4N_5ClO$	C, H, N
IX	H	Cl	H	CH_2CH_2OH	1	A	50	3.5	X	H	$NHOH$	H	93	232	$C_7H_9N_5O_2$	C, H, N
XI	H	Cl	H	H	6.2	B	10	6	XII	H	$NHOCH_3$	H	64	196	$C_6H_7N_5O$	C, H, N
XIII	H	Cl	H	Ribosyl	2	B	15	1	XIV	H	$NHOCH_3$	H	95	202	$C_{11}H_{15}N_5O_5 \cdot 0.5H_2O$	C, H, N
XI	H	Cl	H	H	1.5	C	10	3	XV	H	$N(OH)CH_3$	H	68	265	$C_6H_7N_5O$	C, H, N
XIII	H	Cl	H	Ribosyl	2.9	C	10	1	XVI	H	$N(OH)CH_3$	H	91	202	$C_{11}H_{15}N_5O_5$	C, H, N
XI	H	Cl	H	H	5	D	6	6	XVII	H	$NHNHCH_3$	H	53	251	$C_6H_8N_6$	C, H, N
XIII	H	Cl	H	Ribosyl	2	D	6	2	XVIII	H	$NHNHCH_3$	H	93	224	$C_{11}H_{16}N_6O_4$	C, H, N
XIX	NH_2	Cl	H	H	3.5	D	10	3	XX	NH_2	$NHNHCH_3$	H	95	>300	$C_6H_9N_7 \cdot 1.5H_2O$	C, N; H[c]

[a] A, hydroxylamine; B, methoxyamine; C, methylhydroxylamine; D, methylhydrazine. The reagents were prepared by neutralization of their hydrochlorides with ethanolic KOH or with anhydrous NaOAc. The solutions were approximately molar. Methylhydrazine was used at 3 *M* concentration in EtOH. [b] $NH_2OH \cdot HCl$ as a catalyst was added in $1 \cdot 10^{-3}$ proportion. [c] H: calcd, 5.87; found, 5.06.

Pharmacological studies carried out with 6-hydroxylamino-9-β-D-ribofuranosylpurine in dogs and monkeys showed a toxicity manifested by methemoglobinemia. It is not known whether this toxicity can be attributed to a direct effect of the drug on the red blood cells or to liberation of NH_2OH from the purine derivative. It is possible that the hydroxylaminopurine derivative is hydrolyzed *in vivo* by adenosine deaminase to inosine and NH_2OH; the latter is known to produce methemoglobinemia.[10] Dehydroxylamination of 6-hydroxylamino-9-β-D-ribofuranosylpurine by ox heart adenosine deaminase has been described.[11] An analogous hydrolysis of 1-β-D-arabinofuranosylcytosine to the nontoxic metabolite 1-β-D-arabinofuranosyluracil is also known.[12] It was found by Schaeffer and Bhargava[13] that 6-substituted 9-(hydroxyethyl)purines, especially the 6-amino derivative, were effective inhibitors of adenosine deaminase. We have now synthesized the corresponding 6-hydroxylaminopurine derivative (X) from 6-chloro-9-(hydroxyethyl)purine (IX) for evaluation in this regard and as a potential chemotherapeutic agent.

The N-substituted hydroxylamino compounds were also prepared to test their possible inhibitory growth properties. 6-Methoxyaminopurine (XII) and its ribosyl derivative (XIV) and 6-methyl-N-hydroxylaminopurine (XV) and its ribosyl derivative (XVI) were prepared by reaction of the corresponding chloro compounds (XI, XIII) with methoxyamine and methylhydroxylamine, respectively.

Substituted hydrazines such as 6-methylhydrazinopurine and its 9-ribosyl and 2-amino derivatives were also synthesized (XVII, XVIII, and XX). Aromatic methylhydrazino derivatives have been found to exert a cytotoxic activity and have been widely studied.[14]

The hydroxylamino compounds described were reduced smoothly to the corresponding amino derivatives by treatment with Raney nickel in aqueous ammonia. In the case of the methylthio derivative (VI), a simultaneous desulfurization to 2-aminopurine occurred. 2-Chloro-6-hydroxylaminopurine (VIII) was converted to adenine.

Experimental Section[15]

The data relating to the synthesis of these compounds are listed in Table I and the uv spectra of some of the new derivatives are given in Table II. The remainder exhibit expected λ_{max} values (between 260–270 mμ) at pH 6.8.

Treatment with Raney Nickel of the Hydroxylamino Derivatives.—The hydroxylamino derivative (25 mg) was dissolved in

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(15) Uv spectra were determined with a Cary recording spectrophotometer, Model 11. Ascending paper chromatography was run on Whatman No. 1 paper in the following solvent systems: concentrated NH_4OH–H_2O–*i*-PrOH (10:20:70), *n*-BuOH–H_2O–AcOH (50:25:25), and 1 *M* NH_4OAc–EtOH (35:70). The determination of melting points was carried out with a Mel-Temp melting point apparatus and the temperatures were corrected. Analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. Where analyses are indicated by the symbols of the elements only, analytical results obtained for these elements were within ±0.3% of the calculated values.

TABLE II

UV SPECTRAL PROPERTIES OF SOME HYDROXYLAMINOPURINES

Compd	λ_{max}, mμ (ε × 10^{-3}) 0.1 N HCl	pH 7[a]	0.1 N NaOH	λ_{min}, mμ (ε × 10^{-3}) 0.1 N HCl	pH 7[a]	0.1 N NaOH
II	208 (33.70)	204 (24.15)	324 (7.32)	249 (2.84)	251 (3.32)	253 (2.16)
	295 (32.56)	241 (3.40)				
		285 (14.90)				
IV	208 (41.65)	210 (17.73)	216 (12.40)	243 (9.64)	247 (6.17)	268 (6.50)
	265 (15.94)	265 (7.40)	313 (13.61)	304 (3.87)	297 (1.84)	
	350 (26.96)	342 (17.34)				
VI	210 (24.70)	218 (15.30)	269 (17.71)	228 (20.95)	231 (8.54)	260 (14.92)
	244 (17.60)	246 (12.65)	294 (17.45)	237 (17.18)	275 (4.47)	321 (4.20)
	278 (24.56)	293 (17.17)	352 (4.50)	258 (13.75)		
	317 (21.30)			296 (15.72)		

[a] 0.1 *M* sodium phosphate solution.

aqueous 5% NH_4OH (10 ml), Raney nickel (100 mg) was added, and the mixture refluxed for 1–3 hr. The corresponding amino derivatives were identified by uv spectra (at pH 1, 7, and 11) and paper chromatography. In the case of 2-hydroxylamino-6-methylthiopurine (VI), a simultaneous desulfuration to 2-aminopurine occurred and 2-chloro-6-hydroxylaminopurine (VIII) was converted into adenine.

Biological Activity.—Purine derivatives were evaluated by methods previously described[12c] for their activity against L1210 mouse leukemia (Table III). Compounds were administered intraperitoneally to groups of ten mice daily for ten doses beginning 1 day after intraperitoneal injection of leukemic cells. Results (Table III) are expressed as increased life span (ILS, %) of treated animals as compared to controls. Compounds active against the parent L1210 line of leukemia were tested against a subline resistant to 6-mercaptopurine (L1210/6-MP).

Two compounds, 6-methoxyaminopurine (XII) and 2-hydroxylamino-6-methylthiopurine (VI), were active against L1210 but not L1210/6-MP. The antitumor activity of 6-MP has been ascribed to its conversion to an active nucleotide by IMP–GMP pyrophosphorylase. Since the L1210/6-MP line is deficient in this enzyme activity,[16] it is likely that these two purines are similarly transformed into active nucleotides by a mechanism analogous to that of 6-MP.

8-Hydroxylaminopurine (II) and 9-β-D-ribofuranosyl-6-methylhydroxylaminopurine (XVI) were tested against the Ridgway osteogenic sarcoma. Comparison of tumor growth in treated as compared to control mice showed no significant tumor inhibition.

9-Hydroxyethyl-6-hydroxylaminopurine (X) was evaluated as an inhibitor of adenosine deaminase, using the screening system proposed by Schaeffer and Odin.[17] Incubation of adenosine (0.066 m*M*) in phosphate buffer at pH 7.6 with adenosine deaminase (Sigma), in the presence of X in a concentration three times that of adenosine, resulted in a 20.2% inhibition of deamination of adenosine, measured as loss in optical density at 265 mμ between 1 and 6 min after addition of enzyme. This inhibition of adenosine deaminase is considered as not significant.

TABLE III

ACTIVITY OF PURINE DERIVATIVES AGAINST L1210 MOUSE LEUKEMIA

Compd	Leukemia	Dose, mg/kg[a] daily × 10	Survival, days (treated/control)	ILS, %[b]
II	L1210	50	9.0/8.0	+12
VIII	L1210	25	8.7/8.6	+1
XII	L1210	100	13.8/7.7	+79
	L1210/6-MP[c]	100	8.1/9.3	−13
XIV	L1210	400	8.6/8.1	+6
XV	L1210	100	9.3/8.1	+15
XVI	L1210	200	8.0/8.1	−1
XVII	L1210	50	9.3/9.5	−2
XVIII	L1210	50	8.9/9.5	−6
XX	L1210	50	9.2/9.5	−3
VI	L1210	12.5	14.0/8.6	+63
	L1210/6-MP[c]	25	8.7/9.2	−5
X	L1210	25	9.6/9.4	+2
6-Mercaptopurine	L1210	10	15.9/9.5	+68

[a] Dose reported in highest dose tested that did not result in acute toxicity (weight loss). [b] Increased life span. Values less than 25 are considered not significant; values from 25 to 50 are considered moderately significant; values greater than 50 are considered highly significant. [c] Resistant to 6-mercaptopurine.

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