a single spot on tlc on Brinkmann silica gel GF or polyamide NM_{254}; each gave combustion values for C, H, and N within 0.4% of theory.

***m*-(*o*-[*p*-Aminophenoxypropoxy]benzamidine (12) Dihydrochloride (Method C).**—To a solution of 3.5 g (10.0 mmoles) of **10** in 100 ml of EtOH containing 200 mg of 5% Pd-C was added 0.83 ml (10.0 mmoles) of 12 *N* HCl. The resulting mixture was shaken with H_2 at 2–3 atm; reduction was complete in 2 hr. The filtered solution was evaporated *in vacuo* and the residue recrystallized; yield 3.1 g (85%) of white crystals. See Table II for additional data.

***m*-(*p*-Carboxyphenoxypropoxy)benzamidine Toluenesulfate (9).** Solution of 0.511 g (10.0 mmoles) of **8** in 5 ml of 6 *N* HCl and 5 ml of HOAc was stirred at reflux for 16 hr, then evaporated *in vacuo*. Two recrystallizations from H_2O gave 0.210 g (43%) of white crystals, mp 213–215°. *Anal.* ($C_{24}H_{26}N_2O_8S$) C, H, N.

***m*-(*p*-Benzamidophenoxypropoxy)benzonitrile (41) (Method D).** A solution of 2.85 g (9.55 mmoles) of **31** in 200 ml of EtOH containing 0.30 g of 5% Pd-C was shaken with H_2 at 2–3 atm; reduction was complete in 1 hr. The filtered solution was evaporated *in vacuo* to yield 2.6 g (100%) of a colorless oil suitable for the next reaction.

To 2.0 g (7.45 mmoles) of the crude oil was added 20 ml of $CHCl_3$ and 1.1 ml (8.0 mmoles) of Et_3N followed by 0.92 ml (8.0 mmoles) of benzoyl chloride. The resulting solution was stirred at ambient temperature for 24 hr, then washed successively with three 30-ml portions of 1 *N* HCl, three 30-ml portions of 1 *N* NaOH, and three 30-ml portions of H_2O. The dried solution was evaporated *in vacuo*. Two recrystallizations from C_6H_6 afforded 1.8 g (65%) of white crystals, mp 150–153°. *Anal.* ($C_{30}H_{26}N_4O_3$) C, H, N.

***m*-[*m*-(*p*-Nitrobenzamido)phenoxypropoxy]benzamidine Toluenesulfonate (27) (Method E).**—To a solution of 0.310 g (0.86 mmole) of **12** in 2 ml of DMF was added 1.0 g of 4A Molecular Sieves (Linde) followed by 0.245 ml (1.75 mmoles) of Et_3N. To the resulting mixture was added a solution of 0.209 g (1.41 mmoles) of *p*-nitrobenzoyl chloride in 2 ml of DMF. The mixture was stirred 1 hr at ambient temperature, then poured into 30 ml of H_2O containing 0.380 g (2.0 mmoles) of *p*-toluenesulfonic acid. The crystalline product was collected and recrystallized from H_2O; yield 0.271 g (53%), mp 135–137°. See Table II for additional data.

***m*-(*p*-Phenylureido)phenoxypropoxybenzamidine Toluenesulfonate (26) (Method F).** To a solution of 0.30 g (0.48 mmole) of **5** in 2 ml of DMF was added 0.077 ml (0.48 mmole) of Et_3N followed by 0.129 g (0.50 mmole) of O-(*p*-nitrophenyl) *N*-phenylcarbamate.[9] The resulting solution was stirred at room temperature for 16 hr, then poured into 30 ml of H_2O, and the product was collected. Recrystallization from H_2O gave 0.20 g (72%), mp 134–135°. See Table II for additional data.

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Synthesis and Biological Activity of Some New N^6-Substituted Purine Nucleosides

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The synthesis of N^6-2-phenoxyethyl-, N^6-benzyl-, N^6-*o*-hexyl-, N^6-*o*-pentyl-, N^6-phenyl-, N^6-2-thienyl-, and N^6-2-ethoxyethyladenosines was carried out by quaternization of the N^1 of adenosine with the appropriate halide, followed by rearrangement to the product in aqueous NH_3, or by nucleophilic substitution of 6-chloropurine riboside with the appropriate amine. Also synthesized were the N^6-(Δ^2-isopentenyl) and N^6-allyl derivatives of the antibiotic tubercidin (7-deazaadenosine). The compounds were examined for biological activity in a number of test systems. All of the adenosine derivatives examined showed cytokinin activity in the tobacco pith bioassay. Similarly, at low concentrations (10^{-8} to 10^{-6} *M*), the N^6-substituted adenosines tested stimulated the growth of a human leukemic cell line (6410). At higher concentrations, they decreased the viability of this line of leukemic myeloblasts of line HR1K of Burkitt's lymphoma, and line LKH of leukemic lymphoblasts, whereas they were all ineffective against a culture of normal leukocytes. The N^6-substituted tubercidins on the other hand inhibited the normal leukocytes, but were variably effective against the tumor lines. Most of the compounds interfered with the growth of *Escherichia coli* and some with the growth of Sarcoma 180 cells *in vitro*. A moderate but significant increase in survival time of mice bearing leukemia L1210 was produced by four of the adenosine derivatives.

N^6-(3-Methyl-2-butenyl)adenosine or N^6-(Δ^2-isopentenyl)adenosine (IPA) occurs in sRNA[1] and was originally synthesized by Leonard, *et al.*[2,3] This nucleoside has high cytokinin activity.[3b,3] It also inhibits the growth of human myelogenous leukemic cells and certain mouse tumors[4,5] and has undergone preliminary clinical trials.[6] Because of these findings, several other N^6-substituted adenosines were synthesized and found to have biological activity.[7a] Additional N^6-substituted adenosines have now been prepared[7b] and their biological properties have been studied. The new series of compounds reported in this paper are the N^6-β-D-ribofuranosyl derivatives of those N^6-substituted adenine bases which have shown potent cytokinin activity as reported by Strong[8] and by Skoog, *et al.*[9a] They include the N^6-2-phenoxyethyl- (I), N^6-benzyl- (II), N^6-*n*-hexyl- (III), N^6-*n*-pentyl- (IV), N^6-phenyl- (V), N^6-thienyl- (VI), and N^6-2-

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Table I

Paper Chromatography and R_f Values of N^6-Substituted Purine Nucleosides

Compd	Solvent system[a] A	B	C	D	E	F
N^1-Isopentenyltubercidin	0.08	0.80	0.83	0.84	0.50	0.52
N^6-Isopentenyltubercidin	0.81	0.85	0.85	0.84	0.80	0.76
N^1-Allyltubercidin	0.04	0.67	0.66	0.65	0.48	0.47
N^6-Allyltubercidin	0.70	0.80	0.82	0.72	0.55	0.62
N^1-2-Phenoxyethyladenosine	0.03	0.77	0.85	0.75	0.60	0.61
N^6-2-Phenoxyethyladenosine	0.80	0.85	0.83	0.69	0.47	0.73
N^1-Benzyladenosine	0.07	0.78	0.69	0.61	0.42	0.76
N^6-Benzyladenosine	0.73	0.83	0.86	0.71	0.57	0.79
N^1-*n*-Hexyladenosine	0.02	0.82	0.85	0.73	0.64	0.63
N^6-*n*-Hexyladenosine	0.82	0.87	0.89	0.77	0.75	0.89
N^1-*n*-Pentyladenosine	0.02	0.81	0.82	0.69	0.62	0.55
N^6-*n*-Pentyladenosine	0.79	0.87	0.90	0.73	0.75	0.89
N^6-Phenyladenosine	0.80	0.81	0.88	0.66	0.50	0.82
N^6-2-Ethoxyethyladenosine	0.53	0.85	0.87	0.72	0.49	0.71
N^6-2-Thienyladenosine	0.73	0.82	0.85	0.67	0.51	0.78
Adenosine	0.14	0.52	0.57	0.33	0.25	0.52
Tubercidin (7-deazaadenosine)	0.23	0.60	0.62	0.48	0.34	0.47
N^1-Isopentenyladenosine	0.05	0.78	0.72	0.63	0.68	0.66
N^6-Isopentenyladenosine	0.78	0.86	0.87	0.80	0.78	0.88
N^6-Furfuryladenosine[b]	0.72	0.78	0.79	0.64	0.46	0.73

[a] The solvent systems used for descending chromatography (Whatman No. 1 paper) (measured by volume): A, EtOAc–*n*-PrOH–H_2O (4:1:2) (upper phase); B, *i*-PrOH–H_2O–NH_4OH (7:2:1); C, *i*-PrOH–1% aqueous $(NH_4)_2SO_4$ (2:1); D, *i*-PrOH–concentrated HCl–H_2O (680:170:144); E, *n*-BuOH–H_2O–concentrated NH_4OH (86:14:5); F, *n*-BuOH–AcOH–H_2O (5:3:2). [b] Purchased material.

Table II

Uv-Absorption Spectra of N^6-Substituted Purine Nucleosides

Compd	pH 1.0 λ_{max}, mμ	pH 1.0 $\epsilon \times 10^{-3}$	pH 7.0 λ_{max}, mμ	pH 7.0 $\epsilon \times 10^{-3}$	pH 12.0 λ_{max}, mμ	pH 12.0 $\epsilon \times 10^{-3}$
Tubercidin	271		270		270	
N^1-Isopentenyltubercidin[a]	273		273		272 (sh 265,[c] 295)	
N^6-Isopentenyltubercidin	275, 230	16.0, 20.5	275	16.3	275	16.1
N^1-Allyltubercidin[a]	273		273		272 (sh 265,[c] 295)	
N^6-Allyltubercidin	274.5, 231	13.8, 21.0	275, 210	14.2, 26.0	275	14.3
N^1-2-Phenoxyethyladenosine[a]	261		261		261 (sh 264,[c] 290)	
N^6-2-Phenoxyethyladenosine	263	21.0	268	20.0	268	20.7
N^1-Benzyladenosine[a]	260		260		260 (sh 268,[c] 290)	
N^6-Benzyladenosine	264	20.1	268	19.4	268	19.5
N^1-*n*-Hexyladenosine[a]	259		259		262 (sh 268,[c] 290)	
N^6-*n*-Hexyladenosine	263	20.2	267.5, 210	16.7, 20.0	267.5	18.1
N^1-*n*-Pentyladenosine[a]	260		260		262 (sh 265,[c] 285)	
N^6-*n*-Pentyladenosine	263	18.8	267.5, 210	16.4, 18.4	267.5	17.3
N^6-Phenyladenosine	273	18.3	288	19.1	288	18.9
N^6-2-Ethoxyethyladenosine	263	18.5	267, 211	17.6, 19.6	267	18.1
N^6-2-Thienyladenosine	270	18.7	272, 211	18.6, 20.0	272	19.1
N^6-Furfuryladenosine[b]	265	18.1	268, 211	18.6, 24.1	268	18.7

[a] Values determined on material eluted from chromatographic spots. [b] Material purchased from K & K Laboratories, Inc. [c] Note that the uv spectra of N^1-adenosine analogs exhibit a characteristic shoulder at pH 12 in addition to the maximum peak. In the adenosines this shoulder is to the right of the peak whereas in the tubercidins it is on the left.

ethoxyethyladenosines (VII). The properties and activity of N^6-furfuryladenosine (VIII) purchased commercially were also examined. Because of the potent cytotoxic effect of the antibiotic tubercidin (7-deazaadenosine), and because 7-deazaadenosine is more stable to metabolic degradation than is adenosine,[9b] the N^6-(Δ^2-isopentenyl)- and N^6-allyltubercidins were also prepared and examined.

Synthesis of these compounds followed, in the main, the procedures of Jones and Robins[10] and of Leonard, *et al.*,[3] by which the halide is treated with adenosine to yield the N^1-quaternized nucleoside which is then converted quantitatively to the N^6-substituted derivative by heating in aqueous NH_3. Only those halides in which the CH_2 adjacent to the halogen is activated by unsaturated carbon chains or aromatic rings reacted readily (20 hr) at ordinary temperatures, and gave better than 50% yields. The halides of saturated carbon chains reacted slowly and the temperature had to be elevated for over 72 hr to achieve a yield of 25% or less. Iodobenzene (aromatic ring not bearing a CH_2) did not react at all, even at 100° for 7 days. In this case the N^6 derivative was prepared from aniline and 6-chloropurine riboside. The tubercidin derivatives were prepared by N^1 condensation and rearrangement in the same manner as the adenosine derivatives.

Some halides like *n*-iododecane and cyclohexylmethyl bromide did not react with adenosine when *N*,*N*-dimethylacetamide was used as the solvent, even when heated. However, the addition of anhydrous K_2CO_3 to the reaction mixture at 35° resulted in about a 40–50% yield of the N^1 intermediate. When this

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reaction mixture was heated on a steam bath for 10 min rearrangement to the N^6 derivative took place.

In synthesizing the N^6-substituted adenosines no attempt was made to isolate the N^1 intermediates. Aliquots of the reaction mixtures were, however, chromatographed and the N^1 compounds thus obtained were characterized as to their R_f values and uv spectra. The remaining N^1 intermediates were converted quantitatively to the corresponding N^6-substituted adenosines by treatment with aqueous NH_3 on a steam bath, and these were then isolated and purified. The physical data of all these derivatives are given in Tables I and II.

Recently reported[11] was a group of N^6-alkylated adenines, having cytokinin activity, where the substituents were not hydrocarbons but saturated aliphatic ether chains containing four to six carbon atoms. A ribosyl derivative of one of these adenine derivatives, N^6-2-ethoxyethyladenosine, was also prepared. N^6-Furfuryladenosine[12] (kinetin riboside), because of its known cytokinin activity, was also included in this study.

In view of the fact that the new series of compounds reported in this paper are the nucleosides of those adenine derivatives which possess cytokinin activity, their evaluation as cytokinins was indicated. As is shown in Table III, all the compounds tested showed cytokinin activity. However, the concentrations at which the individual compounds exert their maximal stimulatory effects differ. It is of interest that in all cases where the maximum stimulation is reached at concentrations below the highest level tested (200 μg/l.), a further increase in concentration of the compounds is less effective in promoting growth. Similar optimum concentration ranges were observed with the N^6-substituted adenines examined by Skoog, *et al.*[9a] The reason for this phenomenon of decreasing growth rate after maximum stimulation by cytokinins is obscure.

TABLE III

CYTOKININ ACTIVITY OF SOME REPRESENTATIVE N^6-SUBSTITUTED ADENOSINES IN TOBACCO BIOASSAY[a]

Compd	Rel growth compared to control as 1.0		
	10 μg/l.	25 μg/l.	200 μg/l.
N^6-Furfuryladenine	2.85	6.70	10.85
N^6-Furfuryladenosine	1.43	2.85	10.50
N^6-*n*-Pentyladenine	14.65	7.65	3.57
N^6-*n*-Pentyladenosine	7.65	17.35	15.85
N^6-2-Thienyladenosine	4.42	3.85	8.10
N^6-*n*-Hexyladenosine	2.60	10.00	7.90
N^6-Phenyladenosine	1.30	2.83	3.45
N^6-Benzyladenosine	3.30	9.45	3.92
N^6-2-Ethoxyethyladenosine	1.82	1.50	5.55

[a] According to the method of Murashige and Skoog.[23]

A somewhat similar relationship is observed in a line of human leukemic myeloblasts (cell line RPMI-6410). As shown in Table IV some stimulation of growth of these cells is exerted at low concentrations (10^{-7}–10^{-6} M) of the adenosine analogs, followed by inhibition at increasing concentrations. Adenosine itself shows a stimulatory effect which persists even at those concentrations in which the N^6 derivatives inhibit. It remains to be established whether the two phenomena in the plant and mammalian cell cultures can be equated. The effects of the analogs on various other human cell lines and on Sarcoma 180 cells were examined with respect to loss of viability or inhibition of growth as shown in Tables V and VI. It is to be noted that no stimulation of the growth of S180 cells by low concentrations of these compounds has been observed.

TABLE IV

GROWTH STIMULATION OF HUMAN LEUKEMIC CELLS (6410) *in Vitro*[a] BY N^6-SUBSTITUTED ADENOSINES AT LOW CONCENTRATIONS

Compd	Concn, M	Cell conen[b] per ml × 10^5
Experiment 1[c]		
N^6-Furfuryladenosine	8.7×10^{-8}	6.2
	2.9×10^{-7}	6.5
	8.7×10^{-7}	6.0
	2.9×10^{-6}	4.6
	8.7×10^{-6}	1.7
	2.9×10^{-5}	1.0
N^6-Phenyladenosine	8.7×10^{-8}	5.9
	2.9×10^{-7}	6.5
	8.7×10^{-7}	6.0
	2.9×10^{-6}	6.2
	8.7×10^{-6}	4.1
	2.9×10^{-5}	4.0
N^6-2-Ethoxyethyladenosine	9×10^{-8}	6.9
	3×10^{-7}	6.4
	9×10^{-7}	5.9
	3×10^{-6}	6.1
	9×10^{-6}	4.2
	3×10^{-5}	4.1
Experiment 2[d]		
N^6-Allyladenosine[e]	3.3×10^{-8}	7.0
	3.3×10^{-7}	6.4
	9.8×10^{-7}	6.2
	3.3×10^{-6}	3.0
N^6-*n*-Propyladenosine[f]	3.2×10^{-8}	8.7
	3.2×10^{-7}	9.3
	9.7×10^{-7}	7.0
	3.2×10^{-6}	6.0
N^6-Isopropyladenosine[g]	3.2×10^{-8}	8.5
	3.2×10^{-7}	7.3
	9.7×10^{-7}	7.4
	3.2×10^{-6}	5.5
N^6-Isopentyladenosine[h]	2.9×10^{-7}	10.0
	2.9×10^{-6}	8.3
	8.7×10^{-6}	6.5
	2.9×10^{-5}	5.9

[a] The period of growth was 5 days and the medium in each case was replaced on the third day. In all cultures containing the drugs, cell viability was found to be 87–94%. [b] The synthesis and description of these compounds can be found in ref 7. [c] Inoculum, 5×10^4 cells/ml, viability = 80%; control, 4×10^5 cells/ml, viability = 87%. [d] Inoculum, 1×10^5 cells/ml, viability = 93%; control, 6.4×10^5 cells/ml, viability = 89%. [e] [f] [g] [h] The synthesis and description of these compounds can be found in ref 7.

In the human cell cultures (Table V) the N^6-substituted adenosines showed distinct selectivity. The majority of the compounds affected the viability of the human myeloblastic leukemia (line 6410) and the Burkitt's lymphoma cells (line HRIK), whereas the leukemic lymphoblasts (line LKID) were inhibited by only four of the analogs. The N^6-benzyl-, N^6-thienyl-, and N^6-furfuryladenosines were active in all neoplastic cell lines tested. N^6-Furfuryladenosine has also been found to be active against uterine fibroblasts.[12] Alkyla-

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Table V
Effects of the N⁶-Substituted Adenosines on the Viability of Human Cells in Culture

Compd	Concn, *M*, for 50% loss of viability[a] Cell line 6410 leukemic myeloblasts	Cell line LKID leukemic lymphoblasts	Cell line HRIK Burkitt's lymphoma	Normal human leucocytes cell line (RPMI 5287)
Adenosine	None[b]			
N⁶-Isopentenyladenosine	9×10^{-6}	None	None	*c*
N⁶-2-Phenoxyethyladenosine	None	2.6×10^{-5}	3.6×10^{-5}	None
N⁶-Benzyladenosine	2.8×10^{-6}	8×10^{-6}	4×10^{-6}	Sl growth
N⁶-*n*-Hexyladenosine	8.4×10^{-6}	None	1.1×10^{-5}	None
N⁶-*n*-Pentyladenosine	1.8×10^{-5}	None	None	None (80 hr)
N⁶-Phenyladenosine	None	None	1.1×10^{-5}	None (80 hr)
N⁶-2-Ethoxyethyladenosine	1.1×10^{-5}	None	None	Sl growth
N⁶-2-Thienyladenosine	2.7×10^{-6}	8.1×10^{-6}	2.7×10^{-6}	None
N⁶-Furfuryladenosine	4.3×10^{-6}	5.4×10^{-6}	2.9×10^{-6}	None
Tubercidin	1.8×10^{-6}	1.1×10^{-7}	None	3.7×10^{-5}
N⁶-Allyltubercidin	2.6×10^{-5}	3.6×10^{-6}	3.3×10^{-5}	2.6×10^{-6}
N⁶-Isopentenyltubercidin	None	1.2×10^{-6}	None	6.0×10^{-6}

[a] Viability after 48 hr exposure to the compound was measured by the dye exclusion method using trypan blue. [b] "None" indicates no loss of viability at a concentration of 10^{-4} *M*. [c] In another normal human cell line (RPMI 7666), IPA is moderately active.

Table VI
Growth Inhibitory Activity of N⁶-Substituted Adenosines

Compd	Molar concn for 50% growth inhib of *E. coli*	Molar concn for 50% growth inhib of S180 cells *in vitro*
Adenosine	1×10^{-6}	$>10^{-4}$
N⁶-Allyladenosine	$>10^{-3}$	2×10^{-5}
N⁶-2-Phenoxyethyladenosine	5×10^{-6}	$>10^{-4}$
N⁶-Benzyladenosine	8×10^{-5}	$>10^{-4}$
N⁶-*n*-Hexyladenosine	1×10^{-5}	8×10^{-5}
N⁶-*n*-Pentyladenosine	9×10^{-6}	$>10^{-4}$
N⁶-Phenyladenosine	$>10^{-3}$	$>10^{-4}$
N⁶-2-Ethoxyethyladenosine	$>10^{-3}$	7×10^{-4}
N⁶-2-Thienyladenosine	1×10^{-3}	$>10^{-4}$
N⁶-Furfuryladenosine (kinetin riboside)	8×10^{-6}	5×10^{-6}
N⁶-Furfuryladenine (kinetin)	1×10^{-5}	$>10^{-4}$
N⁶-Isopentenyladenosine	8×10^{-5}	1.7×10^{-5}
Tubercidin	$>10^{-3}$	6×10^{-7}
N⁶-Allyltubercidin	$>10^{-3}$	3.6×10^{-5}
N⁶-Isopentenyltubercidin	2×10^{-4}	3×10^{-5}

tion of the adenosine analog tubercidin (7-deazaadenosine) decreased its activity in the two leukemic cell lines but enhanced it in Burkitt's lymphoma. It is of interest that unlike IPA, which inhibits line 6410 but not LKID, N⁶-isopentenyltubercidin is inactive in line 6410 but is cytotoxic in LKID.

The cultures of normal leukocytes (RPMI-5287) were insensitive to all of the N⁶-substituted adenosines tested. Another normal human leucocyte cell line (RPMI 7666, Gerner) was found to be moderately sensitive to N⁶-isopentenyladenosine.[13] The alkylated tubercidins were more inhibitory in the normal cell line than was tubercidin.

The growth of Sarcoma 180 cells in culture (Table VI) was inhibited by some of these adensoine analogs, the most effective one being N⁶-furfuryladenosine. The adenine derivative, N⁶-furfuryladenine, on the other hand was noninhibitory. Tubercidin was quite effective in this system, while its alkyl derivatives were less inhibitory. The activity of N⁶-isopentenyltubercidin parallels that of IPA and because of the greater metabolic stability of 7-deazaadenosine as compared to adenosine renders it interesting for further study.

A moderate, but significant increase in the survival time of mice bearing leukemia L1210 resulted from treatment with N⁶-2-ethoxyethyl- and N⁶-furfuryladenosine (140 and 138%, respectively, at 200 mg/kg × 6). N⁶-benzyladenosine (141% at 125 mg/kg × 6), and N⁶-phenyladenosine (132% at 100 mg/kg × 6). The remaining compounds produced increases in survival time ranging from 117 to 132%. Some of these compounds also exerted activity against Ehrlich ascites carcinoma and interfered with the replication of the spleen focus forming virus, a member of the Friend leukemia virus complex.[14]

Most of the adenosine analogs inhibited the growth of *Escherichia coli* at 10^{-6} to 10^{-5} *M*, whereas the tubercidin derivatives showed little or no activity. In *Streptococcus faecalis*, however, the N⁶-substituted adenosines showed marked stimulation of growth. Since in this cell system adenosine itself is growth stimulatory it remains to be determined whether the effect of the analogs is exerted following cleavage of the side chain.

Tubercidin is highly inhibitory in *S. faecalis* (2×10^{-8} *M*), while the N⁶-allyl analog is noninihibitory and the N⁶-isopentenyltubercidin is ony moderately inhibitory (5×10^{-4} *M*).

From the point of view of structure–activity relationships it is interesting to note that the N⁶-substituted adenine bases which are good cytokinins yield nucleosides which are inhibitory to mammalian cells.

Experimental Section

Melting points were determined on a Mel-Temp melting point apparatus and are not corrected. Uv spectra were obtained on a Cary Model 14 recording spectrophotometer. Optical rotation was measured on a Jasco Model ORD-UV 5 optical rotatory dispersion recorder. The solvent systems used for descending chromatography are given in Table I below. Whatman paper No. 1 was used. Adenosine was purchased from Nutritional Biochemicals Corp., 6-chloro-9-β-D-ribofuransyl-9H-purine and 6-furfurylamino-9-β-D-ribofuranosylpurine from K & K Laboratories, Inc. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements are within ±0.4% of the theoretical values.

N⁶-2-Phenoxyethyladenosine (I).—Adenosine (2.67 g, 10 mmoles) was stirred and heated at 80° for a few minutes in 50 ml of DMF until dissolved to a clear solution. At 50°, β-bromo-

(13) J. T. Grace, Jr., unpublished data.

(14) R. A. Steeves, personal communication.

phenetole (6.03 g, 30 mmoles) was added and stirring was continued at 50° for 20 hr. Chromatographic tests on a drop of the reaction mixture showed that only about 10% of the N^1 analog was formed. The temperature was raised to 80° and stirring was continued for 24 hr when about 40% reaction had occurred. Further heating gave no improvement in yield.

The reaction mixture was evaporated to a syrup and diluted with H_2O (pH 3.5), an excess of concentrated NH_4OH was added (pH 12) the flask was stirred, and the contents were heated at 80–90° for 2 hr. Periodic additions of NH_3 were made to keep the pH above 10. The solution was evaporated *in vacuo* to remove excess NH_3 and filtered from the tarry residue. The aqueous filtrate was extracted three times with 200-ml portions of EtOAc. The EtOAc solution was dried (Na_2SO_4) and, after filtration, evaporated to dryness. Three 25-ml portions of absolute EtOH were added followed by evaporation. After standing in a refrigerator for 18 hr, the crystals were filtered from the EtOH, washed with cold EtOH, then with a 3:1 mixture of MeCN–EtOH, and finally with Et_2O, and dried: yield 0.42 g (11%), mp 166°, $[\alpha]^{25}D$ −35.6° (*c* 0.1122, H_2O).

Anal. ($C_{18}H_{21}O_5N_5 \cdot 0.5H_2O$) C, H, N. For chromatographic and uv data for this and the other compounds, see Tables I and II.

N^6-Benzyladenosine (6-benzylamino-9-β-D-ribofuranosyl-9H-purine) (II) has been studied by Doree, *et al.*,[15] together with N^6-furfuryladenosine (6-furfurylamino-9-β-D-ribofuranosyl-9H-purine) for conversion to inosine by adenosine deaminase in plants. These compounds are mentioned as having been prepared by the condensation of benzylamine and furfurylamine, respectively, with 6-chloropurine riboside (6-chloro-9-β-D-ribofuranosyl-9H-purine) by the method of Bullock, *et al.*[16] No physical properties were given.

The preparation of the compound is conveniently carried out by the adenosine N^1 quaternization reaction[5,10] as follows. To 140 ml of DMF (freshly distilled) was added adenosine (10.7 g, 40 mmoles) and the mixture was heated with stirring under N_2 at 80° until a clear solution was obtained and then cooled to 30°. Benzyl bromide (20.6 g, 120 mmoles) was added and stirring was continued at 38–40° under N_2 for 24 hr. Chromatographic evaluation showed about an 80% conversion of the adenosine to N^1-benzyladenosine. The reaction mixture, at 25°, was added to 1400 ml of Me_2CO to precipitate a white solid (of the hydrobromide) which was allowed to coagulate in the refrigerator for 18 hr. The clear supernatant solution was decanted and the precipitate was washed with Me_2CO then Et_2O and then dried. It was dissolved in 200 ml of cold water (pH 3), an excess of NH_4OH was added (pH 12), and the mixture was stirred on a steam bath (90–95°) for 3 hr. The pH was maintained between 10 and 12 by additions of NH_4OH. The mixture was treated as described for I except that proportionately greater solvent amounts were used: yield 5.79 g, mp 183°.

The original Me_2CO filtrate was evaporated, heated with an excess of NH_4OH, and after reduction in volume filtered. The filtrate was treated with Dowex 50 to absorb the benzylamine, extracted with EtOAc, and processed as above. An additional 1.42 g of product was obtained, mp 180°, total yield 50.6% based on adenosine, $[\alpha]^{25}D$ −61.7° (*c* 0.227, 95% EtOH). *Anal.* ($C_{17}H_{19}O_4N_5$) C, H, N.

N^6-*n*-Hexyladenosine (III). To 70 ml of DMF was added adenosine (5.34 g, 20 mmoles). The mixture was stirred and heated to 80° until a clear solution was obtained, and then cooled to 30°. Then 9.90 g of 1-bromohexane was added and the reaction mixture was stirred at 80° for 24 hr, following which it was worked up as above: yield 0.85 g (12.1%), mp 130°, $[\alpha]^{25}D$ −71.9° (*c* 0.0834, 95% EtOH). *Anal.* ($C_{16}H_{25}N_5O_4 \cdot 0.33H_2O$) C, H, N. By using an equivalent amount of 1-hexyl iodide, the yield was doubled.

N^6-*n*-Pentyladenosine (IV). To 40 ml of DMF was added adenosine (2.67 g, 10 mmoles) and the mixture was heated to 80° and stirred to dissolve to a clear solution. After cooling to 33°, 4.53 g of 1-bromopentane (30 mmoles) was added, and the reaction mixture was stirred at 80° for 18 hr. The reaction mixture was worked up as above: yield 0.70 g (20%), mp 150°, $[\alpha]^{25}D$ −100.8° (*c* 0.208, 95% EtOH). *Anal.* ($C_{15}H_{23}N_5O_4 \cdot 0.33H_2O$) C, H, N.

By using N,N-dimethylacetamide as the reaction solvent, identical results were obtained. Use of an equivalent amount of 1-iodopentane in the reaction carried out at 33° for 72 hr gave a 30% yield, mp 151°.

N^6-Phenyladenosine (V). None of the phenyl halides (even iodobenzene) reacted with adenosine in DMF at 100° for 6 days to produce N-1 quaternization. Hence, the product was synthesized from 6-chloropurine riboside by nucleophilic displacement.

To 100 ml of absolute EtOH was added 2.00 g (7 mmoles) of 6-chloro-9-β-D-ribofuranosyl-9H-purine, 1.4 g (14 mmoles) of $CaCO_3$, and 1.95 g (21 mmoles) of redistilled aniline. The reaction mixture was refluxed with stirring for 18 hr, after which chromatographic controls indicated no further presence of 6-chloropurine riboside in the reaction. The mixture was filtered hot to remove Ca salts and the product was deposited from the filtrate on cooling. The crystals were filtered off, washed with cold EtOH, then with cold 3:1 MeCN–EtOH, and finally with Et_2O, and dried: yield 1.85 g (77%), mp 199°, $[\alpha]^{25}D$ −110.6° (*c* 0.1446, 95% EtOH). *Anal.* ($C_{16}H_{19}O_4N_5 \cdot 0.25H_2O$) C, H, N.

N^6-2-Thienyladenosine (VI). To 100 ml of EtOH was added 2.00 g of 6-chloropurine riboside (7 mmoles), 1.4 g of $CaCO_3$ (14 mmoles), and 2.37 g of 2-thienylamine (21 mmoles), the latter prepared by the method of Hartough and Meisel.[17] The reaction mixture was refluxed for 18 hr when chromatographic examination revealed the absence of 6-chloropurine riboside. The mixture was filtered hot to remove the Ca salts. The combined filtrate and EtOH wash was evaporated to a gum, which was evaporated three times after additions of absolute EtOH. The clear gum was dissolved in EtOH and MeCN was added to make a 3:1 MeCN–EtOH mixture. On cooling in the refrigerator a white solid crystallized. The crystals were filtered and washed with cold 3:1 MeCN–EtOH mixture then with Et_2O and dried: yield 1.82 g (72%), mp 157°, $[\alpha]^{25}D$ −59.0° (*c* 0.152, 95% EtOH). *Anal.* ($C_{15}H_{17}O_4N_5S \cdot 0.1H_2O$) C, H, N.

N^6-2-Ethoxyethyladenosine (VII). To 100 ml of absolute EtOH were added 2.00 g (7 mmoles) of 6-chloropurine riboside, 1.4 g (14 mmoles) of $CaCO_3$, and 1.87 g (21 mmoles) of 2-ethoxyethylamine. The reaction mixture was treated exactly as described under V: yield 2.305 g (97%), mp 163°, $[\alpha]^{25}D$ −84.2° (*c* 0.178, 95% EtOH). *Anal.* ($C_{14}H_{21}O_6N_5 \cdot 0.33H_2O$) C, H, N.

N^6-Isopentenyltubercidin (N^6-(3-Methyl-2-butenyl)tubercidin) (IX). (a) **1-Bromo-3-methyl-2-butene (2,3-Dimethylallyl Bromide).**—3,3-Dimethylacrylic acid was reduced to the alcohol by the method of Knights and Waight[18] with the important exception that less than 1 mole of LAH was employed (0.95 mole of acid taken), because the use of 1.24 moles invariably gave rise to considerable amounts of saturated derivatives (as determined by nmr), which are difficult to remove by fractional distillation. The distilled alcohol obtained free of saturated isopentyl analog was converted to the bromide according to Kuhn and Schinz.[19] The bromide was fractionally distilled and preserved dry in the cold to prevent decomposition.

(b) **N^1-Isopentenyltubercidin (N^1-(3-Methyl-2-butenyl)-tubercidin).** Tubercidin (7-deazaadenosine)[20] (0.500 g, 1.88 mmoles), 1-bromo-3-methyl-2-butene (0.842 g, 5.65 mmoles), and 7 ml of freshly distilled DMF were stirred under anhydrous conditions at 33° for 21 hr. Chromatographic controls showed about 90% of the tubercidin reacted to form the N^1-isopentenyltubercidin. The control samples heated with a few drops of NH_3 (pH 10–12) at 80–90° showed a quantitative conversion of the N^1 intermediate to N^6-isopentenyltubercidin.

(c) **N^6-Isopentenyltubercidin (N^6-(3-Methyl-2-butenyl)-tubercidin).**—The reaction mixture b containing N^1-isopentenyltubercidin hydrobromide was evaporated to a thick syrup using a high-vacuum pump, removing excess isopentenyl bromide and solvent. The syrup was taken up in 25 ml of cold H_2O and NH_4OH was added to pH 12. The material was stirred and heated at 90–100° to convert the N^1 derivative to the N^6 product. During the heating period, an additional 10 ml of NH_4OH was added. After 2 hr, chromatographic evidence (five systems)

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showed about 80–90% product. Heating 2 hr longer, showed no increase in yield. The aqueous solution was filtered and the filtrate was evaporated to dryness.

A 2.54-cm column was prepared with 150 g of Celite 545[21] and 70 ml of lower phase solvent A (EtOAc–*n*-PrOH–H_2O, 4:1:2) and packed according to the method of Hall.[22] The evaporated product was dissolved in the upper phase of solvent A and absorbed on the column. The column was developed with the upper phase solvent A at a flow rate of 125 ml/hr. The optical density of the effluent was monitored on a Beckman DU-UV spectrophotometer and recorded on a Honeywell recorder. The first fractions totalling 135 ml contained only the product. Unchanged tubercidin was eluted after 550 ml of solvent. The solution containing the product was evaporated to a syrup with a flash evaporator, and reevaporated three times with 25-ml additions of absolute EtOH to dehydrate the gum. The gum was then crystallized using MeCN as the solvent. The crystals were filtered, washed with Et_2O, and dried *in vacuo* at 78° over P_2O_5; yield 0.395 g (63%), mp 160–162°, $[\alpha]^{25}D$ −71.8° (*c* 0.1044, 30% EtOH–H_2O). *Anal.* ($C_{16}H_{22}O_4N_4$) C, H, N.

N⁶-Allyltubercidin (X). (a) Allyl Bromide.—Commercial reagent grade material was fractionated and the fraction boiling at 69–70° was collected. **(b) N¹-Allyltubercidin.**—Tubercidin (0.500 g, 1.88 mmoles), allyl bromide (0.684 g, 5.64 mmoles), and 7 ml of DMF (freshly distilled) were treated as outlined for IX. **(c) N⁶-Allyltubercidin.**—The syrup containing N¹-allyltubercidin was treated as outlined for IX; yield 0.310 g (54%) (based on total tubercidin), mp 130° dec, $[\alpha]^{25}D$ −65° (*c* 0.07675 g, 30% EtOH–H_2O). *Anal.* ($C_{14}H_{18}O_4N_4 \cdot 0.1H_2O$) C, H, N.

Assay of Cytokinin Activity.—The cytokinin effect of the compounds was assayed with callus derived from the pith of Wisconsin 38 tobacco plants using the method and media of Murashige and Skoog.[23] Results are given in Table III.

Microbial Assay Procedures.—The microbial assays were carried out according to techniques published previously.[24] *Escherichia coli* K-12 was grown in the synthetic medium of Gray and Tatum.[25] *S. faecalis* 8043 was grown in the medium of Flynn, *et al.*,[26] in the absence of purines and pyrimidines, but with 1 mμg of folic acid/ml. The compounds were added to the medium at 10^{-3}–10^{-8} *M* for the tubercidins and 10^{-5}–10^{-7} *M* for the N⁶-adenosine analogs. Results are given in Table VI.

Effects on the Growth of Sarcoma 180 Cells *in Vitro*.—The compounds were added in aqueous solution to the cultures of S180 cells grown as monolayers in T-15 flasks in Eagle's[27] medium containing 5% horse serum. The cells were exposed to the compounds for 6 days involving three changes of the medium. During this time the control cultures increased by ten- to fifteenfold. The quantity of cells was estimated by the crystal violet method of Grady, *et al.*,[28] and by protein determinations.[29] The results are given in Table VI.

Effect on Viability of Human Cells *in Vitro*.—The compounds were tested in several human cell lines grown in suspension culture.[30] These included cultures of myeloblastic leukemia (cell line RPMI-6410), lymphoblastic leukemia (cell line RPMI-LKID), Burkitt's tumor lymphoma (cell line RPMI-HRIK), and of normal human leucocytes (free of virus particles) (RPMI cell line 5287). The compounds were dissolved in Dulbecco phosphate-buffered saline[31] and added to RPMI-1634 tissue culture medium (a modification of McCoy 5A medium), containing 10% fetal calf serum, and incubated in 50-ml spinner flasks with 2.5×10^{-5} cells/ml, usually for 48 hr. Cell counts were made using a hemocytometer. Since the growth of these cells in 48 hr was never more than twofold, no definitive conclusions concerning growth inhibition were reached. The effects of the compounds were, therefore, evaluated in terms of their effects on viability (Table V) as measured by dye exclusion, using a 1% solution of trypan blue.[31] The viability of the controls varied with each system studied, being 85–94% for the 6410 cell system, 80–94% for the LKID system, 65–71% for the HRIK system, and 66–82% for the normal cells.

In the studies dealing with the growth-stimulating effects of the compounds (Table IV), the 6410 cell line was grown for 5 days with refeeding at 3 days, resulting in six- to eightfold cell multiplication with rather constant viability. Therefore, actual cell counts were determined.

Effect of N⁶-Substituted Adenosine on Leukemia L1210 *in Vivo*.—Female DBA/2 Ha-DD mice weighing 16–20 g were obtained from the RPMI breeding colony. Each animal was inoculated interperitoneally with 10^6 cells of leukemia L1210 and treated once daily for 6 consecutive days starting the day after tumor inoculation.

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