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N^6-Substituted Adenosines: Synthesis, Biological Activity, and Some Structure–Activity Relationships

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Nucleosides of N^6-substituted adenines, which possess cytokinin activity, inhibit the growth of tumor cells, while the corresponding adenines are relatively inactive. Addnl N^6-substituted adenosines have been prepd and tested to secure information regarding structure–activity relationships, if any. The new compds include N^6-butyl-, N^6-*n*-2-propoxyethyl, N^6-*n*-2-butoxyethyl-, N^6-cyclohexyl-, N^6-cyclopropylmethyl-, N^6-tetrahydrofurfuryl-, N^6-geranyl-, N^6-farnesyl-, and N^6-α-pyridoxyladenosine. They were prepd from 6-chloropurine riboside by nucleophilic substitution with the appropriate amine. The known cytokinin compds, 2-methylthio-N^6-isopentenyladenine, *cis*-6-(β-chloro-2-butenylamino)purine, and *trans*-6-(β-chloro-2-butenylamine)purine and their ribosides, and *trans*-zeatin riboside were examd for other biological activity. The alkylated adenosines show optimal cytokinin activity when the N^6-substituent contains a double bond. In *Escherichia coli* the compds were active at 10^{-6}–10^{-4} *M* with the trans isomers showing greater activity than the cis compds. As inhibitors of mouse adenocarcinoma cells (TA-3) in culture, some of the compds were active at 10^{-5}–10^{-6} *M* but in sarcoma S-180 cells in culture they were all less active. Redn of the double bond in the side chain lowered activity of these compds in the tumor cell cultures. The trans isomers are more active against tumor cells *in vitro* than the cis analogs, paralleling their activity as cytokinins. The presence of an OH group in the side chain diminished antitumor activity. A moderate increase in survival time of mice bearing leukemia L-1210 was produced by the compds bearing an ether linkage in the side chain.

We have previously reported[1,2] the synthesis of a series of N^6-substituted adenine ribosides which are potent cytokinins. Many of these compds inhibited the growth of neoplastic cells *in vitro* at concns of about 10^{-6} *M*. Most of these adenosine analogs had different effects on various leukemic cells and no effects on lymphocytes *in vitro*. It was also noted that at lower concns (e.g, 10^{-8}–10^{-7} *M*) some stimulation of human leukemic (line 6410) cell growth took place in contrast to the inhibitory effects that occurred at higher concns. The corresponding free adenines were relatively inactive against tumor cells. The more active tumor inhibitory nucleosides were found to be N^6-benzyl-, N^6-furfuryl-, N^6-ethoxyethyl-, N^6-phenyl-, and N^6-thenyladenosines. Like N^6-(3-methyl-2-butenyl)adenosine[12] (IPA), these analogs are also active as cytokinins.

The present communication† is an extension of our previous work. In order to secure further information on the structure–activity relationships in this series of adenosine derivatives, addnl analogs were prepd and examd for their biol properties. These include N^6-*n*-Bu- (I), N^6-*n*-2-propoxyethyl- (II), N^6-*n*-2-butoxyethyl- (III), N^6-cyclohexyl- (IV), N^6-cyclopropylmethyl- (V), N^6-tetrahydrofurfuryl- (VI), N^6-geranyl- (VII), N^6-farnesyl- (VIII), and N^6-α^4-pyridoxyladenosines (IX). The compds were tested for cytokinin activity in the tobacco callus bioassay, and in microbial and tumor systems. This series of compds was augmented by *trans*-N^6-4-hydroxymethyl-2-butenyladenosine (zeatin riboside), a potent cytokinin that was prepd in this work by the method of Shaw, *et al.*[4] The compds were chosen for synthesis and examn of properties for the following reasons.

†A portion of this material was presented by the author at the 160th National Meeting of the American Chemical Society.[3]

(I) The *n*-Bu fragment represents the shortest C chain among a series of N^6-substituted adenines resulting in really good cytokinin activity.[5] (II, III) The propoxyethyl and butoxyethyl compds are homologs of N^6-2-ethoxyethyladenosine, a compd with antitumor activity *in vivo*.[2] (IV, VI) The cyclohexyl and tetrahydrofurfuryl compds are satd derivatives of unsatd analogs which showed biol and antitumor activity. (V) The cyclopropylmethyl derivative was prepd to examine the biol effect of the smallest satd ring structure. (VII, VIII) The geranyl and farnesyl side chains were chosen to det the effects of multiple isopentenyl fragments on a single side chain. (IX) The α^4-pyridoxyl side chain was included to evaluate the effect of a strong hydrophilic substituent which contains a biol active moiety.

Zeatin riboside (*trans*-N^6-(4-hydroxymethyl-2-butenyl)-adenosine was included because it is about the most active known cytokinin of the N^6-adenosine series.[6,7] The compds were prepd by condensing 6-chloropurine riboside (6-chloro-9-β-D-ribofuranosyl-9*H*-purine) with the corresponding amines by nucleophilic substitution in boiling EtOH, using $CaCO_3$ or Et_3N as ancillary acid acceptors.[1] The compds were isolated and purified by crystn. The purity of the products was confirmed by chromatog, elemental analyses, and by uv spectra. The physical data are given in Tables I and II.

The new compds were examd for cytokinin activity in a tobacco pith assay system by the method of Murashige and Skoog.[8] As shown in Table III, the compds vary in activity. The N^6-butyl and propoxyethyladenosines have good activity, although somewhat less than that of zeatin riboside. The other compds displayed marginal or no cytokinin activity noted since high concns had to be used for initial response.

Table I. Paper Chromatography and R_f Values of N^6-Substituted Adenosines

Compound	Solvent system[a] A	B	C	D	E
6-Chloropurineriboside	0.53	0.68	0.72	0.57	0.41
N^6-*n*-Butyladenosine	0.74	0.80	0.87	0.72	0.67
N^6-*n*-2-Propoxyethyladenosine	0.72	0.81	0.83		0.63
N^6-*n*-2-Butoxyethyladenosine	0.77	0.86	0.86	0.79	0.72
N^6-Cyclohexyladenosine	0.81	0.87	0.83	0.72	0.70
N^6-Cyclopropylmethyladenosine	0.66	0.81	0.76	0.60	0.59
N^6-Tetrahydrofurfuryladenosine	0.51	0.72	0.80	0.67	0.48
N^6-Geranyladenosine	0.90	0.89	0.89	0.79	0.79
N^6-Farnesyladenosine	0.89	0.86	0.90	0.85	0.83
N^6-α^4-Pyridoxyladenosine	0.29	0.52	0.73		0.63
N^6-(4-Hydroxy-3-methyl-*trans*-butenyl)-adenosine (*trans*-zeatin riboside)[b]	0.46	0.72	0.68		

[a] The solvent systems used for descending chromatog (Whatman No. 1 paper) (measured by vol): 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% aq $(NH_4)_2SO_4$ (2:1), D, *i*-PrOH–concd HCl–H_2O (680:170:144); E, *n*-BuOH–AcOH–H_2O (1000:32.5:300) (cloudy upper phase). [b] See reference 4.

adenines and adenosines were investigated by Hecht, *et al.*[10] They concluded that planar side chains, *i.e.*, trans isomers impart the highest order of cytokinin activity; whereas the cis isomers, possessing more steric bulk, were less active. Because of these cytokinin variations some of these compds were tested in this work in addn to the new ones prepd here.‡

The activities of all the compds discussed as inhibitors of the growth of *E. coli* are summarized in Table IV. The most active inhibitors were N^6-2-propoxyethyladenosine and *trans*-zeatin riboside, followed by the *n*-butyl-, *n*-2-butoxyethyl-, cyclohexyl-, isopentenyl-, and tetrahydrofurfuryladenosines. The α^4-pyridoxyl-, farnesyl-, geranyl-, and cyclopropylmethyladenosines were inactive.

The low water solubility shown by N^6-isopentenyl-2-thiomethyl compds possibly accounts for their minimal activity in this system. The N^6-3-chloro-2-butenyladenosines and adenines are less active than the N^6-isopentenyl analogs but their trans isomers are distinctly more active than the

Table II. Uv Absorption Spectra of N^6-Substituted Adenosines

Compound	pH 1.0 λ max, mμ	pH 1.0 ε × 10^{-3}	pH 7.0 λ max, mμ	pH 7.0 ε × 10^{-3}	pH 12.0 λ max, mμ	pH 12.0 ε × 10^{-3}
N^6-*n*-Butyladenosine	263	19.3	267, 210	18.0, 19.1	267	18.4
N^6-*n*-2-Propoxyethyladenosine	263	18.3	267, 210	18.0, 19.4	267	18.0
N^6-*n*-2-Butoxyethyladenosine	263	16.4	267, 210	16.5, 17.4	267	16.7
N^6-Cyclohexyladenosine	265	19.4	268, 211	18.2, 17.7	268	18.6
N^6-Cyclopropylmethyladenosine	263	18.6	267, 210	18.0, 19.8	267	18.2
N^6-Tetrahydrofurfuryladenosine	265	17.9	267, 210	18.0, 18.1	267	18.1
N^6-Geranyladenosine	266	19.1	268	19.0	268	18.8
N^6-Farnesyladenosine	266	17.7	269	17.3	269	17.3
N^6-α^4-Pyridoxyladenosine	274	20.3	268, 325	20.9, 4.7	270, 309	20.6, 9.5

Table III. Cytokinin Activity of the New N^6-Substituted Adenosines on Tobacco Bioassay[a]

Compound	Relative growth compared to control as 1.00 at concentrations 10 μg/l	25 μg/l	200 μg/l
N^6-*n*-Butyladenosine	2.17	3.80	2.90
N^6-*n*-2 Propoxyethyladenosine	1.73	7.24	7.48
N^6-*n*-2 Butoxyethyladenosine	1.00	1.87	4.00
N^6-Cyclohexyladenosine	1.00	1.00	2.41
N^6-Cyclopropylmethyladenosine	1.28	1.40	5.13
N^6-Tetrahydrofurfuryladenosine	1.13	1.20	4.84
N^6-Geranyladenosine	1.41		3.12
N^6-Farnesyladenosine	1.00	1.59	2.72
N^6-α^4 Pyridoxyladenosine	1.05	1.07	1.52
N^6-(4-Hydroxy-3 methyl-*trans*-butenyl)-adenosine (zeatin riboside)[b]	2.95	5.62	4.72

[a] See reference 8. [b] See reference 4.

Table IV. Growth Inhibition in *E. coli*

N^6-Compound	Molar concn for 50% growth inhibition
N^6-n-Butyladenosine	5×10^{-5}
N^6-*n*-2-Propoxyethyladenosine	5×10^{-6}
N^6-*n*-2-Butoxyethyladenosine	5×10^{-5}
N^6-Cyclohexyladenosine	4×10^{-5}
N^6-Cyclopropylmethyladenosine	None
N^6-Tetrahydrofurfuryladenosine	8×10^{-4}
N^6-Geranyladenosine	8×10^{-4} (in suspension)
N^6-Farnesyladenosine	3×10^{-3} (in suspension)
N^6-α^4-Pyridoxyladenosine	1×10^{-3}
N^6-*trans*-Zeatin riboside	5×10^{-6}
N^6-Isopentenyl-2-thiomethyladenosine[a]	5×10^{-4} (in suspension)
N^6-3-Chloro-*cis*-2-butenyladenosine[b]	1×10^{-3}
N^6-3-Chloro-*trans*-2-butenyladenosine[b]	7×10^{-4}
N^6-Isopentenyl-2-thiomethyladenine[a]	5×10^{-4} (in suspension)
N^6-3-Chloro-*cis*-2-butenyladenine[b]	None
N^6-3-Chloro-*trans*-2-butenyladenine[b]	8×10^{-4}
N^6-Isopentenyladenine[c,d]	1×10^{-3}
N^6-Isopentenyladenosine[c,d]	9×10^{-5}

[a] See reference 9. [b] See reference 10. [c] See reference 11. [d] See reference 12.

The results show the effects of N^6 side chain satn and of very low water solubility (geranyl- and farnesyladenosines). The N^6-α^4-pyridoxyladenosine is inactive.

An N^6-adenosine analog isolated from sol RNA of *E. coli*, wheat germ, and yeast, having cytokinin activity has recently been described by Leonard and his coworkers[9] and identified as N^6-isopentenyl-2-methylthioadenosine. This nucleoside and other 2-substituted N^6-isopentenyl and zeatin ribosides have been synthesized and examd for cytokinin activity.[6] While 2-methylthio, 2-amino, and 2-chloro substituents in the original compd had very little influence on cytokinin effectiveness, the 2-hydroxy substituents greatly lowered cytokinin activity[6] showing that an OH group in the structure has marked effects. In another study, the effects of side-chain planarity in a series of N^6-substituted cis compds paralleling their cytokinin activity. The adenines are less active than the corresponding ribosides, a fact which has been obsd previously.[2]

Against sarcoma S-180 cells (Table V) most of the new compds other than N^6-isopentenyladenosine were inactive paralleling their cytokinin effects. Zeatin riboside is not active in this system, however, this effect may be due to the presence of an OH group in the side chain causing loss of antitumor activity. Pyridoxyladenosine is inactive, perhaps for the same reason. The propoxyethyl- and butoxy-

‡We are indebted to Dr. S. M. Hecht and Professor N. J. Leonard for generous gifts of these materials.

Table V. Growth Inhibitory Activity against Sarcoma 180 Cells and Carcinoma TA-3 Cells *in Vitro*

Compound	Molar concentration for 50% growth inhibition: Sarcoma 180 Cells	TA-3 Cells
N^6-*n*-Butyladenosine	$>10^{-4}$	$>10^{-4}$
N^6-*n*-2-Propoxyethyladenosine	None at 10^{-4}	$>10^{-4}$
N^6-*n*-2-Butoxyethyladenosine	None at 10^{-4}	$>10^{-4}$
N^6-Cyclohexyladenosine	None at 10^{-4}	2.3×10^{-5}
N^6-Cyclopropylmethyladenosine	9×10^{-5}	2.5×10^{-5}
N^6-Tetrahydrofurfuryladenosine	None at 10^{-4}	$>10^{-4}$
N^6-Geranyladenosine	None (suspension)	None at 10^{-5} (suspension)
N^6-Farnesyladenosine	None (suspension)	None at 10^{-5} (suspension)
N^6-α^4 Pyridoxyladenosine	None at 10^{-4}	7.5×10^{-5}
N^6-*trans*-4-Hydroxymethyl-2-butenyladenosine (zeatin riboside)	1.2×10^{-4}	1×10^{-4}
N^6-Isopentenyl-2-methylthioadenosine[a]	None at 2×10^{-5} (suspension)	Slight at 10^{-5}
N^6-3 Chloro-*cis*-2-butenyladenosine[b]	6×10^{-6}	3.3×10^{-6}
N^6-3 chloro-*trans*-2-butenyladenosine[b]	1.2×10^{-5}	2.5×10^{-6}
N^6-Isopentenyl-2-thiomethyladenine[a]	None at 10^{-5} (suspension)	None at 10^{-5} (suspension)
N^6-3 Chloro-*cis*-2-butenyladenine[b]		None at 10^{-4}
N^6-3 Chloro-*trans*-2-butenyladenine[b]	None at 10^{-4}	7.5×10^{-5}
N^6-Isopentenyladenosine[c,d] (IPA)	1.25×10^{-5}	7×10^{-6}
N^6-Isopentenyladenine[c,d]	2.5×10^{-4}	1.9×10^{-4}

[a-d] See footnotes *a-d*, Table IV.

Table VI. *In Vivo* Activity against Mouse Leukemia L-1210

Compound (N^6-adenosine analog)	No. of mice used	Dose, mg/kg per day	Mean life-span, days	% increased life-span (over control)
Control	39		7.4	100
n-Bu	5	75	9.0	122
	8	100	10.0	135
	3	200	8.3	112
n-2-Propoxethyl	8	100	9.3	125
	11	200	9.4	128
	5	300	3.8	Toxic
n-2-Butoxyethyl	8	100	8.6	116
	8	200	6.5	Toxic
Cyclohexyl	6	5	5.3	Toxic
	5	2	6.6	Inactive
Cyclopropylmethyl	5	75	9.2	124
	3	100	8.5	115
Tetrahydrofurfuryl	11	100	9.2	125
	8	200	9.3	125
Geranyl	6	100	6.9	Inactive
Farnesyl	6	100	7.0	Inactive
α^4-Pyridoxyl	6	100	6.9	Inactive
trans-4-Hydroxy-methyl-2-butenyl (zeatin riboside)	11	100	8.0	108
trans-3-Chloro-2-butenyl	5	75	6.2	Inactive
	8	100	8.6	117
	3	200	3.0	Toxic

ethyladenosines are inactive *in vitro*, as was found previously in the case of ethoxyethyladenosine which was active *in vivo*. The 2-thiomethyl-IPA possibly because of its very low water solubility is inactive in this system, but the cis and trans chlorobutenyl adenosines are active, paralleling their high cytokinin activity. The adenosine nucleosides are far more active than the corresponding bases as obsd before[2] in this tumor system. Also in this case the trans analogs are more active than their cis isomers paralleling their cytokinin effects. The geranyl- and farnesyladenosines have very low solubility in aq systems which may be the reason why they are inactive. The satd chain compds have lower effectiveness than their unsatd analogs paralleling their cytokinin effects.

The compds were also tested against mouse mammary carcinoma cells (TA-3) in culture, a more sensitive system, and were found to be more active than against sarcoma 180 (Table V). Cyclohexyladenosine and cyclopropylmethyladenosine are slightly active in this system. Zeatin riboside, as in sarcoma 180, is inactive in this tumor system. The isopentenyl analog (IPA)[11] is more active in this cell line than in sarcoma 180. The sparingly soluble thiomethyl-IPA is slightly active while the cis and trans chlorobutenyl analogs are quite active, paralleling their cytokinin activity. The corresponding adenine bases are relatively inactive, as previously detd.

The effects of these compds on mice injected with 10^6 cells of leukemia L 1210 are shown in Table VI. The compds were given ip daily for 6 days at the dosage indicated. Of the compds tested, N^6-butyl (135% increased life-span), N^6-2-propoxyethyl (128% increased life-span), N^6-cyclopropylmethyl (124% increased life-span), and N^6-tetrohydrofurfuryl (125% increased life-span) analogs have the best activities, paralleling their cytokinin activity. The N^6-2 butoxyethyl derivative is less effective. It may be mentioned that the ether side chain analogs (N^6-alkoxyethyladenosines) show far more antitumor activity *in vivo* than *in vitro*.§

The cyclohexyl analog proved to be extremely toxic. When the dose was reduced to 2 mg/kg per day the compd was inactive.

trans-Zeatin riboside, a very potent cytokinin and mi-

§ See also the case of N^6-2-ethoxyethyladenosine (reference 2).

crobial inhibitor, is virtually inactive in the case of this tumor as in the case of the *in vitro* tumor cell tests. It appears that the introduction of an OH group in the side chain of IPA sharply lowers antitumor activity. Since it was noted above that 2-hydroxy-N^6–IPA had sharply curtailed cytokinin activity *vs.* that of IPA, hydroxylation may effect an unexpected behavior.

α^4-Pyridoxyladenosine contains 2 OH groups in the side chain and is inactive. Geranyl- and farnesyladenosines are too insoluble at levels needed to be active. *trans*-3-Chloro-2-butenyladenosine shows slight activity at 100 mg/kg per day and reduction of dosage to 75 mg/kg per day renders it inactive.

To summarize it may be said that in N^6-substituted adenosine analogs with side chains containing 4–7 C atoms: (1) cytokinin activity is optimal when a double bond is present in the side chain; (2) high cytokinin activity is paralleled by *E. coli* growth inhibition; (3) tumor cell growth inhibition follows cytokinin activity with an exception that the presence of an OH group in the side chain diminishes antitumor effects.

Experimental Section

Mps were detd on a Mel-Temp mp apparatus and are not cor. Uv spectra were obtained on a Cary Model 14 recording spectrophotometer. Optical rotation was measured on a Jasco Model ORD-UV5 optical rotatory dispersion recorder. The solvent systems used for descending chromatog are given in Table I. Whatman paper No. 1 was used. 6-Chloro-9β-D-ribofuranosyl-9*H*-purine (6-chloropurine riboside) was purchased from K & K Laboratories, Inc., Plainview, N. Y. The amines employed are identified under each prepn. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements are within ±0.4% of the theoretical values.

N^6-*n*-Butyladenosine (I). To 100 ml of abs EtOH was added 2.00 g (7 mmoles) of 6-chloro-9-β-D-ribofuranosyl-9*H*-purine (6-chloropurine riboside), 1.4 g (14 mmoles) of $CaCO_3$, and 1.54 g (21 mmoles) of distilled *n*-$BuNH_2$ (Eastman Kodak Co.). The reaction mixt was refluxed with stirring for 18 hr after which chromatog controls indicated no further presence of 6-chloropurine riboside in the reaction. The mixt was filtered hot to remove Ca salts and the product deposited from the filtrate on cooling. The crystals were filtered off and washed with cold EtOH then with Et_2O and dried; yield, 1.90 g (84%); mp 176°; $[\alpha]^{25}D$ −71.4° (*c* 0.216, 95% EtOH). *Anal.* ($C_{14}H_{21}N_5O_4$) C, H, N.

N^6-*n*-2-Propoxyethyladenosine (II). To 100 ml of abs EtOH were added 2.00 g (7 mmoles) of 6-chloropurine riboside, 1.44 g (14 mmoles) of *n*-2-propoxyethylamine,# and 1.415 g of Et_3N (14 mmoles), and the mixt was refluxed 6 hr, when chromatog controls indicated that 6-chloropurine riboside was no longer present in the mixt. The reaction mixt was evapd and redissolved in hot abs EtOH. The product, which crystd on cooling, was filtered, washed with chilled EtOH, and dried: yield, 2.15 g (87%); mp 125°; $[\alpha]^{25}D$ −59.5° (*c* 0.39, 95% EtOH). *Anal.* ($C_{15}H_{23}N_5O_5$) C, H, N. Use of $CaCO_3$ as in the preceding prepn, gave the same results.

N^6-*n*-2-Butoxyethyladenosine (III). A mixt of 100 ml of abs EtOH, 2.00 g (7 mmoles) of 6-chloropurine riboside, 1.40 g (14 mmoles) of $CaCO_3$, and 2.46 g (21 mmoles) of *n*-2-butoxyethylamine** was refluxed and stirred as above for 18 hr after which the reaction chromatog controls indicated the absence of 6-chloropurine riboside. The hot reaction mixt was filtered through Celite and the product crystd from the filtrate on cooling. The crystals were filtered, washed with cold EtOH, and dried: yield, 2.4 g (93%); mp 126.5°; $[\alpha]^{25}D$ −56° (*c* 0.318, 95% EtOH). *Anal.* ($C_{16}H_{25}N_5O_5$) C, H, N.

N^6-Cyclohexyladenosine (IV). The prepn of this compd by the nucleophilic substitution method using either Et_3N or $CaCO_3$ as the ancillary acid binder, presents some difficulty in isolation of the desired product.

A mixt of 100 ml of abs EtOH, 2.00 g (7 mmoles) of 6-chloropurine riboside, 1.39 g (14 mmoles) of cyclohexylamine ($n^{25}D$ 1.4587, Aldrich Chemical Co.) and 1.4 g (14 mmoles) of $CaCO_3$ was refluxed with stirring 18 hr when chromatog controls indicated absence of 6-chloropurine riboside. The Ca salts were removed by filtration of the hot soln. Since no product crystd out on cooling, the filtrate and wash were evapd to about 0.2 of their vol and cooled, whereupon cyclohexylamine hydrochloride pptd in cryst form. It was filtered and dried (0.76 g, mp 208°) and analyzed correctly for $C_6H_{11}NH_2 \cdot HCl$. On adding Et_2O to the mother liquor the product crystd out, was filtered, and washed with Et_2O. It was recrystd from EtOH–Et_2O and dried: yield, 2.06 g (84%); mp 185°; $[\alpha]^{25}D$ −59.0° (*c* 0.312, EtOH). *Anal.* ($C_{16}H_{23}N_4O_4$) C, H, N.

An alternate method of isolation is to take up the evapd filtered material (after removal of cyclohexylamine hydrochloride) in 100 ml of H_2O and ext 3 times with 100-ml portions of $CHCl_3$ (adding 2 ml of HOAc to the first $CHCl_3$ portion to take up any amine). The combined $CHCl_3$ fractions were washed with H_2O and dried (Na_2SO_4). The $CHCl_3$ was evapd, and the residue was crystd from EtOH–Et_2O, as above, yielding the same results. This compd cannot be made *via* the N^1-quaternization procedure[2] since adenosine does not react with iodocyclohexane in DMF nor in *N,N*-dimethylacetamide.

N^6-Cyclopropylmethyladenosine (V). (a) Cyclopropylmethylamine. This amine has been made by Roberts and Mazur[15] by the redn of cyclopropyl cyanide with Na in abs EtOH followed by isolation of the hydrochloride, basifying, ether extn of the amine, and fractionation. The redn of the nitrile could also be effected with LAH in Et_2O. Cyclopropyl cyanide†† (25 g, 373 mmoles) was dissolved in 250 ml of anhyd Et_2O. The soln was added dropwise to a stirred and cooled suspension of 14.17 g of LAH (373 mmoles) in 200 ml of anhyd Et_2O in 3 hr. The suspension was stirred 18 hr allowing the temp to rise to room temp. To the stirred and cooled suspension, cold H_2O (26.9 ml, 1.492 moles) was added dropwise, stirring contd for 1 hr and the mixt allowed to stand for 2 hr. The white suspension was filtered, and the ppt was washed with Et_2O. The combined Et_2O filtrates were sepd and dried (Na_2SO_4). The Et_2O was evapd, and the amine was collected as the fraction which distd at 83–84°: $n^{25}D$ 1.4250; yield, 5.50 g (20.7%).

(b) N^6-Cyclopropylmethyladenosine (V). To 100 ml of abs EtOH was added 3.73 g (13 mmoles) of 6-chloropurine riboside, 3.90 g (38.7 mmoles) of Et_3N, and 2.75 g (38.7 mmoles) of the cyclopropylmethylamine. The reaction mixt was stirred and refluxed for 17 hr when chromatog controls indicated the absence of 6-chloropurine riboside. On cooling to room temp the product crystd out. It was filtered and washed with cold EtOH followed by Et_2O and then dried: yield, 3.84 g (92%); mp 171–172°. The material was recrystd from H_2O to yield 80% silky needles melting at 176°: $[\alpha]^{25}D$ −64.5° (*c* 0.217 95% EtOH). *Anal.* ($C_{14}H_{19}N_5O_4$) C, H, N.

N^6-Tetrahydrofurfuryladenosine (VI). To 100 ml of abs EtOH was added 2.0 g (7 mmoles) of 6-chloropurine riboside, 1.414 g (14 mmoles) of tetrahydrofurfurylamine,‡‡ and 1.41 g (14 mmoles) of Et_3N. The reaction mixt was refluxed with stirring for 4 hr at which time chromatog controls indicated the absence of 6-chloropurine riboside. After filtration the material was crystd from EtOH and dried: yield, 1.98 g (81%); mp 148°; $[\alpha]^{25}D$ −59° (*c* 0.306 95% EtOH). *Anal.* $C_{15}H_{21}N_5O_5$) C, H, N.

N^6-Geranyladenosine (VII). To 50 ml of abs EtOH was added 1.0 g (3.5 mmoles) of 6-chloropurine riboside, 1.07 g (7 mmoles) of geranylamine,§§ and 1.41 g (14 mmoles) of Et_3N. The reaction mixt was refluxed under N_2 with stirring 6 hr when chromatog controls showed the absence of 6-chloropurine riboside. It was cooled, and the product was filtered off and washed with chilled EtOH then Et_2O and dried. The material was recrystd from MeOH: yield, 1.04 g (74%); mp 108°; $[\alpha]^{25}D$ −55° (*c* 0.342, 95% EtOH). *Anal.* ($C_{20}H_{29}N_5O_4$) C, H, N. The use of $CaCO_3$ as an ancillary condensing agent practically abolished reactivity.

N^6-Farnesyladenosine (VIII). To 30 ml of abs EtOH were added 0.644 g (2.25 mmoles) of 6-chloropurine riboside, 0.50 g (2.26 mmoles) of farnesylamine,§§ and 0.455 g (4.50 mmoles) of Et_3N, and the mixt was refluxed under N_2 with stirring for 5 hr when chromatog controls showed the absence of 6-chloropurine riboside. The reaction mixt was filtered and evapd to dryness. The solids were crystd from MeOH–H_2O (14:1), filtered, and washed with chilled MeOH–H_2O and sucked dry. Recrystn from MeOH yielded

#Supplied by K & K Laboratories Inc. The material had a bp 129° (750 mm) and $n^{25}D$ 1.4127 comparing with the same lit. values of Harder, *et al.*[14] The bp was detd by Siwoloboff's method.[13]

**Supplied by K & K Laboratories. The material had bp 152° (750 mm) and $n^{25}D$ 1.4196 comparing with the same lit. values of Harder, *et al.*[14] boiling point determined by Siwoloboff's method.

††Supplied by Aldrich Chemical Co., $n^{25}D$ 1.4207

‡‡Supplied by Aldrich Chemical Co., $n^{25}D$ 1.4570.

§§This amine was generously donated by Hoffman-LaRoche, Inc., through the courtesy of Dr. W. E. Scott.

0.447 g (42%): mp 98°; $[\alpha]^{25}D$ −63.1° (*c* 0.646, 95% EtOH). *Anal.* ($C_{25}H_{37}N_5O_4$) C, H, N. Use of $CaCO_3$ or Li_2CO_3 as ancillary condensing agents resulted in poor reactivity.

N^6-Pyridoxyladenosine(α^4-pyridoxyl-N^6-adenosine) (IX). To 100 ml of abs EtOH was added 2.86 g (10 mmoles) of 6-chloropurine riboside, 3.00 g (12.5 mmoles) of pyridoxamine dihydrochloride (Sigma Chemical Co.), and 6.06 g (60.0 mmoles) of Et_3N. On refluxing a white ppt deposited initially which later dissolved leaving an opalescence in the reaction mass. With further refluxing a white solid deposited out of soln. After 20 hr at reflux temp, the reaction mass was filtered hot, and the ppt was washed with EtOH and dried at room temp: yield, 3.90 g (93.5%); mp 249°; $[\alpha]^{25}D$ −51.6° (*c* 0.155, 35% EtOH). *Anal.* ($C_{18}H_{22}O_6 \cdot H_2O$) C, H, N.

Assay of Cytokinin Activity. The cytokinin effect of the new compds was assayed with the callus derived from the pith from apical stems of Wisconsin 38 tobacco plants using the method and media of Murashige and Skoog.[8] Results are given Table III.

Microbial Assay Procedure. The microbial assays were carried out according to techniques previously published.[16] *E. coli* (K-12) was grown in the synth medium of Gray and Tatum.[17] The compds were added to the medium at 10^{-3}–10^{-7} *M*. Results are given in Table IV.

Effects on the Growth of Sarcoma-180 and Carcinoma TA-3 Cells *in Vitro*. (a) Sarcoma-180 Cells. The compds were added in aq soln (or as a suspension if the solubility was low) to cultures of S-180 cells grown as monolayers in T-15 flasks in Eagle's[18] medium containing 5% horse serum. The cells were exposed to the compds for 6 days during which time there were 3 changes of medium. The control cultures increased by 10- to 15-fold. The quantity of cells was estimated by the crystal violet method of Grady, *et al.*,[19] and by protein detns.[20] (Table V).

(b) Carcinoma TA-3 Cells. This mammalian cell line (mouse mammary adenocarcinoma TA-3) was grown in RPMI 1640 medium[21] supplemented with 10% horse serum. The test system consisted of tube cultures (12 × 75 mm) inoculated with 5×10^4 cells in 1 ml of medium, supplemented with another milliliter of medium containing the compd and then incubated at 36° in upright position. All the concns of the compds were tested in 5 tubes each. The medium was replaced with fresh medium on the second day by centrifuging the cells for 10 min at 500 rpm and by aspirating off the supernatant. The growth of the controls as estd by protein assay[22] was 12- to 15-fold in 3 days (Table V).

Effect of N^6-Substituted Adenosines on Leukemia L 1210, *in vivo*. Female DBA_2/HA mice (6–8 weeks old) (18–20 g) were obtd from the RPMI breeding colony. Each animal was injected ip with 1×10^6 cells of leukemia L 1210, and treated with the drug once daily for 6 consecutive days starting the day after tumor inoculation. The compds were administered in a homogenized 1% CMC emulsion. Survival time was noted on each animal (Table VI).

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