Synthesis of Potential Anticancer Agents. XXX. (1-Aziridinyl)purines[1]

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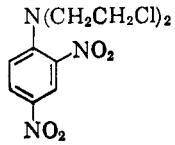
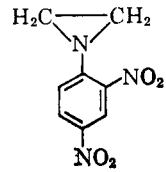
Eight (1-aziridinyl)purines have been prepared by the nucleophilic displacement of a chlorine atom of the corresponding chloropurines. Initial screening results have shown that the 6-(1-aziridinyl)purines have moderate activity against Ca 755 and in one case good activity against Walker 256.

In our search for better anticancer agents, we have investigated the synthesis of purines containing potential alkylating groups in an effort to produce compounds that will combine irreversibly with enzymes important to cellular metabolism. Such compounds might interfere with *de novo* synthesis of nucleic acids and selectively attack rapidly dividing cancer cells.

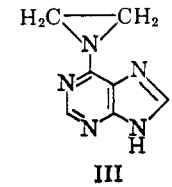
Certain derivatives of nitrogen mustard such as *N*,*N*-bis-(2-chloroethyl)-2,4-dinitroaniline (I) which contain groups that drastically reduce the basicity of the amino group and, therefore, the chemical reactivity of chlorine atoms, are known to be biologically inert.[2] The reverse is true for the 2,4-dinitrophenyl derivative (II) of aziridine, and, in fact, II is an example of so-called "one-armed" alkylating agents that possess anticancer activity (*viz.*, against Walker rat carcinoma 256).[2] Since the ring nitrogen atoms of a purine affect



I



II



III

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(2) W. C. J. Ross, *Adv. Cancer Research*, **1**, 397 (1953).

substituents in a manner similar to the nitro groups of the 2,4-dinitrophenyl group, it would appear that the aziridinyl group attached to the purine ring, particularly in the 6-position (III), would possess the desired chemical reactivity, and, therefore, biological activity. Furthermore, a variety of types of 1-aziridinyl compounds have shown significant anticancer activity, for example, triethylene phosphoramide (TEPA),[3] 2,5-diethylenimino-3,6-dipropoxy-1,4-benzoquinone (E39),[4] and triethylene melamine (TEM).[5]

Initial efforts to prepare 6-(1-aziridinyl)purine (III) from the reaction of ethylenimine and 6-chloropurine (IV) were unsuccessful, probably because of the reaction of ethylenimine with the ring nitrogens of the imidazole moiety of 6-chloropurine to give V or more complicated structures. To avoid this difficulty, the reaction of 6-chloro-9-ethyl-9*H*-purine[6] (VIIa) with ethylenimine was studied, and 6-(1-aziridinyl)-9-ethyl-9*H*-purine (VIIIa) was prepared in good yield. This reaction was then extended to 9-benzyl-6-chloro-9*H*-purine[7] (VIIb) in the hope that the resulting 6-(1-aziridinyl)-9-benzyl-9*H*-purine (VIIIb) could be debenzylated to the desired 6-(1-aziridinyl)purine, since it is known in other cases that the reduction of other functional groups can be accomplished without rupture of the aziridinyl ring.[8,9] In this case, probably because the aziridinyl ring is activated by attachment to the 6-position of the purine ring, it was not possible to debenzylate without affecting the aziridinyl ring. Sodium in liquid ammonia[10,11] gave N^6-ethyladenine (VI) contaminated with adenine, whereas catalytic reduction using palladium on charcoal gave only 9-benzyl-N^6-ethyladenine (IX).[7] We then turned our attention to the synthesis of 6-(1-aziridinyl)-9-β-D-ribofuranosyl-9H-purine (VIIIc) This compound, prepared from 6-chloro-9-β-D-ribofuranosyl-9*H*-purine[12] (VIIc) in the manner described above for VIIIa and VIIIb, gave a weaker color test[13]

(3) S. M. Buckley, C. C. Stock, R. P. Parker, M. L. Crossley, E. Kuh, and D. R. Seeger, *Proc. Soc. Exp. Biol. and Med.*, **78**, 299 (1951).

(4) G. Domagk, *Ann. N. Y. Acad. Sci.*, **68**, 1197 (1958).

(5) S. M. Buckley, C. C. Stock, M. L. Crossley, C. P. Rhoads, *Cancer Research*, **10**, 207 (1950).

(6) J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **79**, 5238 (1957).

(7) J. A. Montgomery and C. Temple, Jr., *ibid.* **83**, 630 (1961).

(8) D. S. Tarbell and D. K. Fukushima, *ibid.* **68**, 2499 (1946).

(9) J. A. Hendry and R. F. Homer, *J. Chem. Soc.*, 328 (1952).

(10) V. du Vigneaud and O. K. Behrens, *J. Biol. Chem.*, **117**, 27 (1937).

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

(12) B. R. Baker, K. Hewson, H. J. Thomas, and J. A. Johnson, *J. Org. Chem.*, **22**, 954 (1957).

(13) J. Epstein, R. W. Rosenthal, and R. J. Ess, *Anal. Chem.*, **27**, 1436 (1955).

for the aziridinyl group than did VIIIa or VIIIb indicating the possibility that some reaction may have occurred between the aziridinyl group of VIIIc and the hydroxyl groups of its ribose moiety. Paper chromatography did not resolve this problem.

Because of the necessity for blocking the imidazole nitrogen of chloropurines before allowing them to react with ethylenimine, the benzylation of 2,6-dichloropurine (Xa) was undertaken prior to attempts to prepare a 2,6-bis-(1-aziridinyl)purine. This benzylation, carried out as previously described,[7] gave a mixture of the 7- and 9-isomers from which only the 9-isomer could be isolated pure. The identity of the material was established by its conversion to 9-benzylguanine (XIVb) *via* 9-benzyl-2-chlorohypoxanthine (XIVa) and a comparison of this material with a sample[14] prepared in an unambiguous manner.[15] Although 7-benzyl-2,6-dichloro-7*H*-purine[16] (XIIa) could not be obtained free of 9-isomer, the impure material was converted to the corresponding hypoxanthine (XVa) which was obtained free of 9-isomer as shown by paper chromatography. This compound (XVa) was converted to 7-benzylguanine (XVb) by treatment with alcoholic ammonia in a bomb. The ultraviolet spectrum of XVb was practically identical with that of 7-methylguanine.[18]

Reaction of XIVa and XVa with ethylenimine gave 2-(1-aziridinyl)-9-benzylhypoxanthine (XIVd) and 2-(1-aziridinyl)-7-benzylhypoxanthine (XVc), both guanine analogs. N^2,N^2-Dimethyl-9-benzylguanine also was prepared from XIVa.

Reaction of 9-benzyl-2,6-dichloro-9*H*-purine with ethylenimine or its lithium salt gave only 6-(1-aziridinyl)-9-benzyl-2-chloro-9*H*-purine (XIIIa): di-replacement could not be effected employing a variety of conditions.

9-Benzyl-6,8-dichloro-9*H*-purine (XVII) was prepared by the benzylation of 6,8-dichloropurine (XVI) in the usual manner. The identity of XVII was established by a two step sequence. First, XVII was allowed to react with dimethylamine and the resulting 9(7)-benzyl-8-chloro-N^6,N^6-dimethyladenine (XIXa) catalytically dechlorinated using palladium on charcoal. The product proved to be identical with 9-benzyl-N^6,N^6-dimethyladenine (XX).[7] Reac-

(14) Kindly supplied by Dr. R. K. Robins.
(15) H. C. Koppel, D. E. O'Brien, and R. K. Robins, *J. Am. Chem. Soc.*, **81**, 3046 (1959).
(16) This compound has now been prepared by us in another manner.[17]
(17) J. A. Montgomery and K. Hewson, *J. Org. Chem.*, **26**, in press (1961).
(18) P. D. Lawley, *Proc. Chem. Soc.*, 290 (1957).

tion of XVII with ethylenimine gave 6-(1-aziridinyl)-9-benzyl-8-chloropurine (XIXb) only.

Benzylation of 2-amino-6-chloropurine (Xb) gave 2-amino-9-benzyl-6-chloro-9*H*-purine (XIb) the identity of which was confirmed by acid hydrolysis to 9-benzylguanine (XIVb). The 7-isomer contaminated with 9-isomer was obtained in this case also. 2-Amino-6-(1-aziridinyl)-9-benzyl-9*H*-purine (XIIIb) was prepared from XIb by reaction with ethylenimine.

Preliminary results have shown that the 6-(1-aziridinyl)purines inhibit the growth of carcinoma 755 moderately well. In addition, 6-(1-aziridinyl)-9-benzyl-9*H*-purine (VIIIb) inhibited the growth of Walker carcinoma 256 to a significant degree. The fact that VIIIb inhibits both Ca 755, a purine-sensitive tumor, and Walker 256, a tumor sensitive to alkylating agents but not purines, underlines the hopeful conjecture that these compounds may be a truly new class of tumor-inhibitory compounds that owe their activity to irreversible enzyme antagonism, resulting from the fact that structurally they combine an alkylating function with a metabolite carrier.

Experimental

Melting points below 260° were determined on a Kofler Heizbank and are corrected; those above 260° were determined in a capillary tube in an aluminum block and are uncorrected. The ultraviolet absorption spectra were determined with a Beckman DK-2 spectrophotometer (optical densities at the maxima with a Beckman DU) or a Cary Model 14.

Preparation of the (1-Aziridinyl)purines. General Procedure.—Ethylenimine was added in portions to a heated, well-stirred mixture of one equivalent of chloropurine and one equivalent of sodium bicarbonate in purified dioxane. After the reaction was complete, the reaction mixture was filtered and the insoluble residue triturated with dioxane. The combined filtrate and washings were taken to dryness *in vacuo* and the glass that remained was recrystallized from the appropriate solvent. In the preparation of VIIIa and VIIIb, triethylamine was used in place of sodium bicarbonate as the acid acceptor and benzene instead of dioxane as the solvent. Details of these preparations are given in Table I. The ultraviolet spectra of these compounds are listed in Table II.

Reduction of 6-(1-Aziridinyl)-9-benzyl-9*H*-purine. A. Catalytic.—A solution of 6-(1-aziridinyl)-9-benzyl-9*H*-purine (100 mg., 0.398 mmole) in ethanol (10 ml.) was hydrogenated at room temperature and atmospheric pressure with a 5% palladium on charcoal catalyst (50 mg.). A total of 8.5 ml. of hydrogen (87% of theor.) was taken up during 18 hr. The catalyst was removed by filtration, the filtrate evaporated to dryness and the residue dissolved in 0.1 *N* hydrochloric acid (10 ml.). The solution was evaporated to dryness *in vacuo* and the residual oil

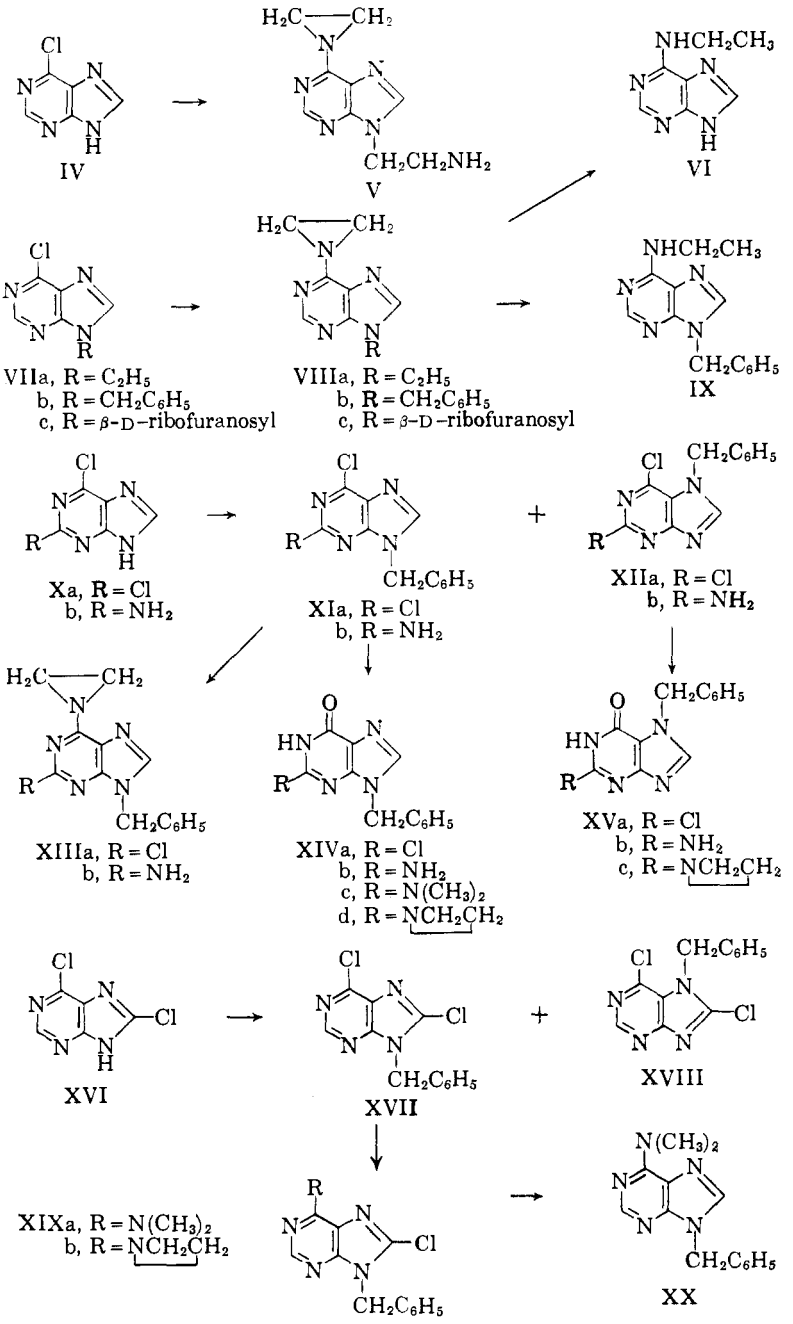
IV
V
VI
VIIa, R = C2H5
b, R = CH2C6H5
c, R = β-D-ribofuranosyl
VIIIa, R = C2H5
b, R = CH2C6H5
c, R = β-D-ribofuranosyl
IX
Xa, R = Cl
b, R = NH2
XIa, R = Cl
b, R = NH2
XIIa, R = Cl
b, R = NH2
XIIIa, R = Cl
b, R = NH2
XIVa, R = Cl
b, R = NH2
c, R = N(CH3)2
d, R = NCH2CH2
XVa, R = Cl
b, R = NH2
c, R = NCH2CH2
XVI
XVII
XVIII
XIXa, R = N(CH3)2
b, R = NCH2CH2
XX

TABLE I

1-(AZIRIDINYL)PURINES

Compound No.	Reactants[a] Ml./g.	Mole ratio	Dioxane, ml.	Reaction Temp., °C.	Time, hr.	Recrystn. solvent	M.p., °C.	Yield G.	Yield %	Analyses Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
VIIIa	1.1/1.00	3.7	20[b]	60–65	2	Skellysolve B	126	0.54	52	57.12	56.98	5.86	5.84	37.02	37.07
VIIIb	1.5/1.00	7.1	25[b]	80	2	CCl_4	137[c]	0.77	69	66.91	66.53	5.21	5.23	27.87	27.79
VIIIc	2.4/3.00	4.4	75	70	1	Acetone[d]	...[e]	1.85	60	49.96[f]	50.36	6.11[f]	6.72	19.43[f]	19.27
XIIIa	0.2/0.50	2.1	5	65–70	1	Benzene–Skellysolve C	128	0.26	50	58.95	59.17	4.25	4.39	24.56	24.61
XIIIb	1.0/0.73	7.2	25	90–95	3	Ethyl alcohol	108	0.50	57	61.54[g]	62.08	6.47[g]	6.46	26.92[g]	26.90
XIVd	5.0/1.00	2.6	100	95–100	6	Ethyl alcohol	92	0.24	23	62.91	62.79	4.90	4.97	26.20	25.82
XVc	0.3/0.20	7.1	30	90	1.5	$CHCl_3$	275	0.10	48	62.91	62.74	4.90	4.87	26.20	26.25
XIXb	0.5/0.50	5.1	5	75–85	1.25	Cyclohexane	307	0.29	55	58.95	58.75	4.25	4.40	24.56	24.66

[a] Ethylenimine/chloropurine. [b] Benzene. [c] With decomposition. [d] Trituration. [e] Indefinite. [f] Calcd. for $C_{12}H_{15}N_5O_4 \cdot C_3H_6O \cdot 0.5H_2O$. [g] Calcd. for $C_{10}H_{14}N_6 \cdot C_2H_5OH$.

TABLE II

ULTRAVIOLET SPECTRA OF THE (1-AZIRIDINYL)PURINES

	pH 1 λmax, mμ	pH 1 ε × 10⁻³	pH 7, 13 λmax, mμ	pH 7, 13 ε × 10⁻³		pH 1 λmax, mμ	pH 1 ε × 10⁻³	pH 7, 13 λmax, mμ	pH 7, 13 ε × 10⁻³
VIIIa	266	16.7	270	14.8	XIVd	254.5	12.5	253.5	13.7
b	267	17.5	270	16.0		283	6.4	275(sh)	
c	265	12.6	268	13.1	XVc	251	11.5	288	5.7
XIIIa	273	17.4	276	16 3		282.5	5.0		
			284(sh)		XIXb	268	17.4	272	16.6
b	252	10.3	289	10.8				279(sh)	
	290	11.5							

triturated with acetone (10 ml.). This trituration converted the oil into a crystalline solid which was collected by filtration and dried *in vacuo;* yield, 90 mg. (74%). The ultraviolet and infrared spectra of this sample, as well as its R_f values in four solvent systems, were practically identical with those of an authentic sample of 9-benzyl-N^6-ethyladenine hydrochloride.[7]

B. Sodium.—Metallic sodium (*ca.* 90 mg.) was added in small pieces to a stirred suspension of 6-(1-aziridinyl)-9-benzyl-9*H*-purine (300 mg., 1.19 mmoles) in liquid ammonia (50 ml.) until a blue color that lasted more than 10 min. was produced. When the addition of sodium was complete, ammonium chloride (210 mg.) was added, and the resulting orange-yellow solution was concentrated (10 ml.) in a stream of nitrogen. Chloroform (50 ml.) was added and the mixture further concentrated (25 ml.) until the vapors were neutral. At this point an additional amount of chloroform (25 ml.) was added, the insoluble inorganic materials were removed, and the filtrate was evaporated to dryness. The residue, N^6-ethyladenine, was triturated with ether, collected by filtration, and dried *in vacuo* over phosphorus pentoxide; yield 95 mg. (49%).

A sample of the above material was recrystallized from benzene–cyclohexane; m.p. 227–228° dec. (lit.[19] m.p. 238–239°). Comparison of the ultraviolet spectrum of this sample with that of authentic N^6-ethyladenine[19] indicated that the sample was impure. Paper chromatograms, developed in four systems, showed that the sample was contaminated with adenine.

9-Benzyl-2,6-dichloro-9*H*-purine (XIa) and 7-Benzyl-2,6-dichloro-7*H*-purine (XIIa).—A mixture of 2,6-dichloropurine (5.7 g., 30 mmoles), benzyl chloride (6.9 ml., 61 mmoles), and potassium carbonate (4.2 g., 30 mmoles) in dimethyl sulfoxide (50 ml.) was stirred vigorously for 18 hr. and then filtered. The filtrate was concentrated to one-quarter volume *in vacuo* before it was poured into ice water. The oil that separated from the ice water solidified on standing and the solid was collected by filtration, washed with water, and air dried. Recrystallization of this material from 75% aqueous ethyl alcohol with Norite treatment gave 5.9 g. (70%) of a mixture of the 7- and 9-isomers; m.p. 122–130°. The mixture was recrystallized from methyl alcohol (90 ml.) to give 1.06 g. (12.5%) of pure 9-isomer (XIa); m.p. 148°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 7, 13 276 (10.0). The product was shown to be pure 9-isomer (XIa) by paper chromatography and by conversion to 9-benzylguanine as described below.

Anal. Calcd. for $C_{12}H_8Cl_2N_4$: C, 51.81; H, 2.91; N, 20.05. Found: C, 51.74; H, 2.91; N, 20.06.

The mother liquors from the recrystallization described above gave a 30% yield of the 7-isomer (XIIa) contaminated with some 9-isomer (XIa). Further recrystallizations reduced the amount of 9-isomer present but did not remove it completely. The isomer content of the mixtures could be estimated by infrared spectroscopy.

2-Amino-9-benzyl-6-chloro-9*H*-purine (XIb) and 2-Amino-7-benzyl-6-chloro-7*H*-purine (XIIb).—A mixture of 2-amino-6-chloropurine (2.30 g., 1.36 mmoles), benzyl chloride (3.1 ml., 2.7 mmoles), and potassium carbonate (1.88 g., 1.36 mmoles) in dimethyl sulfoxide (25 ml.) was stirred vigorously at room tempera-

(19) G. B. Elion, E. Burgi, and G. H. Hitchings, *J. Am. Chem. Soc.*, **74**, 411 (1952).

ture for 20 hr. before it was poured, with stirring, into cold water (100 ml.). The solid that formed was removed by filtration, washed with water, and dried on the funnel by trituration with absolute alcohol. This crude product (3 g.) was extracted with boiling chloroform which was filtered and allowed to cool to room temperature. The solid that precipitated was collected by filtration and dried; yield 0.64 g. (18%); m.p. 212°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 313 (7.5); pH 7, 13, 223 (25.4), 247 (5.2), 308 (7.8). This material was shown by acid hydrolysis to be 9-benzylguanine and by chromatography to be pure 9-isomer.

Anal. Calcd. for $C_{12}H_{10}ClN_5$: C, 55.60; H, 3.81; N, 27.03. Found: C, 55.13; H, 3.86; N, 26.81.

Careful fractional crystallization, first from chloroform and then from water, of the second and third crops from the above chloroform extraction gave an additional 0.81 g. (23%) of 9-isomer making the total yield 1.45 g. (41%). From the chloroform insoluble material was obtained 0.85 g. (24%) of the 7-isomer; m.p. > 260°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 319 (7.0); pH 7, 13, 316 (5.6).

Anal. Calcd. for $C_{12}H_{10}ClN_5$: C, 55.60; H, 3.81; Found: C, 55.75; H, 4.06.

9-Benzyl-2-chlorohypoxanthine (XIVa).—A suspension of 9-benzyl-2,6-dichloropurine (278 mg., 1.00 mmole) in 0.2*N* sodium hydroxide (10 ml.) was refluxed with continuous stirring until essentially complete solution was effected. The hot reaction mixture was filtered and the filtrate acidified with glacial acetic acid. The white solid that precipitated was collected by filtration, washed with dilute acetic acid, and dried *in vacuo;* yield 233 mg. (90%); m.p. 245°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 252.5 (12.8); pH 7, 257 (14.0); pH 13, 256.5 (14.3).

Anal. Calcd. for $C_{12}H_9ClN_4O$: C, 55.39; H, 3.50; Cl, 13.46. Found: C, 55.46; H, 3.71; Cl, 13.42.

9-Benzylguanine[15] **(XIVb). A.**—A suspension of 9-benzyl-2-chlorohypoxanthine (100 mg., 0.39 mmole) in absolute ethanol (15 ml.) was placed in a 40 ml. glass-lined Parr bomb and saturated with dry ammonia gas at 5°. The resulting solution was heated at 150° for 6 hr. The bomb was chilled, opened and the content concentrated to 10 ml. in a stream of dry nitrogen. The solid that precipitated was collected by filtration, washed with ethanol and dried *in vacuo;* yield 53 mg. (57%); m.p. 303° (lit.[15] m.p. 300–302°); λ_{max} mμ ($\epsilon \times 10^{-3}$): pH 1, 254 (11.4), 278–9 (7.6); pH 7, 254 (11.4), 278–9 (7.6); pH 13, 269 (10.7).

B. A suspension of 2-amino-9-benzyl-6-chloropurine (259 mg., 1 mmole) in 1 *N* hydrochloric acid (20 ml.) was refluxed for 1 hr. The reaction solution was concentrated *in vacuo* to half volume, and the concentrate was neutralized with sodium bicarbonate (1.68 g., 20 mmoles). The solid that precipitated was collected by filtration, washed with water and dried *in vacuo;* yield 203 mg. (85%); m.p. 302°; λ_{max} in mμ: pH 1, 254 (11.1), 278 (7.3); pH 7, 253 (11.9), 270 (sh); pH 13, 258 (sh), 268 (10.0).

The products obtained in A and B were chromatographically identical with authentic 9-benzylguanine.

9-Benzyl-N^2,N^2-dimethylguanine (XIVc).—9-Benzyl-2-chlorohypoxanthine (0.29 mmole) and 25% aqueous dimethylamine (0.2 ml.) were dissolved in dioxane (10 ml.) and the reaction mixture was heated at 95–100° for 20 hr. with a second addition of aqueous dimethylamine at the end of the first 2 hr. The reaction mixture was evaporated to dryness *in vacuo*. Trituration of the resulting residue with

ether gave 82 mg. of crude product which was recrystallized from ethanol to give the pure product; yield 57 mg. (73%); m.p. 282°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 264 (16.8), 295 (6.9); pH 7, 260 (16.8); pH 13, 261 (11.5).

Anal. Calcd. for $C_{14}H_{15}N_5O$: C, 62.45; H, 5.76; N, 26.02. Found: C, 62.14; H, 5.66; N, 25.78.

7-Benzyl-2-chlorohypoxanthine (XVa).—A suspension of 7-benzyl-2,6-dichloropurine (1.11 g., 4.00 mmoles) (contaminated with some 9-isomer), in 1*N* sodium hydroxide (25 ml.) was heated at 115° for 1.5 hr. The reaction solution was cooled to room temperature and then allowed to stand overnight in the refrigerator to complete precipitation of the product. The solid that formed was collected by filtration and dissolved in water, and the water solution was acidified with glacial acetic acid. The solid that precipitated from the acid solution was collected by filtration and dried *in vacuo* to give 757 mg. (72%) of crude product. Recrystallization of a sample (277 mg.) of the crude material from absolute ethyl alcohol (50 ml.) gave analytically pure 7-benzyl-2-chlorohypoxanthine; yield 60 mg.; m.p. 285°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 255 (10.8); pH 7, 13, 264 (10.1).

Anal. Calcd. for $C_{12}H_9ClN_4O$: C, 55.39; H, 3.50; N, 21.54. Found: C, 55.26; H, 3.48; N, 21.40.

Additional material obtained from the concentration and chilling of the ethanol filtrate was contaminated with 9-isomer.

9-Benzyl-6,8-dichloro-9*H*-purine (XVII).—A well-stirred mixture of 6,8-dichloropurine (1.06 g., 5.00 mmoles), anhydrous potassium carbonate (0.69 g., 5.0 mmoles), and benzyl chloride (1.4 ml., 10 mmoles) in *N*,*N*-dimethylformamide (10 ml.) was heated at 70–85° for 1 hr. and then concentrated to half-volume *in vacuo*. The concentrate was poured into ice water and the oil that formed was removed by extraction with ether. Dilution of the concentrated ether extract with ethyl alcohol gave a solid which was collected by filtration, washed with a small amount of ether and dried; yield 378 mg.; m.p. 90°. This crude material was extracted with Skellysolve C and the extract evaporated to a thick oil which was dissolved in aqueous ethyl alcohol (1:1). The solid that crystallized from this solution was removed by filtration, washed with water and dried *in vacuo* over phosphorus pentoxide at room temperature; yield 203 mg. (15%). One recrystallization from aqueous ethyl alcohol gave the analytical sample; m.p. 92°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 7, 269.5 (13.0), 275 (sh); pH 13, 269.5 (11.9), 276 (sh).

Anal. Calcd. for $C_{12}H_8Cl_2N_4$: C, 51.81; H, 2.91; N, 20.15. Found: C, 51.73; H, 3.11; N, 19.86.

A second run using 6.18 g. of 6,8-dichloropurine gave a 57% yield of crude product which was recrystallized from Skellysolve C to give a 30% yield of pure material.

Although chromatographic evidence of the presence of 7-benzyl-6,8-dichloro-7H-purine was obtained, none of this material was isolated.

9-Benzyl-6-dimethylamino-9*H*-purine[7] **(XX).**—A stirred suspension of 9-benzyl-6,8-dichloropurine (500 mg., 1.8 mmoles) in 25% aqueous dimethylamine (20 ml.) was heated under reflux conditions in an oil-bath at 90–95° for 5 hr. The reaction mixture was cooled to room temperature and the solid collected by filtration to give 478 mg. (92%) of crude product. Recrystallization from ethanol gave chro-

matographically homogeneous material; yield 410 mg. (79%); m.p. 117°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 271.5 (20.7); pH 7, 278 (20.2); pH 13, 278 (20.2).

A mixture of some of the 9-benzyl-6-dimethylamino-8-chloropurine (307 mg., 1.07 mmoles) whose preparation is described above, magnesium oxide (43 mg., 1.07 mmoles), and 5% palladium on charcoal (34 mg.) in absolute alcohol (30 ml.) was hydrogenated at atmospheric pressure. Reduction was complete in 6 hr. The catalyst was removed by filtration and the filtrate evaporated to dryness. Trituration of the resulting residue with ethyl alcohol gave a solid which was shown by its ultraviolet spectrum to be 9-benzyl-6-dimethylaminopurine; yield 135 mg. (50%); m.p. 128° (lit.[7] m.p. 131°); λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 268 (18.2); pH 7, 276 (18.5); pH 13, 277 (18.6).

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Complex Esters of Thioinosinic-(5′) Acid[1]

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Several dinucleoside phosphates, derivatives of thioinosinic-(5′) acid, have been prepared for evaluation of their biologic activity, particularly their ability to inhibit organisms resistant to the action of 6-mercaptopurine.

The basis for our program on the synthesis of derivatives of thioinosinic acid (I) has been adequately discussed.[2,3] Briefly, there is good reason to hope that a derivative of I can be synthesized that will inhibit 6-mercaptopurine-resistant neoplasms that are unable to perform the "lethal synthesis" that converts 6-mercaptopurine to its

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(3) J. A. Montgomery, H. J. Thomas, and H. J. Schaeffer, *ibid.*, **26**, 1929 (1961).