was stirred at room temperature for 1 hr, heated at reflux for 10 hr, and cooled. The ester hydrochloride (145 g, 96%, mp 145.8–146.7°) obtained by filtration was used without further purification in the preparation of **7**.

3-(Undecanoyloxy)propyltrimethylammonium Iodide (7). Compound **11** (25.0 g, 0.081 mole) was treated with 150 ml of 10% aqueous Na_2CO_3, and the mixture was extracted with C_6H_6 (five 100-ml portions). Removal of the C_6H_6 by distillation under reduced pressure afforded the oily liquid free base of **11**. The latter was dissolved in 200 ml of Me_2CO, 34.6 g (0.244 mole) of MeI was added, and the mixture was heated at reflux for 12 hr. The product, obtained by filtration, was recrystallized four times (Me_2CO); yield 15.1 g (45%), mp 127.0–127.4°.

Evaluation of Insect-Repellent Activity.—Repellency against *Aedes aegypti* mosquitoes was evaluated by Mr. I. H. Gilbert and Mr. H. K. Gouck of the Entomology Research Division, U. S. Department of Agriculture, Gainesville, Fla.[12] Female *Aedes aegypti* mosquitoes, 7–8 days old, were confined in small cylindrical cages (4 × 12 cm). The sides of the cages were clear plastic; one end was covered with gauze and the other end was fitted with a plastic slide closure. Mosquitoes in stock cages were immobilized by a low temperature, and six females were placed in each small cage. The cages were then held in a warm room for at least 1 hr to permit the mosquitoes to recover before tests were begun. Tests were made by placing the end of the cage equipped with the slide in contact with a treated area on a human arm and opening the slide to give the mosquitoes direct access to the treated skin for a period of 1 min. Treated areas of the skin were sprayed with water every hour to simulate the wetting which would occur under sweating conditions; the water was applied with an atomizer to provide wetting but no runoff. In each test period (*cf.* Table II) cages of mosquitoes were exposed to untreated areas of the skin to provide checks on the percentage of mosquitoes biting.

Acknowledgments.—The authors are indebted to Dr. C. N. Smith, Mr. I. H. Gilbert, and Mr. H. K. Gouck of the Entomology Research Division, U. S. Department of Agriculture, Gainesville, Fla., for evaluating the mosquito repellency of the compounds reported in this communication. We wish to thank Dr. Andrew Lasslo for helpful suggestions in connection with this work, and Mr. John H. Buckner for assistance in the syntheses.

(12) Communication from the Entomology Research Division, U. S. Department of Agriculture, Beltsville, Md. (Gainesville, Fla.), Sept 1968.

Synthesis and Antitumor Activity of 9-Substituted Nitrogen Mustard Derivatives of Naturally Occurring Purines[1]

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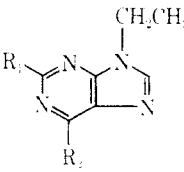
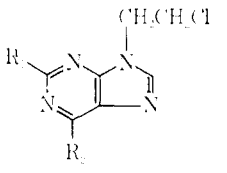
Substitution at position 9 of a number of purines often provided compounds with interesting antitumor activity.[3] That this activity does not result from the *in vivo* dealkylation of the 9-substituted derivatives to the parent purines is illustrated by the confirmed activity of 9-(tetrahydrofur-2-yl)adenine against Ca755.[4] A number of 9-substituted purines were found to inhibit nucleoside cleavage, thus potentiating the antitumor activity of compounds such as thioguanosine.[5] In the area of purine nitrogen mustard derivatives, 9-[bis(β-chloroethyl)aminopropyl]hypoxanthine[6] was found to be active against Ehrlich ascites 6C3HED and several other experimental tumors in mice.[7] Since 9-(β-chloroethyl)adenine was reported to inhibit the growth of the C1300 experimental tumor[8] and since a number of 6-S-substituted 9-(β-hydroxyethyl)- and 9-(β-chloroethyl)purines are active against Ca755, solid Friend virus leukemia, and cell culture testing systems,[9] naturally occurring purines bearing a 9-bis(β-chloroethyl)aminoethyl moiety should be studied. In connection with our previous work in this area,[3,10,11] synthesis of compounds of this type was investigated.

The present investigation includes the preparation of 9-bis(β-chloroethyl)aminoethyl derivatives of adenine (Ia), hypoxanthine (Ib), and guanine (Ic).

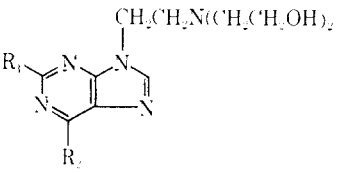


Ia, $R_1 = H$; $R_2 = NH_2$
b, $R_1 = H$; $R_2 = OH$
c, $R_1 = NH_2$; $R_2 = OH$

Treatment of 9-(β-chloroethyl)adenine (IIa) with diethanolamine in refluxing 2-ethoxyethanol, followed by reaction of the resulting solution with anhydrous HCl gave the dihydrochloride salt of 9-[bis(β-hydroxyethyl)aminoethyl]adenine (IIIa). Subsequent treatment of IIIa with $SOCl_2$ under reflux conditions yielded the desired Ia.



IIa, $R_1 = H$; $R_2 = NH_2$
b, $R_1 = NH_2$; $R_2 = SCH_3$



IIIa, $R_1 = H$; $R_2 = NH_2$
b, $R_1 = H$; $R_2 = SCH_3$
c, $R_1 = H$; $R_2 = OH$
d, $R_1 = NH_2$; $R_2 = SCH_3$
e, $R_1 = NH_2$; $R_2 = OH$

Careful treatment of 6-methylthio-9-[bis(β-hydroxyethyl)aminoethyl]purine[3] (IIIb) with dilute aqueous H_2O_2 and HCl yielded the hydrochloride salt of the corresponding hypoxanthine derivative IIIc. Treatment of IIIc with $SOCl_2$ gave Ib, isolated as a dihydrochloride salt.

(1) This investigation was supported in part by the Cancer Chemotherapy National Service Center, National Cancer Institute of the National Institutes of Health, Public Health Service, Contract PH-43-65-94; and in part by research grant CY-4008(C3) from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

(2) Taken in part from the M.S. thesis of James D. Westover, submitted to the Department of Chemistry, Arizona State University, Tempe, Ariz. 1961.

(3) R. K. Robins, *J. Med. Chem.*, **7**, 186, (1964).

(4) W. A. Bowles, F. H. Schneider, L. R. Lewis, and R. K. Robins, *ibid.*, **6**, 471 (1963).

(5) G. A. LePage and I. G. Junga, *Cancer Res.*, **23**, 739 (1963).

(6) H. H. Lin and C. C. Price, *J. Org. Chem.*, **26**, 266 (1961).

(7) R. J. Rutman, F. S. Lewis, S. Buckner, C. C. Price, F. Idewell*n*, and E. Owen, *Cancer Res. (Suppl)*, **22**, 559 (1962).

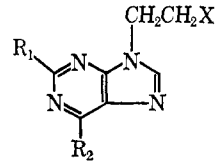
(8) (a) J. H. Lister and G. M. Timmis, *J. Chem. Soc.*, 327 (1960); (b) S. S. Epstein and G. M. Timmis, *Biochem. Pharmacol.*, **11**, 743 (1962).

(9) D. E. O'Brien, J. D. Westover, R. K. Robins, and C. C. Cheng, *J. Med. Chem.*, **8**, 182 (1965).

(10) H. C. Koppel, R. H. Springer, R. K. Robins, F. H. Schneider, and C. C. Cheng, *J. Org. Chem.*, **27**, 2173 (1962).

(11) H. C. Koppel, R. H. Springer, G. D. Daves, Jr., and C. C. Cheng, *J. Pharm. Sci.*, **52**, 81 (1963).

TABLE I
ANTITUMOR TEST RESULTS OF 9-SUBSTITUTED DERIVATIVES OF NATURALLY OCCURRING PURINES[a]



R_1	R_2	X	Dose,[b] mg/kg	Test/control Ca755	S180	L1210	LZ	WA	WC	WM	DA	DL	MP	Cell culture ED_{50},[c] μg/ml
NH_2	H	OH	200.0			0.89								
			25.0	0.52										
NH_2	H	Cl	46.0					0.14						
			25.0		0.43							1.00		
			23.0					0.64						
			12.5	0.09							1.00			
			11.5					0.71						
			6.2	0.33										
			5.7					1.20						
			5.0									0.88		
			3.1	0.31										
														1.0×10^2 (KB)
NH_2	H	$N(CH_2CH_2OH)_2$	75.0	1.46										
NH_2	H	$N(CH_2CH_2Cl)_2$	23.0				1.31							
			14.0				1.37							
			10.0									2.19		
			8.4				1.12							
			7.5	0.21		2.04								
			5.0		0.74	1.69						1.11		
			3.7	0.60										
			3.3			1.54							1.91[d]	
			2.5					0.00[e]				1.00		
			2.0							0.03			1.66	
			1.3			1.39		0.00[e]						
			1.0							0.34			1.40	
			0.8			1.08								
			0.6					0.00						
			0.5							0.57			1.28	
			0.3					0.34						
			0.13						1.16					
			0.06						0.70					
			0.03						0.94					
			0.02						1.34					
														1.0×10^2 (KB)
														1.0×10^2 (H2)
														1.0×10^2 (HR)
OH	H	OH	43.8	0.40										
OH	H	Cl	75.0	1.50										
OH	H	$N(CH_2CH_2Cl)_2$	200.0							0.25				
			32.0			1.03								
														3.1 × 10 (KB)
OH	NH_2	$N(CH_2CH_2Cl)_2$	50.0					1.00						

[a] The biological testing was performed by the screening contractors of the Cancer Chemotherapy National Service Center. Ca755 = Adenocarcinoma 755, S180 = Sarcoma 180, L1210 = lymphoid leukemia L1210, LZ = delayed L1210, WA = Walker carcinosarcoma 256 (subcutaneous), WC = Walker 256 resistant to cytoxan, WM = Walker 256 (intramuscular), DA = Dunning leukemia (ascites), DL = Dunning leukemia (solid), MP = L1210 resistant to 6-mercaptopurine, KB = human epidermoid carcinoma of the nasopharynx, H2 = HEp 2 human epidermoid carcinoma, HR = HEp 2 resistant to 6-mercaptopurine. [b] Below toxicity level. [c] ED_{50} = the dose that inhibits growth to 50% of control growth. [d] Cures, 2/6. [e] Cures, 7/7.

Diethanolamine in refluxing 2-ethoxyethanol converted 2-amino-6-methylthio-9-(β-chloroethyl)purine[9] (IIb) to 2-amino-6-methylthio-9-[bis(β-hydroxyethyl)-aminoethyl]purine (IIId) in 71% yield. Although the hydrolysis of the methylthio group to the hydroxyl group in the hypoxanthine series was smoothly carried out by a mixture of H_2O_2 and HCl, application of the same reaction conditions to IIId resulted in the rupture of the purine ring. The conversion of IIId to the acetate salt of 9-[bis(β-hydroxyethyl)aminoethyl]guanine (IIIe) was eventually accomplished by the method of Gerster and Robins,[12] and the resulting hygroscopic acetate salt of IIIe was treated with a weakly basic ion-exchange resin to afford the free base IIIe. The latter, upon treatment with $SOCl_2$, yielded the desired nitrogen mustard derivative of guanine Ic.

The antitumor screening results of these compounds in a variety of animal test systems are listed in Table I. Of the three target compounds, the adenine derivative Ia was found to be active against leukemia L1210, Carcinoma 755, Walker carcinosarcoma 256 (intramuscular), Walker 256 (subcutaneous), and leukemia L1210 resistant to 6-mercaptopurine. The hypoxanthine derivative Ib was inhibitory against Walker 256 (intramuscular). The guanine derivative Ic was only tested against Walker 256 (subcutaneous) and was found to be inactive. Among the intermediates synthesized, 9-(β-chloroethyl)adenine (IIa) possessed activity against both Carcinoma 755 and Walker 256 (subcutaneous).

(12) J. F. Gerster and R. K. Robins, *J. Am. Chem. Soc.*, 87 3752 (1965).

Experimental Section[13]

9-[Bis(β-hydroxyethyl)aminoethyl]adenine (IIIa).—A solution of 3.95 g (0.02 mole) of 9-(β-chloroethyl)adenine[8a] (IIa) and 4.2 g

(13) All melting points (corrected) were taken on a Thomas–Hoover melting point apparatus. The uv absorption spectra were determined with a Beckman DK-2 spectrophotometer. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

(0.04 mole) of diethanolamine in 125 ml of 2-ethoxyethanol was refluxed for 18 hr. The hot reaction mixture was treated with charcoal, diluted with 50 ml of EtOH, and filtered. The hot filtrate was adjusted to pH 1 by addition of ethanolic HCl. The acidic solution was then chilled and the resulting precipitate was collected by filtration and air dried. The yield of IIIa, isolated as a dihydrochloride salt, was 2.1 g (30% yield), mp 205° dec. An analytical sample was obtained by recrystallization from absolute EtOH, mp 205° dec. *Anal.* ($C_{11}H_{18}N_6O_2 \cdot 2HCl \cdot H_2O$) C, H, N, Cl.

9-[Bis(β-chloroethyl)aminoethyl]adenine (Ia).—A finely powdered suspension of 1.79 g (0.005 mole) of IIIa·2HCl·H_2O and 150 ml of $SOCl_2$ was heated on the steam bath for 1 hr. Excess $SOCl_2$ was then removed from the reaction mixture under reduced pressure as heating was continued on the steam bath. The residue was recrystallized from absolute EtOH to give 1.18 g (68% yield) of analytically pure dihydrochloride salt of Ia, mp 177–179°, λ_{max}^{EtOH} 260 mμ (ε 15,000). *Anal.* ($C_{11}H_{16}Cl_2N_6 \cdot 2HCl \cdot H_2O$) C, H, Cl, N.

9-[Bis(β-hydroxyethyl)aminoethyl]hypoxanthine (IIIc).—A solution of 2.97 g (0.01 mole) of 6-methylthio-9-[bis(β-hydroxyethyl)aminoethyl]purine[9] (IIIb), 10 ml of 30% H_2O_2, 10 ml of concentrated HCl, and 100 ml of H_2O was heated on the steam bath for 3 hr. The unreacted H_2O_2 was then decomposed by the slow addition of 10 g of MnO_2. After permitting to stand overnight, the solid was filtered and the filtrate was evaporated to dryness. The resulting yellow syrupy residue was covered with 100 ml of 95% EtOH, heated to boiling, and allowed to cool slowly. The white crystalline product IIIc, on cooling, separated as the analytically pure monohydrochloride salt, mp 124–126°. *Anal.* ($C_{11}H_{17}N_5O_3 \cdot HCl \cdot H_2O$) C, H, N.

9-[Bis(β-chloroethyl)aminoethyl]hypoxanthine (Ib).—A fine suspension of 1.60 g (0.005 mole) of IIIc·HCl·H_2O in 150 ml of $SOCl_2$ was refluxed for 6 hr. During this time a complete solution was obtained, followed by the precipitation of crystalline solids. The reaction mixture was evaporated to dryness and the residue was recrystallized from 40 ml of absolute EtOH to give 1.40 g (75% yield) of analytically pure Ib·2HCl, mp 205° dec, $\lambda_{max}^{H_2O}$ 250 mμ (ε 11,300). *Anal.* ($C_{11}H_{15}Cl_2N_5O \cdot 2HCl$) C, H, Cl^-, N.

2-Amino-6-methylthio-9-[bis(β-hydroxyethyl)aminoethyl]-purine (IIId).—A solution of 12.2 g (0.05 mole) of 2-amino-6-methylthio-9-(β-chloroethyl)purine[9] and 10.5 g (0.1 mole) of diethanolamine in 250 ml of 2-ethoxyethanol was refluxed for 18 hr. The red solution was evaporated *in vacuo* on a steam bath. The resulting red residue was covered with 75 ml of absolute EtOH and allowed to stand overnight at 5°. The light tan solid was collected, washed with cold EtOH, and air dried to give 11.1 g (71% yield) of crude product, mp 157–159°. An analytical sample of IIId was obtained by recrystallization from absolute EtOH; mp 161–162°. *Anal.* ($C_{12}H_{20}N_6O_2S$) C, H, N.

9-[Bis(β-hydroxyethyl)aminoethyl]guanine (IIIe).—A solution of 6.24 g (0.02 mole) of IIId, 20 ml of 30% H_2O_2, and 200 ml of glacial AcOH was stirred at room temperature for 24 hr. The resulting light yellow solution was added to 1 l. of Me_2CO with vigorous stirring. The mixture was then allowed to stand for 30 min and the supernatant liquid was separated by decantation. The resulting white residue was stirred with 200 ml of absolute EtOH for 30 min and the resulting hygroscopic solid was collected by rapid filtration. The solid was then dissolved in 100 ml of H_2O and the solution was stirred with 8 g of Amberlite IR-45 for 30 min. The weakly basic ion-exchange resin was then separated by filtration. The process was repeated three times. The resulting aqueous filtrate was evaporated to dryness and the residue was recrystallized from absolute EtOH to give 2.1 g (37% yield) of IIIe, mp 192–194° dec. *Anal.* ($C_{11}H_{18}N_6O_3 \cdot H_2O$) C, H, N.

9-[Bis(β-chloroethyl)aminoethyl]guanine (Ic).—A finely divided suspension of 1.50 g (0.005 mole) of IIIe·H_2O in 200 ml of $SOCl_2$ was refluxed for 5 hr. Although a complete solution was not formed during this period, a definite change in the appearance of the suspended solids was noted. The reaction mixture was evaporated to dryness under reduced pressure. The resulting off-white solid product was covered with 100 ml of absolute EtOH, heated to reflux, and then again evaporated to dryness. The crude product was recrystallized from 50 ml of absolute EtOH to give 1.10 g (62% yield) of analytically pure Ic·HCl, mp 218–220° dec, $\lambda_{max}^{H_2O}$ 253 mμ (ε 11,000), $\lambda_{max}^{H_2O}$ 269 mμ (ε 8200). *Anal.* ($C_{11}H_{16}Cl_2N_6O \cdot HCl$) C, H, N.

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Antineoplastic Agents. XXV. 1,4-Diazabicyclo[2.2.1]heptanes[1]

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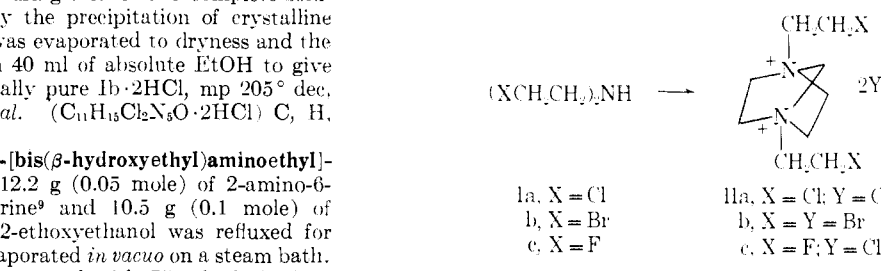
Mannich-type reactions employing bis(2-chloroethyl)amine have been used to prepare a number of nitrogen mustards derived from acetophenones,[3a,b] acetylenic carbinols,[3c] natural products,[3d] coumarins,[3e] thiophenes,[3b] benzimidazoles,[3f] and cyclic ketones.[3g] During the course of our initial studies in this area, condensing 1 mole of formaldehyde with 2 moles of bis(2-chloroethyl)amine was found to yield a new quaternary ammonium salt, shown to be 1,4-bis(2-chloroethyl)-1,4-diazabicyclo[2.2.1]heptane diperchlorate (IIa).[1,4] The new heterocyclic compound IIa demonstrated signifi-



Ia, X = Cl; IIa, X = Cl; Y = ClO_4
b, X = Br; b, X = Y = Br
c, X = F; c, X = F; Y = ClO_4

cant activity against the Walker 256 carcinoma.[4] To provide further examples of this new heterocyclic system for evaluation as possible cancer chemotherapeutic agents and to determine the scope of the condensation reaction, an analogous study was extended to several N-alkyl- and N-benzyl-substituted 2-haloethylamines.

Treating ethanol solutions of amines Ia–c and IIIa–h with 37% formalin at room temperature led to quaternary ammonium salts IIa–c and IVa–h (Table I) in good yields. Generally, reaction was complete within 24 hr, but the less reactive[5] fluoro derivative Ic required 144 hr. Attempted condensation with the relatively poor nucleophile N-[2-chloroethyl-3,5-bis(trifluoromethyl)benzyl]amine led only to recovery of starting amine, even at extended reaction times. Structures assigned to each new compound were supported by pmr studies.[1]

(1) Part XXIV: G. R. Pettit, D. C. Fessler, and J. A. Settepani, *J. Org. Chem.*, in press.

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(3) (a) G. R. Pettit and J. A. Settepani, *J. Med. Pharm. Chem.*, **5**, 296 (1962); (b) W. Werner and M. Mühlstädt, *Justus Liebigs Ann. Chem.*, **693**, 197 (1966); (c) G. R. Pettit and B. J. Dauley, *Can. J. Chem.*, **46**, 792 (1968); (d) G. R. Pettit and J. A. Settepani, *J. Org. Chem.*, **27**, 1714 (1962); (e) R. C. Elderfield and A. C. Meuta, *J. Med. Chem.*, 10, 922 (1967); (f) G. R. Revankar and S. Siddappa, *Monatsh. Chem.*, **98**, 169 (1967); (g) W. Werner, *J. Prakt. Chem.*, **37**, 154 (1968).

(4) (a) G. R. Pettit and J. A. Settepani, *Chem. Ind.* (London), 1805 (1964); (b) H. Böhme and H. Orth, *Arch. Pharm.*, **300**, 148 (1967).

(5) G. R. Pettit, J. A. Settepani, and R. A. Hill, *Can. J. Chem.*, **43**, 1792 (1965).