reduced pressure. The remaining oil was dissolved in ether. The ether solution was extracted with 5% $NaHCO_3$ solution. The sodium bicarbonate extract was acidified with concentrated H_2SO_4. The cloudy mixture was extracted with ether, and the ether solution was washed with water and dried ($MgSO_4$). On filtration and removal of the solvent there remained an oil which crystallized from benzene–petroleum ether. One further recrystallization from the same solvent yielded 126 mg. (13.6%) of crystals, m.p. 114–116.5°. An analytical sample prepared from a similar run melted at 116–117°.

Anal. Calcd. for $C_{14}H_{13}NO_6$: C, 57.73; H, 4.50; N, 4.81. Found: C, 57.56; H, 4.47; N, 4.42, 4.74.

Methyl DL-3-Phthalimido-5-oxo-6-diazohexanoate (XII).—This compound was prepared in the same manner as VI was prepared from its precursor. From 1 g. (0.0036 mole) of 3-phthalimido-4-carbomethoxybutanoic acid and 0.75 g. (0.0036 mole) of phosphorus pentachloride in 75 ml. of ether the intermediate acid chloride was obtained. After removing the $POCl_3$ in the usual manner, the remaining oil was dissolved in chloroform and added dropwise to a solution of diazomethane. After refrigeration there were obtained crystals which, after one recrystallization from benzene–petroleum ether, weighed 279 mg. (24.6%), m.p. 102.5–105°. An analytical sample melted at 103–106°.

Anal. Calcd. for $C_{15}H_{13}N_3O_5$: C, 57.14; H, 4.16; N, 13.33. Found: C, 57.14; H, 3.87; N, 13.24.

Attempted Preparation of 3-Amino-5-oxo-6-diazohexanoic Acid. A 2-g. quantity of XII was dissolved in 25 ml. of CH_2Cl_2 and cooled in an ice-salt bath. The mixture was stirred and a solution of 0.46 g. (2 equiv.) of 95+% hydrazine in 7 ml. of CH_2Cl_2 was added from a dropping funnel. After stirring and cooling for 2 hr. the mixture was kept at −10° overnight. It was then stirred and cooled in an ice-salt bath for another 5 hr. and kept overnight again at −10°. The resulting solid (0.754 g.) was collected by filtration. The filtrate was reduced to about 8 ml. under reduced pressure. An ice-cold solution of 70 ml. of methanol and 14 ml. of 1 *N* NaOH was added. The solution was then stored at −10° overnight. The pH was then very carefully adjusted to 6.5 (Beckman zeromatic pH meter) with 2 *N* HCl. Most of the methanol was removed under reduced pressure. The remaining solution was shell-frozen and lyophilized. The oil was placed on a refrigerated chromatographic column 10 mm. in diameter and 7 cm. in length packed with equal weights of N.F. activated charcoal and Celite 545. It was eluted with 1% acetone. Fractions (10 ml.) were collected and checked for a diazo compound by adding HI solution to samples. The two fractions that gave an iodine color with HI were shell-frozen and lyophilized. There remained in each fraction a white solid. In an attempt to purify the material from a typical fraction, 0.5 ml. of water was added leaving a sticky mass; it was decanted. Ethanol (3 ml.) was added to the decantate. This yielded a white solid with the characteristics of NaCl. The mother liquor, after filtration, yielded an oil which decomposed at room temperature.

The sticky mass referred to above likewise yielded only NaCl when treated with absolute ethanol, and the mother liquor gave an oil which decomposed at room temperature.

Methyl DL-Phthalimidobutanedioate (VII).—Two grams (0.0072 mole) of IV was converted to the corresponding acid chloride as described in the preparation of VI. After the $POCl_3$ had been removed, 13 ml. of absolute methanol were added and the mixture was heated for a few minutes. On refrigeration white crystals were formed which were collected by filtration. One recrystallization from methanol yielded 1.1 g. (52.2%) of product melting at 95–99°. An analytical sample melted at 97.5–100°.

Anal. Calcd. for $C_{14}H_{13}NO_6$: C, 57.73; H, 4.50; N, 4.81. Found: C, 57.92; H, 4.25; N, 4.60.

Acknowledgment.—We acknowledge the assistance of Charles O. Garrison, Thomas H. Henscheid, and Boyd D. Nash.

Enzyme Inhibitors. VIII. Studies on the Mode of Binding of Some 6-Substituted 9-(Hydroxyalkyl)purines to Adenosine Deaminase[1,2]

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The syntheses of some 6-substituted 9-(2-hydroxypropyl)purines and some 6-substituted 9-(2,3-dihydroxypropyl)purines have been accomplished by the condensation of 5-amino-4,6-dichloropyrimidine with the appropriate amino alcohols, followed by ring closure of the resultant substituted pyrimidines to give the desired 6-chloro-9-substituted purines. Displacement of the 6-chloro group by certain nucleophilic reagents gave a variety of 6-substituted derivatives. Enzymatic evaluation of these compounds established that the 6-amino and the 6-methylamino derivatives inhibited adenosine deaminase. Comparison of the 6-aminopurines which were substituted at the 9-position by *n*-propyl, 3-hydroxypropyl, 2-hydroxypropyl, and 2,3-dihydroxypropyl groups established that there is only one hydroxyl binding site on adenosine deaminase in the area two to three carbons removed from the 9-position of the purine nucleus.

Several previous studies on the determination of the sites on the substrate which are important for binding to adenosine deaminase have revealed that rather large changes in the substituent at the 9-position of the purine nucleus can be made without markedly altering the capacity of the compound to bind to the enzyme.[3,4] Thus, it would appear that the 9-position of a 6-aminopurine would be a suitable area for attempting the preparation of active-site-directed irreversible inhibitors.[5] Before such active-site-directed irreversible inhibitors can be designed in a rational manner, it is necessary to know as much as possible about the binding sites, as well as the areas on the enzyme which have a large bulk tolerance. In an attempt to learn more about the nature of the binding by the substituent at the 9-position of the purine nucleus, we have synthesized and studied, as potential reversible inhibitors of adenosine deaminase, those purines which have at the

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(2) For the previous paper of this series, see H. J. Schaeffer and E. Odin, *J. Pharm. Sci.*, **54**, 421 (1965).

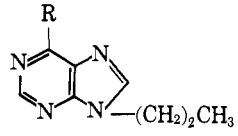
(3) H. J. Schaeffer, S. Marathe, and V. Alks, *ibid.*, **53**, 1368 (1964).

(4) H. J. Schaeffer and P. S. Bhargava, *Biochemistry*, **4**, 71 (1965).

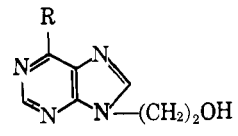
(5) B. R. Baker, *J. Pharm. Sci.*, **53**, 347 (1964).

9-position a *n*-propyl group, a 3-hydroxypropyl group, a 2-hydroxypropyl group, and a 2,3-dihydroxypropyl group.

Chemistry.—The synthesis of 6-amino- and 6-methylamino-9-*n*-propylpurine (I and II) was accomplished by the method of Temple, *et al.*,[6] and the preparation of 6-amino- and 6-methylamino-9-(3-hydroxypropyl)purine (III and IV) was effected by the method described previously.[4,7] The syntheses of the 6-substituted 9-(2-hydroxypropyl)purines and the 6-



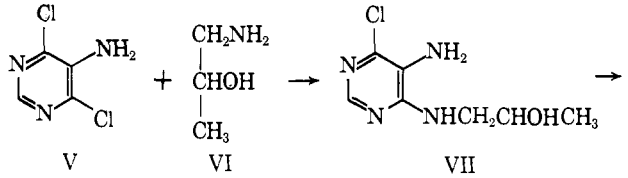
I, R = NH_2
II, R = NHMe



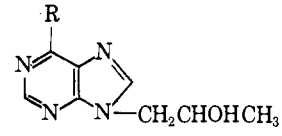
III, R = NH_2
IV, R = NHMe

substituted 9-(2,3-dihydroxypropyl)purines were accomplished by the routes outlined in Charts I and II, respectively. Thus, when 5-amino-4,6-dichloropyrimidine (V) was allowed to react with 1-amino-2-propanol (VI), a good yield of the corresponding substituted pyrimidine VII was obtained. Cyclization of VII by means of ethyl orthoformate and hydrochloric acid gave the desired 6-chloropurine derivative VIII. The 6-substituted 9-(2-hydroxypropyl)purines (IX–XIV) were prepared by allowing VIII to react with the appropriate nucleophilic reagent.

CHART I



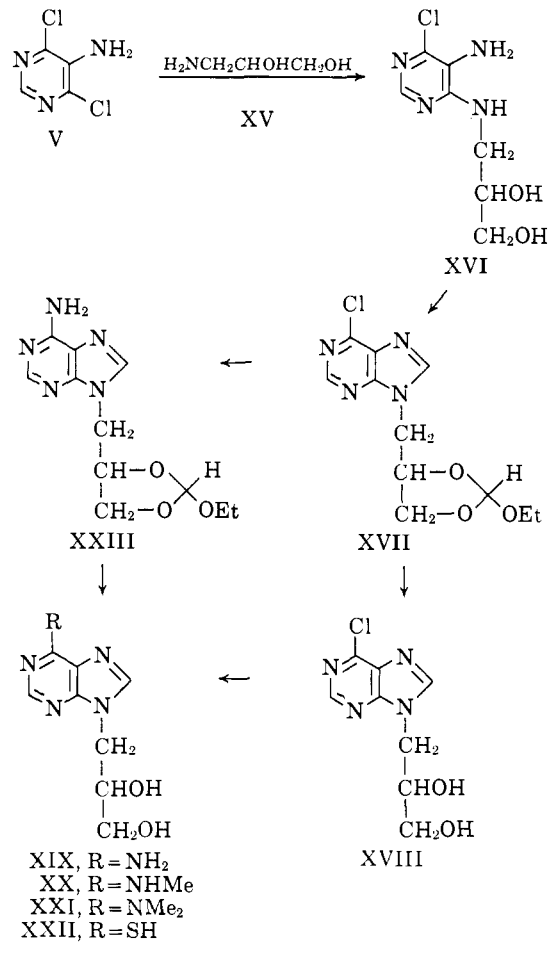
V VI VII



VIII, R = Cl
IX, R = NH_2
X, R = NHMe
XI, R = NMe_2
XII, R = SH
XIII, R = OH
XIV, R = OMe

A similar sequence of reactions was employed for the preparation of the 6-substituted 9-(2,3-dihydroxypropyl)purines (Chart II). Condensation of V with 1-amino-2,3-dihydroxypropane (XV) gave 5-amino-4-chloro-6-(2,3-dihydroxypropylamino)pyrimidine (XVI) in an excellent yield. Attempted cyclization of XVI to produce 6-chloro-9-(2,3-dihydroxypropyl)purine (XVIII) by means of ethyl orthoformate and HCl or by means of diethoxymethyl acetate gave a compound which on the basis of its ultraviolet spectrum was a 6-chloropurine derivative but which did not exhibit absorption in its infrared spectrum for hydroxyl groups. However, on the basis of its subsequent conversion into XVIII and XXIII, we have assigned structure XVII to the initial cyclization product. Thus, when XVII was converted into XVIII by means of hydrogen chloride, and XVIII allowed to react with methanolic ammonia, 6-amino-9-(2,3-dihydroxypropyl)-purine (XIX) was produced. This 6-amino derivative (XIX) was identical in all respects with the product formed by allowing XVII to react with methanolic ammonia to give XXIII which on treatment with HCl gave XIX. This sequence of reactions to produce XIX is consistent with the assignment of the orthoester structure to XVII. The formation of an orthoester from a vicinal diol on a substituent at the 9-position of a purine nucleus suggests that this reaction may find utilization in the formation of a new type of blocking group for ribonucleosides and perhaps for ribonucleotides. Recently, it has been reported that certain ribofuranosylpurines are, in fact, converted into the corresponding orthoesters by reaction with ethyl orthoformate.[8,9]

CHART II



V XV XVI XXIII XVII XIX, R = NH_2
XX, R = NHMe
XXI, R = NMe_2
XXII, R = SH
XVIII

Experimental[10]

5-Amino-4-chloro-6-(2-hydroxypropylamino)pyrimidine (VII).—A solution of 4.43 g. (27.0 mmoles) of V, 2.07 g. (27.5 mmoles) of 1-amino-2-propanol, and 3.04 g. (30.0 mmoles) of triethylamine in 30 ml. of 1-butanol was heated under reflux for 21 hr. The volatile materials were removed *in vacuo* and the oily product was dis-

(6) C. Temple, Jr., C. L. Kussner, and J. A. Montgomery, *J. Med. Pharm. Chem.*, **5**, 866 (1962).

(7) M. Ikehara, E. Ohtsuka, S. Kitagawa, K. Yagi, and Y. Tonomura, *J. Am. Chem. Soc.*, **83**, 2679 (1961).

(8) J. Zemlicka, *Chem. Ind.* (London), 581 (1964).

(9) M. Jarman and C. B. Reese, *ibid.*, 1493 (1964).

(10) The infrared spectra were determined on a Perkin-Elmer Model 137 spectrophotometer; the ultraviolet spectra and enzyme rates were determined on a Perkin-Elmer Model 4000A spectrophotometer. The melting points, unless noted otherwise, were determined on a Kofler Helzbank and are corrected.

solved in 10 ml. of water. After chilling, the white solid which formed was collected by filtration; yield 4.99 g., m.p. 155–156°. The crude material was recrystallized from water and dried at 100°; yield of VII, 4.47 g. (81.4%); m.p. 160–161°; λ_{max} in mμ ($\epsilon \times 10^{-3}$), at pH 1, 305 (13.2), at pH 7, 290 and 263 (9.40 and 9.40), at pH 13, 290 and 263 (10.0 and 10.0); ν in cm.$^{-1}$ (KBr), 3390 (OH), 3200–3100 (NH_2), 1640 and 1570 (C=N and C=C).

Anal.[11] Calcd. for $C_6H_{11}ClN_4O$: C, 41.47; H, 5.47; N, 27.64. Found: C, 41.29; H, 5.43; N, 27.78.

6-Chloro-9-(2-hydroxypropyl)purine Hydrochloride (VIII).—To a suspension of 3.94 g. (19.4 mmoles) of VII in 50 ml. of triethyl orthoformate was added 25.2 mmoles of concentrated HCl in small portions. A white precipitate formed, and the suspension was stirred for 20 hr. at room temperature. The reaction mixture was chilled, and the white solid was collected by filtration; yield 2.79 g., m.p. 174–176° (oil bath). The filtrate was chilled and saturated with HCl gas. A second fraction precipitated and was collected by filtration (1.60 g.); m.p. 174–176° (oil bath); total yield of VIII, 90.7%. Recrystallization of 500 mg. of the crude product from acetonitrile gave 447 mg. of the analytical sample: m.p. 174–176° (oil bath); λ_{max} in mμ ($\epsilon \times 10^{-3}$), at pH 1, 265 (9.70), at pH 7, 265 (9.70), at pH 13, 265 (9.70); ν in cm.$^{-1}$ (KBr), 3400 (OH), 2600–2350 (C=N$^+$), 1610 and 1580 (C=N and C=C).

Anal. Calcd. for $C_8H_{10}Cl_2N_4O$: C, 38.57; H, 4.05; N, 22.49. Found: C, 38.62; H, 4.20; H, 4.20; N, 22.25.

6-Amino-9-(2-hydroxypropyl)purine (IX).—A solution of 300 mg. (1.21 mmoles) of VIII in 20 ml. of 20% methanolic NH_3 was heated in a steel bomb at 95° for 18 hr. Removal of the volatile materials *in vacuo* gave 391 mg. of a white solid mixture. The mixture was triturated with two 15-ml. portions of hot acetone and filtered. The acetone was evaporated *in vacuo* and gave the crude product, yield 218 mg., m.p. 182–184°. One recrystallization from acetone–hexane gave the analytical material IX: yield 164 mg. (70.1%); m.p. 192–193°; λ_{max} in mμ ($\epsilon \times 10^{-3}$), at pH 1, 260 (7.42), at pH 7, 261 (8.05), at pH 13, 261 (8.05); ν in cm.$^{-1}$ (KBr), 3350 (OH), 3200 and 1670 (NH_2), 1600 and 1580 (C=N and C=C).

Anal. Calcd. for $C_8H_{11}N_5O$: C, 49.73; H, 5.73; N, 36.25. Found: C, 49.64; H, 5.81; N, 36.01.

6-Methylamino-9-(2-hydroxypropyl)purine (X).—A solution of 300 mg. (1.21 mmoles) of VIII in 15 ml. of 40% methylamine in water was heated under reflux for 4 hr. The volatile materials were removed *in vacuo* and gave a white solid mixture. The methylamine hydrochloride was removed by dissolving the crude mixture in absolute ethanol and precipitating the salt by the addition of small amounts of ether. After all of the hydrochloride salt was removed by filtration, the filtrate was evaporated *in vacuo*, and the crude material was crystallized from acetone–hexane; yield of X, 193 mg. (70.9%); m.p. 114–116° (oil bath); λ_{max} in mμ ($\epsilon \times 10^{-3}$), at pH 1, 263 (1.99), at pH 7, 267 (1.94), at pH 13, 267 (1.94); ν in cm.$^{-1}$ (KBr), 3300 (OH), 1640 and 1575 (C=N and C=C).

Anal. Calcd. for $C_9H_{13}N_5O \cdot H_2O$: C, 47.98; H, 6.71; N, 31.09. Found: C, 47.78; H, 6.74; N, 31.30.

6-Dimethylamino-9-(2-hydroxypropyl)purine Dihydrochloride (XI).—A solution of 300 mg. (1.21 mmoles) of VIII in 20 ml. of 25% dimethylamine in water was heated under reflux for 3 hr. Removal of the volatile materials *in vacuo* gave an oil which would not crystallize. The crude mixture was dissolved in $CHCl_3$–methanol (5:1) and HCl was passed through the chilled solution. The addition of a small amount of ether caused the precipitation of a white solid which was collected by filtration; yield 335 mg. (99.9%), m.p. 188–190° (oil bath). One recrystallization from methanol–ether, saturated with HCl gave 280 mg. of the pure product XI: m.p. 188–190°; λ_{max} in mμ ($\epsilon \times 10^{-4}$), at pH 1, 268 (2.06), at pH 7, 275 (2.13), at pH 13, 275 (2.06); ν in cm.$^{-1}$ (KBr), 3400 (OH), 2700–2400 (–NH$^+$), 1670 (C=N$^+$H), 1600 and 1535 (C=N and C=C).

Anal. Calcd. for $C_{10}H_{17}Cl_2N_5O$: C, 40.83; H, 5.83; N, 23.84. Found: C, 40.93; H, 5.93; N, 23.63.

6-Mercapto-9-(2-hydroxypropyl)purine (XII).—A solution of 300 mg. (1.21 mmoles) of VIII and 184 mg. (2.42 mmoles) of thiourea in 10 ml. of 1-propanol was heated under reflux for 2 hr. The clear reaction mixture was chilled and the resulting precipitate was collected by filtration; yield 143 mg., m.p. 304–308° (aluminum block). The filtrate was evaporated *in vacuo* and crystallization of the residue from water gave an additional 50 mg. of crude product, m.p. 304–308°. The two fractions were combined and recrystallized from water to give the pure product XII: yield 167 mg. (65.7%); m.p. 314–316°; λ_{max} in mμ ($\epsilon \times 10^{-4}$), at pH 1, 322 (2.20), at pH 7, 320 (2.37), at pH 13, 310 (2.12); ν in cm.$^{-1}$ (KBr), 3500 (OH), 2650 (SH), 1600 and 1540 (C=N and C=C).

Anal. Calcd. for $C_8H_{10}N_4OS$: C, 45.68; H, 4.75; S, 15.24. Found: C, 45.41; H, 4.65; S, 14.92.

6-Hydroxy-9-(2-hydroxypropyl)purine Hydrochloride (XIII).—A solution of 350 mg. (1.41 mmoles) of VIII in 10 ml. of 1 *N* HCl was heated under reflux for 2.5 hr. Removal of the volatile materials *in vacuo* gave the crude product as the hydrochloride salt, m.p. 174–182° (oil bath). Recrystallization from methanol–ether gave the pure product XIII: 253 mg. (77.6%); m.p. 198–200° (oil bath); λ_{max} in mμ ($\epsilon \times 10^{-4}$), at pH 1, 249 (1.36), at pH 7, 249 (1.36), at pH 13, 253 (1.38); ν in cm.$^{-1}$ (KBr), 3450 (OH), 1710 (C=O enol), 1680 (C=N$^+$), 1570 and 1540 (C=N and C=C).

Anal. Calcd. for $C_8H_{10}N_4O_2 \cdot HCl$: C, 41.66; H, 4.80; N, 24.29. Found: C, 41.39; H, 4.88; N, 24.09.

6-Methoxy-9-(2-hydroxypropyl)purine (XIV).—A solution of 200 mg. (0.808 mmole) of VIII and 175 mg. (3.23 mmoles) of sodium methoxide in 10 ml. of anhydrous methanol was heated under reflux for 2 hr. The solution was acidified to pH 6 with glacial acetic acid and evaporated *in vacuo*. The residual solid was triturated with $CHCl_3$ (20 ml.) and filtered to remove the insoluble salts. The addition of hexane to the filtrate caused the separation of the solid product which was collected by filtration; yield 133 mg. (79.1%), m.p. 130–132° (oil bath). Recrystallization from $CHCl_3$–hexane gave 86 mg. of the analytical sample XIV: m.p. 130–132° (oil bath); λ_{max} in mμ ($\epsilon \times 10^{-4}$), at pH 1, 252 (1.20), at pH 7, 252 (1.20), at pH 13, 252 (1.20); ν in cm.$^{-1}$ (KBr), 3350 (OH), 1600 and 1580 (C=N and C=C), 1230 and 1060 (=C–O–C).

Anal. Calcd. for $C_9H_{12}N_4O_2$: C, 51.43; H, 5.81; N, 26.91. Found: C, 51.59; H, 5.90; N, 26.78.

5-Amino-4-chloro-6-(2,3-dihydroxypropylamino)pyrimidine (XVI).—A solution of 6.00 g. (36.6 mmoles) of V, 3.36 g. (37.0 mmoles) of XV, and 3.70 g. (36.6 mmoles) of triethylamine in 40.0 ml. of 1-butanol was heated under reflux for 24 hr. The reaction mixture was evaporated *in vacuo* to a tan oil and was crystallized from water. Two recrystallizations from water followed by drying at 100° *in vacuo* gave the analytical material XVI: yield 5.57 g. (69.3%); m.p. 155–157° (oil bath); λ_{max} in mμ ($\epsilon \times 10^{-4}$), at pH 1, 302 (1.18), at pH 7, 260 (89.9) and 290 (89.9), at pH 13, 260 (89.9) and 290 (89.9); ν in cm.$^{-1}$ (KBr), 3400 (OH), 3300–3200 (NH_2), 1640 (NH_2), 1580 (C=N).

Anal. Calcd. for $C_7H_{11}ClN_4O_2$: C, 38.44; H, 5.07; N, 25.62. Found: C, 38.43; H, 5.13; N, 25.45.

6-Chloro-9-(2,3-dihydroxypropyl)purine Hydrochloride (XVIII).—To a suspension of 5.50 g. (25.0 mmoles) of XVI in 100 ml. of triethyl orthoformate was slowly added 2.14 ml. (26.0 mmoles) of concentrated HCl. The solid slowly dissolved, and the clear mixture was left at room temperature overnight. Removal of the volatile materials *in vacuo* at 35° gave 7.10 g. of a tan oil which did not exhibit hydroxyl absorption in the infrared. The crude oil was dissolved in $CHCl_3$ (25 ml.) and ether (25 ml.). Hydrogen chloride was bubbled through the chilled solution, and the white precipitate was collected by filtration[12]; yield, 6.57 g. (98.5%), m.p. 145–150° (oil bath). Recrystallization from methanol–ether saturated with HCl gave 5.01 g. (75.5%) of the pure product (XVIII): m.p. 155–160° (oil bath); λ_{max} in mμ ($\epsilon \times 10^{-3}$), at pH 1, 260 (9.84), at pH 7, 260 (9.84), at pH 13, 262 (11.6); ν in cm.$^{-1}$ (KBr), 3400 (OH), 2600–2350 (C=N$^+$H), 1580 (C=C).

Anal. Calcd. for $C_8H_{10}Cl_2N_4O_2$: C, 36.24; H, 3.80; Cl, 26.75. Found: C, 36.50; H, 4.00; Cl, 26.41.

Cyclization of 3-(5-Amino-6-chloro-4-pyrimidinylamino)-1,2-propanediol with Diethoxymethyl Acetate.—A mixture of 4.50 g. (20.6 mmoles) of XVI in 10.0 ml. of diethoxymethyl acetate was heated under reflux for 25 min. The solvent was removed *in vacuo* at 50° and gave 5.61 g. of a tan oil which lacked OH absorption in the infrared spectrum. The crude material was purified by passing it through a neutral alumina column (40 g.) with 60 ml. of $CHCl_3$. Removal of the chloroform gave 3.93 g. of an oil which had an identical infrared spectrum and thin layer chro-

(11) The analyses reported in this paper were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn.

(12) No attempt was made to keep the reaction mixture anhydrous; it is possible that the removal of the orthoester occurred because of moisture which could have been absorbed from the atmosphere.

matography R_f values as the oil obtained by the cyclization procedure employing triethyl orthoformate. A small amount of this material was dissolved in $CHCl_3$ and converted to 3-(6-chloro-9-purinyl)-1,2-propanediol hydrochloride by bubbling HCl gas through the chilled solution and collecting the precipitate, m.p. 155–160°. XVIII was identical in every respect with an authentic sample prepared by a method previously described.

3-(6-Amino-9-purinyl)-1,2-O-dihydroxypropyl Ethyl Orthoformate (XXIII).—A mixture of 219 mg. (1.00 mmole) of XVI, 6.0 ml. of triethyl orthoformate, and 1.30 mmoles of concentrated HCl was stirred overnight at room temperature. The volatile materials were removed *in vacuo* at 35° and 20 ml. of 19% methanolic NH_3 was added. The solution was placed in a steel bomb and heated overnight at 55°. Removal of the volatile materials gave a soft white solid. Chloroform (10 ml.) was added, and the hot mixture was filtered to remove NH_4Cl. The filtrate was treated with hexane until cloudy and chilled overnight, which gave a fine white solid that was removed by filtration; yield 108 mg., m.p. 137–140° (oil bath). Recrystallization from ethyl acetate–hexane gave 63.5 mg. (24%), m.p. 162–164°. A second run gave 37% of the pure material: λ_{max} in mμ ($\epsilon \times 10^{-4}$), at pH 1, 261 (1.49), at pH 7, 261 (1.54), at pH 13, 261 (1.54); ν in cm.$^{-1}$ (KBr), 3300 (NH_2), 1660 and 1590 (C=N and C=C).

Anal. Calcd. for $C_{11}H_{15}N_5O_3$: C, 49.80; H, 5.69; N, 26.40. Found: C, 49.65; H, 5.75; N, 26.46.

3-(6-Amino-9-purinyl)-1,2-O-dihydroxypropyl Ethyl Orthoformate (XXIII).—The 3-(6-chloro-9-purinyl)-1,2-O-dihydroxypropyl ethyl orthoformate (XVII, 1.07 g., 3.74 mmoles) obtained from the diethoxymethyl acetate cyclization was dissolved in 20 ml. of 20% methanolic NH_3. The mixture was heated in a steel bomb at 55° for 18 hr. Removal of the volatile materials *in vacuo* gave a semisolid material. Chloroform (20 ml.) was added and the NH_4Cl was removed by filtration. The addition of hexane to the filtrate caused the separation of 382 mg. (38.4%) of pure material, m.p. 160–163° (oil bath). The ultraviolet and infrared spectra were identical with those of an authentic sample prepared by a different procedure.

Anal. Calcd. for $C_{11}H_{15}N_5O_3$: C, 49.80; H, 5.69; N, 26.40. Found: C, 49.95; H, 5.81; N, 26.73.

6-Amino-9-(2,3-dihydroxypropyl)purine Hydrochloride (XIX).—A solution of 250 mg. (0.942 mmole) of XXIII in 3 ml. of 10% HCl was left at room temperature for 2.5 hr. Removal of the volatile constituents *in vacuo* at 50° followed by the addition of methanol and ether gave the pure hydrochloride salt (XIX); yield 212 mg. (91%); m.p. 216–219°; λ_{max} in mμ ($\epsilon \times 10^{-4}$), at pH 1, 260 (1.36), at pH 7, 261 (1.38), at pH 13, 261 (1.42); ν in cm.$^{-1}$ (KBr), 3350 (OH), 1670 (C=N$^+$H), 1580 (C=C).

Anal. Calcd. for $C_8H_{12}ClN_5O_2$: C, 39.10; H, 4.92; N, 28.50. Found: C, 38.84; H, 5.18; N, 28.10.

6-Amino-9-(2,3-dihydroxypropyl)purine Hydrochloride (XIX).—A solution of 500 mg. (1.88 mmoles) of XVIII in 20 ml. of 20% methanolic NH_3 was heated in a steel bomb at 80° for 20 hr. Removal of the volatile materials *in vacuo* gave 588 mg. of a soft yellow solid. The crude solid was dried to a brittle material *in vacuo*, but quickly softened on exposure to air. A hydrochloride salt was prepared by dissolving the crude material in anhydrous methanol and passing dry HCl through the chilled solution. The white precipitate was collected by filtration and two recrystallizations from methanol–ether gave the analytical material XIX, yield 260 mg. (56.1%), m.p. 215–218°. The melting point and infrared and ultraviolet spectra were identical with those of an authentic sample prepared by a different procedure.

6-Methylamino-9-(2,3-dihydroxypropyl)purine Dihydrochloride (XX).—A solution of 500 mg. (1.88 mmoles) of XVIII in 20 ml. of 40% methylamine in water was heated under reflux for 3 hr. The volatile materials were removed *in vacuo* and gave a yellow oil. The oily mixture was dissolved in 10 ml. of absolute ethanol and methylamine hydrochloride was removed by adding ether or chloroform and removing the salt by filtration. To the filtrate was added $CHCl_3$ (10 ml.) and HCl was bubbled through the chilled solution until a precipitate formed. Ether (20 ml.) was then added, and the white solid was collected by filtration; yield 492 mg., m.p. 183–186° (oil bath). The material was purified by dissolving in methanol (10 ml.) and bubbling HCl through the chilled solution until a precipitate formed. Ether (15 ml.) was added, and the pure product XX was collected by filtration: yield 394 mg. (70.8%); m.p. 190–196° (oil bath); λ_{max} in mμ ($\epsilon \times 10^{-4}$), at pH 1, 263 (1.74), at pH 7, 266 (1.65), at pH 13, 266 (1.65); ν in cm.$^{-1}$ (KBr), 3350 (OH), 3000–2750 (NH_3^+), 1675 (C=N$^+$H), 1585 (C=C).

Anal. Calcd. for $C_9H_{15}Cl_2N_5O_2$: C, 36.49; H, 5.10; N, 23.65. Found: C, 36.25; H, 5.26; N, 23.75.

6-Dimethylamino-9-(2,3-dihydroxypropyl)purine Dihydrochloride (XXI).—A mixture of 500 mg. (1.88 mmoles) of XVIII in 20 ml. of 25% aqueous dimethylamine was heated under reflux for 3 hr. The volatile materials were removed *in vacuo* and methanol (5 ml.) was added to the residue. After the chilled solution was saturated with HCl gas, $CHCl_3$ (10 ml.) was added to the mixture. The white salt was removed by filtration, washed with $CHCl_3$, and gave 439 mg. (74.6%) of XXI, m.p. 164–167° (oil bath). A small sample was recrystallized from methanol–ether saturated with HCl; m.p. 164–167°; λ_{max} in mμ ($\epsilon \times 10^{-4}$), at pH 1, 269 (1.54), at pH 7, 275 (1.57), at pH 13, 276 (1.57); ν in cm.$^{-1}$ (KBr), 3400 (OH), 2750 and 2350 (NH^+), 1665 (C=N$^+$H), 1565 (C=C).

Anal. Calcd. for $C_{10}H_{17}Cl_2N_5O_2$: C, 38.71; H, 5.53; N, 22.58. Found: C, 38.72; H, 5.67; N, 22.31.

6-Mercapto-9-(2,3-dihydroxypropyl)purine (XXII).—A mixture of 500 mg. (1.88 mmoles) of XVIII and 152 mg. (2.00 mmoles) of thiourea in 20 ml. of *n*-propyl alcohol was heated under reflux for 2 hr. The reaction mixture was evaporated to about 5 ml. *in vacuo* and $CHCl_3$ (10 ml.) was added. After being chilled, the mixture was filtered to remove the crude product and gave 319 mg. of a soft yellow solid. Recrystallization from 1-propanol gave 154 mg. (36.4%) of the pure material XXII: m.p. 285–288°; λ_{max} in mμ ($\epsilon \times 10^{-4}$), at pH 1, 323 (1.96), at pH 7, 321 (2.16), at pH 13, 310 (2.03); ν in cm.$^{-1}$ (KBr), 3400 (OH), 2600 (SH), 1600 and 1540 (C=N and C=C).

Anal. Calcd. for $C_8H_{10}N_4O_2S$: C, 42.46; H, 4.45; S, 14.17. Found: C, 42.19; H, 4.68; S, 13.91.

Reagents and Assay Procedure.—Adenosine and adenosine deaminase were purchased from the Sigma Chemical Co. The general method of assay has been described previously.[13] All enzymatic reactions were run in phosphate buffer (0.05 *M*) at pH 7.6 and at 25°. Solutions of the enzyme, substrate, and inhibitors were all prepared in 0.05 *M* phosphate buffer at pH 7.6. During each experiment, the cells contained a total volume of 3.1 ml. which was 0.066 m*M* with respect to adenosine. Sufficient amounts of enzyme were used so that the initial rate of reaction gave a change of approximately 0.8 optical density units/min. Compounds which did not show significant inhibition at concentrations 2–3 times that of substrate were classified as noninhibitory. Inhibitors were compared by means of the inhibition index $[I/S]_{0.5}$, *i.e.*, the ratio of the m*M* concentration of inhibitor to the m*M* concentration of substrate for 50% inhibition. In order to determine the concentration of an inhibitor required for 50% inhibition, a plot of V_0/V *vs.* I was made where V_0 = initial velocity of the uninhibited enzymatic reaction, V = initial velocity of the inhibited enzymatic reaction at various inhibitor concentrations, and I = the various concentrations of inhibitor.[14]

Results

Enzymatic evaluation of these purine derivatives revealed that for any given 9-substituent, those compounds with an amino group at the 6-position were more inhibitory than those compounds with a 6-methylamino group which in turn were more inhibitory than those compounds with a 6-dimethylamino group. Those compounds with a 6-chloro, a 6-mercapto, a 6-hydroxy, or a 6-methoxy group were essentially noninhibitory. These results are in agreement with the data previously found concerning the ability of various groups at the 6-position of the purine nucleus to bind to adenosine deaminase.[15] However, when a comparison is made for a given substituent at the 6-position of the purine nucleus, then it is found that the inhibition decreases in the following order for the 9-substituent: 2-hydroxypropyl > 3-hydroxypropyl > 2,3-dihydroxypropyl > propyl. The constants for the various compounds are given in Table I.

(13) N. O. Kaplan in "Methods in Enzymology," Vol. II, S. P. Colowick and N. O. Kaplan, Ed., Academic Press Inc., New York, N. Y., 1955, p. 473.
(14) B. R. Baker and H. S. Sachdev, *J. Pharm. Sci.*, **52**, 933 (1963).
(15) H. J. Schaeffer and R. Vince, *J. Med. Chem.*, **8**, 33 (1965).

TABLE I

INHIBITION INDEX AND PARTIAL INHIBITION OF ADENOSINE DEAMINASE BY SOME 6-SUBSTITUTED 9-(HYDROXYALKYL)PURINES

Compound	mM reqd. for 50% inhibition[a]	$[I/S]_{0.5}$	% inhibition[a,b]
A. 9-Substituted 6-Aminopurines			
9-Propyl (I)	0.220 ± 0.008	3.3 ± 0.1	35
9-(3-Hydroxypropyl) (III)	0.046 ± 0.005[c]	0.70 ± 0.08	74
9-(2-Hydroxypropyl) (IX)	0.016 ± 0.002	0.25 ± 0.02	87
9-(2,3-Dihydroxypropyl) (XIX)	0.087 ± 0.002	1.3 ± 0.1	59
B. 9-Substituted 6-Methylaminopurines			
9-Propyl (II)	0.465 ± 0.001	7.0 ± 0.1	18
9-(3-Hydroxypropyl) (IV)	0.127 ± 0.007[c]	1.9 ± 0.1	50
9-(2-Hydroxypropyl) (X)	0.086 ± 0.005	1.3 ± 0.1	58
9-(2,3-Dihydroxypropyl) (XX)[d]	...	...	18

[a] The concentration of adenosine in all experiments was 0.066 mM. [b] The concentration of the inhibitor was 0.12 mM. [c] Data taken from ref. 4. [d] The degree of inhibition by this compound was too low to allow an accurate measurement of the inhibition index.

Discussion

In a previous study it was shown that adenosine deaminase has little bulk tolerance for groups on the 6-amino group of the purine nucleus[15]; the present study confirms those findings since it has been found that the effectiveness of inhibition in a given series of compounds decreases in following order: 6-amino > 6-methylamino > 6-dimethylamino. In most cases the 6-dimethylamino analogs are practically noninhibitory. In addition, it is known that if a hydroxyl group is properly situated on an alkyl chain at the 9-position of a 6-aminopurine, it can increase binding to the enzyme.[4] For example, 9-(3-hydroxypropyl)-6-aminopurine (III) has $[I/S]_{0.5} = 0.7$, whereas 9-(2-hydroxyethyl)-6-aminopurine has $[I/S]_{0.5} = 1.1$, and 9-(4-hydroxybutyl)-6-aminopurine has $[I/S]_{0.5} = 1.9$. It is apparent that the hydroxyl group of the 3-hydroxypropyl and the 2-hydroxyethyl compounds can form stronger bonds to the binding site than can the 4-hydroxybutyl compound. If one assumes that the hydroxyl group of these compounds is binding to the same site of the enzyme as does the hydroxyl group of *trans*-2-[9-(6-aminopurinyl)]cyclopentanol, it is obvious that the shorter chain hydroxylated derivatives should bind more tightly since only small conformational changes would be required. In the case of 9-(4-hydroxybutyl)-6-aminopurine, the butyl chain must undergo considerable folding, which is energetically unfavorable, to allow the terminal hydroxyl group to bind to the site which the 2′-hydroxyl group of the substrate utilizes. This unfavorable conformation of a folded chain is reflected by the lower amount of binding and, therefore, of inhibition.

In order to establish that the hydroxyl group of III and 9-(2-hydroxyethyl)-6-aminopurine are binding to the same site on the enzyme, we decided to synthesize 9-(2,3-dihydroxypropyl)-6-aminopurine (XIX). If there are two different binding sites for a 2- and 3-hydroxyl group on an alkyl chain, XIX could bind even more tightly than either of the monohydroxylated compounds. It was found, however, that XIX is bound to a lesser extent to the enzyme than either of the monohydroxylated derivatives. This result can be rationalized by assuming that there is only a single site in this region of the enzyme for hydroxyl binding. The reason that XIX binds more weakly than either of the monohydroxylated compounds can be explained by the fact that the vicinal diol system in XIX will have strong intramolecular hydrogen bonds.[16] Because the hydroxyl groups in XIX are intramolecularly hydrogen bonded, there will be less driving force for the formation of an enzyme–inhibitor complex which would result in a smaller amount of inhibition.

A second fact that also points to the concept of one binding site for the hydroxyl group in the region of the enzyme two or three carbons removed from the 9-position of the purine nucleus is the observation that 9-(2-hydroxypropyl)-6-aminopurine (IX) is strongly bound to the enzyme. Thus, the movement of a hydroxyl group from the 3-position in III to the 2-position in IX increases binding by a factor of 2.8. It appears that the strongest bonds can be formed from the hydroxyl group to the enzyme when the hydroxyl group is at the 2-position of the 9-alkyl group. An examination of Table I reveals that similar conclusions can be drawn from the inhibition studies on the 9-substituted 6-methylaminopurines.

Finally, on the basis of these arguments it might be suggested that 9-(2-hydroxyethyl)-6-aminopurine should be as effective an inhibitor as III since it appears to have an ideally located hydroxyl group on the alkyl chain. However, we believe that two different types of forces are involved in binding the 9-substituent to adenosine deaminase. The binding by the hydroxyl group is one type and binding by means of hydrophobic bonds in the second type. 9-Propyl-6-aminopurine (I) has been found to have $[I/S]_{0.5} = 3.3$. Recently we have found[17] that 9-ethyl-6-aminopurine has $[I/S]_{0.5} = 6.2$. This decrease in hydrophobic bonding by an ethyl group relative to a propyl is probably the cause of the decrease binding of 9-(2-hydroxyethyl)-6-aminopurine relative to the 9-(3-hydroxypropyl)-6-aminopurine. The results of a more complete study of hydrophobic bonding will be the subject of a future paper.

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(16) For a discussion of hydrogen bonds in related compounds, see L. P. Kuhn, *J. Am. Chem. Soc.*, **80**, 5950 (1958).

(17) H. J. Schaeffer and D. Vogel, to be published.