Enzyme Inhibitors. V. The Syntheses of 6-Substituted-(9-hydroxyalkyl)purines and Their Evaluation as Inhibitors of Adenosine Deaminase*

Howard J. Schaeffer and Parmatma S. Bhargava

ABSTRACT: In order to determine which atoms and functional groups of purine nucleosides are important for binding to enzymes that utilize nucleosides as their substrates, a variety of nucleoside analogs have been synthesized as potential inhibitors of these enzymes. The nucleoside analogs which have been prepared are the 9-(2-hydroxyethyl)-, 9-(3-hydroxypropyl)-, and 9-(4-hydroxybutyl)-6-substituted purines. These compounds were prepared by the condensation of the appropriate amino alcohol with 5-amino-4,6-dichloropyrimidine followed by ring closure of the resultant substituted pyrimidine to give the 6-chloro-9-(hydroxyalkyl)-purines. Displacement of the 6-chloro group by a number of nucleophilic reagents led to a variety of nucleoside analogs. Evaluation of these compounds as inhibitors of adenosine deaminase revealed that those compounds with an amino or methylamino group at the 6 position were inhibitory. Based on the inhibition observed with these and other compounds, it is suggested that there is a relatively "tight fit" by the enzyme around the ribofuranosyl portion of the substrate, adenosine.

In the past twenty years the chemical and biochemical properties of purine nucleosides and nucleotides have been extensively studied (Buchanan, 1960; Handschumacher and Welch, 1960). The pathways are relatively well known by which a cell synthesizes nucleosides and nucleotides and incorporates them into their polymeric forms which constitute the genetic apparatus of all cells. Although the over-all pathway of biosynthesis has been described, little information is available concerning the nature of the atoms or functional groups in purine nucleosides and -tides which are required for binding to the active site of the various enzymes. Such information would be of considerable value, not only in understanding the mechanism of the reaction per se, but in the preparation of potential growth inhibitors of malignant cells. This is of special interest since certain derivatives of purines and purine nucleosides which are capable of inhibiting the growth of cancer cells have been synthesized or have been isolated from natural sources (Welch, 1961). One major problem in this area has been the cleavage of purine ribonucleosides and -tides to purine and carbohydrate moiety by intracellular enzymes, which thus render impotent potential inhibitors (Roll *et al.*, 1956).

In our previous studies we have described the synthesis and enzymatic evaluation of a variety of nucleoside analogs which are sterically similar to purine nucleosides but which are stable toward hydrolytic and, presumably, enzymatic cleavage. These nucleoside analogs are purines which are substituted at the 9 position by a cycloaliphatic group which in turn may be hydroxylated so that it sterically resembles the carbohydrate moiety of a purine nucleoside. In such cyclic compounds, the number of conformations that the 9 substitutent can occupy is considerably less than in compounds with an acyclic 9 substituent because of the restrictions imposed by the cycloaliphatic nucleus. Consequently, the nucleoside analogs with a hydroxylated cycloaliphatic substituent at the 9 position of the purine nucleus were considered ideal for the initial studies in our investigation on the determination of the mode of binding by the 9 substituent. In order to determine the effect on binding to the enzyme by substituents at the 9 position of the purine nucleus which have more conformational freedom than the cyclic analogs, we have prepared a series of 6-substituted-9-hydroxyalkylpurines. The present paper describes the synthesis and enzymatic evaluation of these compounds as inhibitors of adenosine deaminase.

Chemistry

The method which we selected for the synthesis of the 6-substituted-9-hydroxyalkylpurines is a modification of the procedure employed by Ikehara and Ohtsuka (1961) and Ikehara *et al.*, (1961) and is based on the general method of Montgomery and Temple (1957). The initial reaction involved the condensation of 5-amino-4,6-dichloropyrimidine (compound I) with an appropriate amino alcohol, i.e., 2-aminoethanol, 3-aminopropanol, or 4-aminobutanol. The resulting 4-(hydroxyalkyl)-5-amino-6-chloropyrimidines (compounds III, XII, and XX) were converted into the corre-

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sponding 6-chloro-9-hydroxyalkyl)purines (compounds IV, XIII, and XXI) by means of either ethyl orthoformate and acetic anhydride mixtures or diethoxymethyl acetate. It was found that during the preparation of compound IV, a small amount of the 6-hydroxy analog (compound V) was formed. Nucleophilic displacement of the 6-chloro group of compound IV by ammonia, methylamine, dimethylamine, hydrazine, and thiourea resulted in the formation of compounds VI through X. Catalytic hydrogenolysis of the 6-chloro group of compound IV using a palladium-on-charcoal catalyst and magnesium oxide as the acid acceptor gave a good yield of compound XI. By the use of similar procedures, compound XIII was converted into compounds XIV through XIX and XXI was converted into XXII and XXIII as outlined in Chart I.

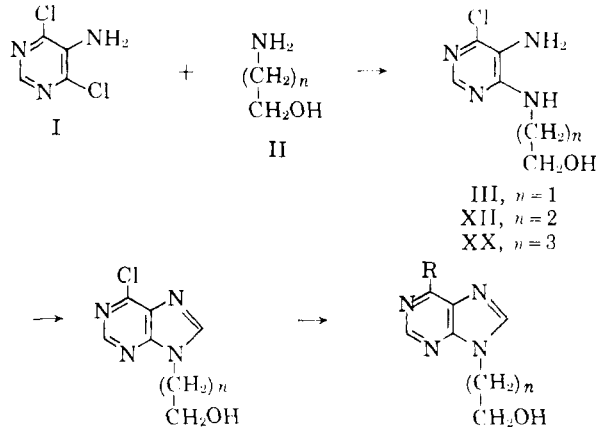


CHART I

Experimental[1]

9-(2-Hydroxyethyl)-6-chloropurine (IV). To 3.39 g (18.0 mmoles) of compound III was added 13.5 ml triethyl orthoformate and 13.5 ml of acetic anhydride. The reaction solution was heated gradually and at about 100° all the solid dissolved. The reaction mixture was heated under reflux for 3 hours and then the volatile materials were evaporated *in vacuo*. The residual liquid was kept at 0° in 18% methanolic ammonia for 12 hours. The methanol was evaporated *in vacuo* and the remaining liquid was extracted with acetone. The acetone-insoluble material was removed and was shown to be the 6-hydroxy analog (compound V), as described later. Concentration of the extract gave a white crystalline substance, 0.58 g (15.4%), mp 146°. Further concentration of the mother liquor gave an additional crop of crystals, 1.20 g (32.0%), mp 146°. One recrystallization of the combined crops gave the pure material, mp 150°. The reported melting point is 148–149° (Ikehara and Ohtsuka, 1961). $\bar{\nu}$ in cm^{-1} (KBr): 3350 (OH); 1590 and 1550 (C=C and C=N); λ_{max}^{EtOH} in $m\mu$ ($\epsilon \times 10^{-3}$) 265 (9.6).

9-(2-Hydroxyethyl)-6-hydroxypurine (V). The acetone-insoluble material (0.09 g, mp 265°) obtained during the preparation of compound IV was recrystallized from a mixture of methanol and acetone. Two recrystallizations of the crude product gave the analytical sample (compound V), mp 275° (aluminum block). $\bar{\nu}$ in cm^{-1} (KBr): 3230 (OH); 1675 (C=O); 1590 and 1540 (C=C and C=N); λ_{max}^{EtOH} in $m\mu$ ($\epsilon \times 10^{-3}$) 250 (11.1).

Anal.[2] Calcd for $C_7H_8N_4O_2$: C, 46.51, H, 4.48; N, 31.01. Found: C, 46.38; H, 4.64; N, 31.29.

9-(2-Hydroxyethyl)-6-aminopurine (VI). To 0.23 g (1.2 mmoles) of compound IV in a stainless-steel bomb was added 5 ml of liquid ammonia. The reaction mixture was heated at 65–70° for 23 hours. On evaporation of the liquid ammonia, a white crystalline material precipitated. The crude material on recrystallization from a mixture of ethanol and water gave the pure sample (compound VI), 0.13 g (72.5%), mp 238°. The reported mp is 233–235° (Ikehara and Ohtsuka, 1961). $\bar{\nu}$ in cm^{-1} (KBr): 3300 (OH); 1690 (NH); 1600 and 1560 (C=C and C=N); $\lambda_{max}^{H_2O}$ in $m\mu$ ($\epsilon \times 10^{-3}$) 260 (13.9).

9-(2-Hydroxyethyl)-6-methylaminopurine (VII). A solution of 0.17 g (0.89 mmole) of compound IV in 7 ml of ethanol and 7 ml of 40% methylamine in water was heated under reflux for 3 hours. Concentration of the reaction mixture gave a white residue which was extracted with hot chloroform. The chloroform extracts on concentration gave a white crystalline material, 0.16 g (94.2%), mp 176°. One recrystallization of the crude product from chloroform gave the analytical sample (compound VII), mp 176°. $\bar{\nu}$ in cm^{-1} (KBr): 3300 shoulder (NH), 3200 (OH); 1625 (NH); 1560 and 1540 (C=C and C=N); $\lambda_{max}^{H_2O}$ in $m\mu$ ($\epsilon \times 10^{-3}$) 265 (10.8).

Anal. Calcd for $C_8H_{11}N_5O$: C, 49.75; H, 5.70; N, 36.20. Found: C, 49.68; H, 5.67; N, 35.93.

[1] 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 4000 A spectrophotometer. The melting points were determined on a Kofler Heizbank and are corrected.

[2] The analyses reported in this paper were performed by Galbraith Microanalytical Laboratory, Knoxville, Tenn.

9-(2-Hydroxyethyl)-6-dimethylaminopurine (VIII). To a solution of 0.24 g (1.23 mmoles) of compound IV in 5 ml ethanol was added 5 ml of 25% dimethylamine in water. The reaction mixture was heated under reflux for 1 hour. Concentration of the reaction solution gave a white crystalline material, 0.21 g (82.0%), mp 136°. One recrystallization of the crude product from a mixture of water and ethanol gave the analytical sample (compound VIII), mp 136°. $\bar{\nu}$ in cm^{-1} (KBr): 3300 (OH); 1590 (C=C and C=N); $\lambda_{max}^{H_2O}$ in mμ ($\epsilon \times 10^{-3}$) 274 (20.2).

Anal. Calcd for $C_9H_{13}N_5O$: C, 52.18; H, 6.32; N, 33.80. Found: C, 52.04; H, 6.16; N, 33.58.

9-(2-Hydroxyethyl)-6-hydrazinopurine (IX). To 0.28 g (1.42 mmoles) of compound IV was added 2 ml of hydrazine. The mixture was cooled for 1 minute in an ice bath and then stirred for 2.5 hours at room temperature. The solid was collected from the cold reaction mixture by filtration, washed with cold *n*-propyl alcohol, and recrystallized from a mixture of water and ethanol. There was obtained 0.15 g (54.5%), mp 217°, of the desired product. One recrystallization of the crude product from a water and ethanol mixture gave the analytical sample (compound IX), mp 217°. $\bar{\nu}$ in cm^{-1} (KBr): 3350 (NH), 1600 (NH), 1575 (shoulder) and 1525 (C=C and C=N); $\lambda_{max}^{pH7.6}$ 267 mμ (ε 12,590).

Anal. Calcd for $C_7H_{10}N_6O$: C, 43.25; H, 5.20; N, 43.24. Found: C, 43.09; H, 5.37; N, 43.09.

9-(2-Hydroxyethyl)-6-mercaptopurine (X). To a stirred solution of 0.25 g (1.26 mmoles) of compound IV in 10 ml *n*-propyl alcohol was added 0.09 g (1.26 mmoles) of thiourea. The reaction mixture was heated under reflux for 40 minutes and then cooled in an ice bath. The white solid was collected by filtration, washed with 10 ml cold *n*-propyl alcohol, and dissolved in 5 ml of ice-cold 1.0 N sodium hydroxide. The solution was filtered, chilled in an ice bath, and acidified to *p*H 6 by addition of glacial acetic acid. The white product was collected by filtration, 0.23 g (93.0%), mp 280°. Two recrystallizations of the crude material from a mixture of methanol and water gave the analytical sample (compound X), mp 282°. $\bar{\nu}$ in cm^{-1} (KBr): 3300 (OH); 1580 and 1550 (C=C and C=N); $\lambda_{max}^{pH11.0}$ in mμ ($\epsilon \times 10^{-3}$) 315 (10.7).

Anal. Calcd for $C_7H_8N_4OS$: C, 42.80; H, 4.08; N, 28.60. Found: C, 42.94; H, 4.15; N, 28.84.

9-(2-Hydroxyethyl)purine (XI). To 60 mg of a 5% palladium-on-charcoal catalyst and 70 mg of magnesium oxide was added a solution of 110 mg (0.56 mmole) of compound IV in 200 ml of ethanol. The mixture was hydrogenated at room temperature at an initial pressure of 45.8 psi; the theoretical amount of hydrogen (0.56 mmole) was absorbed within 20 minutes. The reaction mixture was filtered through a Celite pad, and the filter cake was washed with hot ethanol (3 × 50 ml). The filtrate was concentrated to 25 ml and then 10 ml of 5% sodium carbonate solution was added. The mixture was evaporated to dryness *in vacuo* and extracted with ether (3 × 50 ml). Evaporation of the solvent gave a white crystalline material (90 mg, 98.0%), mp 92°. Two recrystallizations of the crude product from a mixture of acetone and benzene gave the analytical sample (compound XI), mp 96°. $\bar{\nu}$ in cm^{-1} (KBr): 3300 (OH); 1580 and shoulder at 1550 (C=C and C=N); $\lambda_{max}^{pH7.6}$ in mμ ($\epsilon \times 10^{-3}$) 264 (7.55).

Anal. Calcd for $C_7H_8N_4O$: C, 51.15; H, 4.91; N, 34.18. Found: C, 51.10; H, 4.94; N, 33.96.

9-(3-Hydroxypropyl)-6-chloropurine (XIII). To 5.85 g (31.0 mmoles) of compound XII was added 45 ml of ethyl orthoformate and 45 ml of acetic anhydride. The reaction mixture was heated under reflux for 3 hours and then the volatile materials were evaporated *in vacuo*. The residual syrup was kept at 0° in 50 ml of 18% methanolic ammonia for 24 hours. Evaporation of methanol *in vacuo* gave a viscous liquid which could not be crystallized. Column chromatography on neutral alumina (40 g) using chloroform as the eluent gave a white crystalline substance (1.75 g, 37.2%), mp 120°. Two recrystallizations of the crude material from a mixture of chloroform and benzene gave the analytical sample (compound XIII), mp 120°. $\bar{\nu}$ in cm^{-1} (KBr): 3340 (OH); 1590 and 1560 (C=C and C=N); λ_{max}^{EtOH} in mμ ($\epsilon \times 10^{-3}$) 265 (9.03).

Anal. Calcd for $C_8H_9N_4ClO$: C, 45.05; H, 4.28; N, 26.40. Found: C, 45.31; H, 4.15; N, 26.19.

9-(3-Hydroxylpropyl)-6-hydroxypurine (XIV). To 0.16 g (0.75 mmole) of compound XIII was added 2.5 ml of 1 N hydrochloric acid. The reaction mixture was heated under reflux for 40 minutes and then it was evaporated to dryness *in vacuo*. Recrystallization of the residue from a mixture of ethanol and acetone gave 0.08 g (55.0%) of the desired product, mp 220°. Two recrystallizations of the crude product from a mixture of ethanol and acetone gave the analytical sample (compound XIV), mp 221.° $\bar{\nu}$ in cm^{-1} (KBr): 3500 (OH); 1700 (C=O); 1600 and 1560 (C=N and C=C); $\lambda_{max}^{pH7.6}$ 250 mμ (ε 12,420).

Anal. Calcd for $C_8H_{10}N_4O_2$: C, 49.48; H, 5.15; N, 28.82. Found: C, 49.19; H, 5.38; N, 28.60.

9-(3-Hydroxypropyl)-6-aminopurine (XV). This compound was prepared by the method of Ikehara *et al.* (1961) and gave a 72% yield of compound XV, mp 210°. One recrystallization of the crude product from ethanol gave the analytical sample, mp 210°. The reported mp is 204–206°. $\bar{\nu}$ in cm^{-1} (KBr): 3305 (OH); 1670 (NH_2); 1600 (C=C); $\lambda_{max}^{H_2O}$ in mμ ($\epsilon \times 10^{-3}$) 259 (16.8).

Anal. Calcd for $C_8H_{11}N_5O$: C, 49.75; H, 5.70; N, 36.20. Found: C, 49.91; H, 5.48; N, 36.00.

The following compounds were prepared by procedures similar to those which were employed for the preparation of the corresponding 9-(2-hydroxyethyl)-6-substituted purines. Therefore only the yield and spectral and analytical data will be recorded.

9-(3-Hydroxypropyl)-6-methylaminopurine (XVI). Yield, 0.15 g (76.5%). One recrystallization of the crude product from chloroform gave the analytical sample (compound XVI), mp 144°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3300 (OH); 1625 (NH); 1570 and 1550 (C=C and C=N); $\lambda_{max}^{H_2O}$ in mμ ($\epsilon \times 10^{-3}$) 265 (15.8).

Anal. Calcd for $C_9H_{13}N_5O$: C, 52.15; H, 6.32; N, 33.80. Found: C, 52.37; H, 6.08; N, 33.53.

9-(3-Hydroxypropyl)-6-dimethylaminopurine (XVII). Yield, 0.10 g (48.3%), mp 114°. One recrystallization

TABLE I: The K_i and 50% Inhibition of Adenosine Deaminase by Certain 6-Substituted-9-(hydroxyalkyl)purines.

Compound[a]	$K_i \times 10^5$ M	mM Concentration for 50% Inhibition[b]	$[I/S]_{0.5}$
VI	3.8 ± 0.3	0.070 ± 0.004	1.1 ± 0.05
VII	34 ± 2	0.348 ± 0.062	5.3 ± 0.93
XV	3.0 ± 0.5	0.046 ± 0.005	0.70 ± 0.08
XVI	9.8 ± 0.8	0.127 ± 0.007	1.92 ± 0.107
XXII	10 ± 2	0.128 ± 0.009	1.94 ± 0.140
XXIII	12 ± 1	0.170 ± 0.0016	2.58 ± 0.024

[a] The K_m of adenosine was 7.3×10^{-5} M. [b] The concentration of adenosine in these experiments was 0.066 mM.

of the crude product from benzene gave the analytical sample (compound XVII), mp 114°. $\bar{\nu}$ in cm^{-1} (KBr): 3260 (OH); 1595 (C=C and C=N); $\lambda_{max}^{H_2O}$ in mμ ($\epsilon \times 10^{-3}$) 275 (20.0).

Anal. Calcd for $C_{10}H_{15}N_5O$: C, 54.27; H, 6.83; N, 31.65. Found: C, 53.99; H, 6.90; N, 31.43.

9-(3-Hydroxypropyl)-6-mercaptopurine (XVIII). Yield, 0.28 g (64.5%), mp 292°. One recrystallization from a mixture of water and methanol gave the pure product (compound XVIII), mp 294°. $\bar{\nu}$ in cm^{-1} (KBr): 3430 (OH); 2800–2600 (acidic H); 1590 and 1540 (C=C and C=N); $\lambda_{max}^{pH7.6}$ in mμ ($\epsilon \times 10^{-3}$) 315 (24.3).

Anal. Calcd for $C_8H_{10}N_4OS$: C, 45.60; H, 4.80; N, 26.62. Found: C, 45.44; H, 4.80; N, 26.71.

9-(3-Hydroxypropylpurine) (XIX). Yield, 110 mg (40.0%), mp 88°. One recrystallization of the crude product from acetone and benzene gave the analytical sample (compound XIX), mp 89°. $\bar{\nu}$ in cm^{-1} (KBr): 3400 (OH), 1580 (C=N); $\lambda_{max}^{pH7.6}$ 264 mμ (ϵ 8000).

Anal. Calcd for $C_8H_{10}N_4O$: C, 53.98; H, 5.62; N, 31.40. Found: C, 53.72; H, 5.72; N, 31.16.

9-(4-Hydroxybutyl)-6-aminopurine (XXII). This compound was prepared by the method of Ikehara *et al.* (1961), mp 196–197°. $\bar{\nu}$ in cm^{-1} (KBr): 3325 (OH); 3125 (NH); 1680 (NH); 1610 and 1565 (C=C and C=N).

9-(4-Hydroxybutyl)-6-methylaminopurine (XXIII). A mixture of crude compound XXI (519 mg) in 15 ml of 40% aqueous methylamine and 8 ml ethanol was heated in a stainless-steel bomb at 75° for 20 hours. Evaporation of the reaction mixture *in vacuo* gave a solid which was extracted with hot chloroform. After the chloroform solution was evaporated *in vacuo*, the residual solid was crystallized from benzene and recrystallized by benzene-hexane; yield, 52.8 mg (10.3%), mp 110.5°. $\bar{\nu}$ in cm^{-1} (KBr): 3375 (OH); 3200 (NH); 1635 (NH); 1575 and 1530 (C=C and C=N).

Anal. Calcd for $C_{10}H_{15}N_5O$: C, 54.28; H, 6.83; N, 31.66. Found: C, 54.07; H, 6.61; N, 31.41.

Reagents and Assay Procedure. Adenosine and adenosine deaminase were purchased from the Sigma Chemical Co. The general method of assay has been described by Kaplan (1955) and involves measuring the rate of disappearance of the absorption band of adenosine at 265 mμ. All reactions were run at 25° in 0.05 M phosphate buffer at *p*H 7.6. The stock solutions of all reagents were prepared in 0.05 M phosphate buffer at *p*H 7.6. For the assay, the cell contained a total volume of 3.1 ml which was 0.066 mM with respect to adenosine. A sufficient amount of enzyme was used so that the initial rate of reaction gave a change of approximately 0.8 optical density unit per minute. Compounds that did not exhibit significant inhibition of the enzymatic reaction at concentrations two to three times that of substrate were classified as noninhibitory.

Results

The initial evaluation of the 6-substituted-9-(hydroxyalkyl)purines revealed that those compounds with an amino or methylamino group (VI, VII, XV, XVI, XXII, and XXIII) at the 6 position of the purine nucleus were inhibitors of adenosine deaminase whereas those compounds with a chloro, hydroxy, dimethylamino, hydrazino, mercapto, or hydrogen group at the 6 position were essentially noninhibitory at concentrations two to three times that of the substrate.

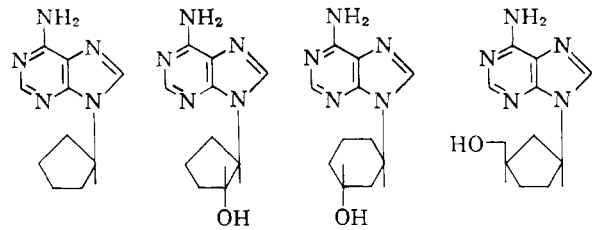
The compounds which were inhibitory were further evaluated by two different procedures. The K_i values were determined by the reciprocal-plot method developed by Lineweaver and Burk (1934). Compounds VI, VII, XV, XVI, XXII, and XXIII were all found to be competitive inhibitors of adenosine. In addition, the compounds were also evaluated by measurement of the index of inhibition $[I/S]_{0.5}$, i.e., the ratio of the millimolar concentration of inhibitor to the millimolar concentration of substrate for 50% inhibition. In order to determine the concentration of inhibitor required for 50% inhibition, a plot of V_0/V versus 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 (Baker and Sachdev, 1963). The results of both types of determinations are given in Table I. Finally, it was found that none of these compounds served as substrates for adenosine deaminase; i.e., they were inhibitors exclusively.

Discussion

Previous studies on adenosine deaminase, in which an attempt had been made to determine which atoms and functional groups of adenosine are important for binding to the enzyme, have intentionally employed 9-cycloaliphatic purines so that the effect of the stereochemistry of substituents on the cycloaliphatic group could be studied (Schaeffer *et al.*, 1964a,b,c). From these investigations it has been found that the functional group at the 6 position of the purine nucleus is critical for binding to the enzyme and that, in general, it must be an amino group. It has been found that the effectiveness of binding by the group at the 6 position of the purine nucleus decreases in the following order: 6-amino > 6-methylamino > 6-dimethylamino. In most cases, the dimethylamino group exhibits only a small amount of inhibition when its concentration is two to three times that of substrate.

In the present study, we have also found that compounds with an amino or methylamino group at the 6 position of the purine nucleus were inhibitory. An examination of Table I reveals that those compounds with a 6-amino group were more effective inhibitors than the corresponding compounds with a 6-methylamino group. Those compounds with a hydroxy, dimethylamino, hydrazino, mercapto, or hydrogen group at the 6 position of the purine nucleus had little or no inhibitory activity against adenosine deaminase. These results are consistent with our previous suggestion that the mode of binding by the group at the 6 position on the purine nucleus is either through its unshared electrons to a group on the enzyme, possibly by a hydrogen bond from the enzyme to the amino group, or by means of the inductive effect that the group at the 6 position has on the purine nucleus (Shah *et al.*, 1964). The fact that the 6-amino group contributes to binding is shown by the observation that those compounds with a hydrogen at the 6 position of the purine nucleus were noninhibitory, even though this substituent is much smaller than an amino group and, therefore, should not prevent binding because of a steric effect. The reason that the 6-methylamino analogs (compounds VII, XVI, XXIII) are weaker inhibitors than the corresponding 6-amino derivatives (compounds V, XV, XXII) may be steric in origin and consequently it follows that the enzyme has little bulk tolerance for any group much larger than a 6-amino group. A study of such bulk effects is in progress and will be the subject of a future paper.

The determination of the mode of binding to adenosine deaminase by the ribofuranosyl group at the 9 position of adenosine is considerably more complicated than the determination of the mode of binding by the 6-amino group because of the number of functional groups present and because of the stereochemistry of these groups in the ribose moiety. Therefore, we elected to attack the problem by preparing a number of 6-substituted purines which contained at the 9 position a monohydroxylated cyclopentyl or cyclohexyl group whose stereochemistry was elucidated during the synthetic program. In this way, it should be possible to evaluate the relative importance to binding of each of the hydroxyl groups of ribose in the substrate, adenosine. The key compounds which were prepared are shown here (Schaeffer *et al.*, 1964a,b,c). Evaluation of



XXIV XXV XXVI XXVII

these compounds as inhibitors of adenosine deaminase revealed that compound XXV was more inhibitory than compounds XXIV, XXVI, or XXVII, all of which were approximately equal in their ability to inhibit the enzymatic reaction. Therefore it follows that the hydroxy group at C_2' makes a significant contribution to the binding whereas the hydroxy group at C_3' and the hydroxymethyl group at C_4' makes only a small contribution to binding to this enzyme.

In order to determine what the effect would be by reducing the bulk of the group at the 9 position but concurrently retaining a hydroxy group to assist in the binding to the enzyme, we prepared the 6-substituted-9-(hydroxyalkyl)purines. A comparison of those compounds with a 6-amino group (compounds VI, XV, XXII) established that the compound with a hydroxypropyl group at the 9 position of the purine nucleus (compound XV) is a slightly better inhibitor than the compound with a hydroxyethyl group (compound VI) which in turn is slightly better than the compound with a hydroxybutyl group (compound XXII). The difference between compounds XV and VI is small, however, and may, in fact, be identical within experimental error.[3] Nevertheless, the most significant point is that all three of the compounds (VI, XV, XXII) have a smaller K_i than does compound XXV ($K_i = 13.5 \times 10^{-5}$ M). One explanation for this increased binding by the 6-amino-9-(hydroxyalkyl)purines is that the hydroxy group on the 9-alkyl group can reach the binding site on the enzyme much more readily than can the hydroxy group of compound XXV. A second explanation, which we prefer, is based on an assumption that the enzyme has a relatively "tight fit" for the ribofuranosyl portion of the substrate. Thus substrate analogs that have groups at the 9 position which are larger than the ribofuranosyl moiety will bind only weakly, if at all. Substrate analogs which are of approximately the same size as adenosine but which have only one hydroxy group on the 9 substituent could be moderate to good inhibitors whereas substrate analogs with a small group at the 9 position which contains one hydroxy group could bind rather tightly since there would be no steric hindrance to the approach of the inhibitor

[3] At the present time it is not known whether the hydroxy group of compounds VI, XV, and XXII binds to the same point or to different points on the enzyme.

to the enzyme. This hypothesis allows us to correlate the ability of a variety of compounds to complex with adenosine deaminase. For example, Weinbaum *et al.* (1964) have shown that adenylic acid and 9-(α- and β-D-fructofuranosyl)adenine act neither as substrates nor inhibitors. We have found that 9-(β-D-glucofuranosyl)-adenine is neither a substrate nor an effective inhibitor. From these data one may conclude that the enzyme has little bulk tolerance at either the C_1' or the C_5' position of the carbohydrate moiety. The fact that smaller groups can be accommodated at the C_5' position was established when it was found that 6-amino-9-(5-deoxy-β-D-xylofuranosyl)purine was a substrate of adenosine deaminase. Similarly, Weinbaum *et al.* (1964) found that 2-deoxyadenosine, 3'-amino-3'-deoxyadenosine, and cordycepin were all substrates of adenosine deaminase. Thus, one can correlate the ability of a nucleoside or a nucleoside analog to complex with adenosine deaminase by the relative size of the group at the 9 position of the purine nucleus and by the number and stereochemistry of the binding groups attached to that 9 substituent. If it is assumed that these compounds bind at the "active site" of the enzyme, and certainly this is true for those compounds which act as substrates, then it follows that there is relatively little bulk tolerance for the 9 substituent over and beyond the large size of a ribofuranosyl group, at least along the periphery of the group. It may be that there is bulk tolerance at the 9 position for groups which can assume a conformation that projects away from the binding sites of the ribofuranosyl ring. Such a proposal would be worthy of further investigation.

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