may follow the expected pure form II behavior for much longer times.

The methylprednisolone form II data of Hamlin *et al.* (3) have been replotted in Fig. 13 and compared with theory (dotted lines) assuming a constant K value (Eq. 12). The corresponding h ratios and the intercept ratios are given in columns 6 and 8 of Table IV.

Because the use of a constant K value is subject to question here, the agreement of the theory with data must be regarded as only qualitatively satisfactory. However, the authors believe that the present arguments are the most satisfactory ones for explaining this phenomenon.

A quantitative model that can account for simultaneous reversion and dissolution is presently being investigated. Results of these studies will be reported at a later date.

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Enzyme Inhibitors XVI

Mode of Binding of Some 9-(2-Hydroxyalkyl)-6-(substituted)-purines to Adenosine Deaminase

By HOWARD J. SCHAEFFER and CHARLES F. SCHWENDER

The syntheses of a variety of 6-substituted-9-(2-hydroxybutyl)-, 9-(2-hydroxyheptyl)-, and 9-(2-hydroxyoctyl)purines have been completed. Those compounds with a 6-amino or a 6-methylamino group were inhibitors of adenosine deaminase, the compounds with a 6-amino group being more active than those compounds with a 6-methylamino group. For a series of 9-substituted adenines, the decreasing order of binding was: 9-(2-hydroxypropyl) > 9-(2-hydroxyethyl) ≅ 9-(2-hydroxybutyl) >9-(2-hydroxyheptyl) > 9-(2-hydroxyoctyl). From the data, it is concluded that there is a specific binding site for the terminal methyl group in 9-(2-hydroxypropyl)adenine and that the hydroxyl binding site and the main hydrophobic site of adenosine deaminase cannot be bridged by adenine derivatives which are substituted at the 9-position by straight-chain alkyl group bearing a hydroxyl group on carbon 2.

RECENTLY EVIDENCE was presented that there exists on the enzyme, adenosine deaminase, a nonpolar area which is involved in binding, by means of hydrophobic interactions, the alkyl group of some 9-alkyladenines (1). In addition, evidence was presented that if a hydroxyl group is attached to the alkyl group of a 9-alkyladenine, the hydroxyl group may either increase or decrease the binding of the inhibitor to the enzyme depending on the position of the hydroxyl group on the alkyl chain (1, 2). On the basis of these studies, it was suggested that in the reversible complex between a 9-(hydroxyalkyl)adenine and adenosine deaminase there is only one hydroxyl binding site on adenosine deaminase, and that this site on the enzyme is an area two to three carbon atoms removed from the site on the enzyme which binds the 9-position of the purine nucleus of the inhibitor (2). In order to study further the binding sites of this enzyme, it is apparent that it should be possible to prepare compounds which would occupy the hydrophobic binding region as well as the hydroxyl binding site of adenosine deaminase; such compounds should be bound more tightly to the enzyme. Compounds which may be able to occupy both binding sites on this enzyme are the 9-(2-hydroxyalkyl)-6-substituted purines. The present paper describes the synthesis and enzymatic evaluation of these compounds as inhibitors of adenosine deaminase.

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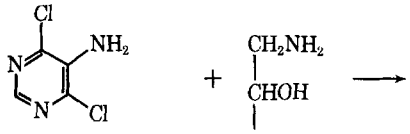
CHEMISTRY

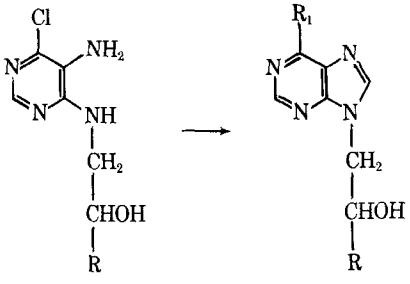
The compounds which were selected for synthesis are the 6-substituted purines which are substituted at the 9-position by either a 2-hydroxybutyl, a 2-hydroxyheptyl, or a 2-hydroxyoctyl group. The general method of synthesis is a modification of the

procedure previously utilized to prepare 9-alkyl-6-chloropurines (3, 4) and is outlined in Scheme I. Condensation of 5-amino-4,6-dichloropyrimidine (I) with the appropriate amino alcohol; *i.e.*, 1-amino-2-butanol (II*a*), 1-amino-2-heptanol (II*b*), or 1-amino-2-octanol (II*c*) gave the corresponding 5-amino-6-chloro-4-(2-hydroxyalkylamino)pyrimidine which on treatment with ethyl orthoformate and hydrochloric or ethanesulfonic acid produced the desired 6-chloro-9-(2-hydroxyalkyl)purine. When 6-chloro-9-(2-hydroxybutyl)- and 6-chloro-9-(2-hydroxyheptyl)purines were allowed to react with ammonia, methylamine, dimethylamine, 1 *N* hydrochloric acid, or thiourea, the desired 6-substituted derivatives were obtained. In the case of 6-chloro-9-(2-hydroxyoctyl)purine, only the 6-amino and the 6-mercapto derivatives (V*c*, IX*c*) were prepared.



I II

series *a*, R=C_2H_5
series *b*, R=C_5H_{11}
series *c*, R=C_6H_{13}



III

IV, R_1=Cl VII, R_1=NMe_2
V, R_1=NH_2 VIII, R_1=OH
VI, R_1=NHMe IX, R_1=SH

Scheme I

EXPERIMENTAL[1]

5 - Amino - 4 - chloro - 6 - (2 - hydroxybutylamino)pyrimidine (IIIa).—A mixture of 1.08 Gm. (6.62 mmoles) of I, 650 mg. (7.29 mmoles) of 1-amino-2-butanol (5), and 739 mg. (7.30 mmoles) of triethylamine in 50 ml. of 1-butanol was heated at reflux temperature for 22 hr. After the reaction mixture was evaporated *in vacuo* to a syrup, the crude product was crystallized and recrystallized from water and gave the crystalline product, 743 mg. (51.6%), m.p. 160°. ν in cm.$^{-1}$ (KBr): 3425 (OH); 3275 (NH); 1670 (NH); 1585 (C=C, C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): 0.1 *N* HCl, 305 (1.22); H_2O, 265 (0.830) and 292 (0.848); 0.1 *N* NaOH, 265 (0.839) and 292 (0.882).

Anal.[2]—Calcd. for $C_8H_{13}ClN_4O$: C, 44.34; H, 6.05; N, 25.86. Found: C, 44.40; H, 6.15; N, 26.14.

6-Chloro-9-(2-hydroxybutyl)purine (IVa).—A mixture of 5.66 Gm. (25.1 mmoles) of III*a* and 2.09 ml. (25.1 mmoles) of concentrated hydrochloric acid in 100 ml. of triethyl orthoformate was stirred at room temperature for 2.5 hr. After the reaction mixture was evaporated *in vacuo* to a crude liquid product, crystallization of the syrup from chloroform–hexane and recrystallization yielded 3.09 Gm. (54.3%) of solid product, m.p. 125°. ν in cm.$^{-1}$ (KBr): 3275 (OH); 1590 and 1555 (C=C, C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): 0.1 *N* HCl, 267 (0.832); H_2O, 267 (0.816); 0.1 *N* NaOH, 266 (0.860).

Anal.—Calcd. for $C_9H_{11}ClN_4O$: C, 47.68; H, 4.89; N, 24.71. Found: C, 47.88; H, 5.01; N, 24.76.

6-Amino-9-(2-hydroxybutyl)purine (Va).—In a stainless steel bomb a mixture of 305 mg. (1.34 mmoles) of IV*a* in 20 ml. of liquid ammonia was heated at 50° for 21 hr. After the ammonia had evaporated, the residual solid was recrystallized from 5% methanol in chloroform and hexane to give the white solid product; yield, 184 mg. (66.4%), m.p. 184°. ν in cm.$^{-1}$ (KBr): 3350 (OH); 3200 and 1710 (NH); 1630 and 1570 (C=C, C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): 0.1 *N* HCl, 259 (1.08); H_2O, 261 (1.21); 0.1 *N* NaOH, 261 (1.17).

Anal.—Calcd. for $C_9H_{13}N_5O$: C, 52.16; H, 6.32; N, 33.80. Found: C, 51.96; H, 6.20; N, 33.53.

6-Methylamino-9-(2-hydroxybutyl)purine (VIa).—A mixture of 472 mg. (2.08 mmoles) of IV*a* and 20 ml. of aqueous methylamine (40%) was heated at 85° in a steel bomb for 68 hr. After the reaction mixture had been evaporated *in vacuo*, the residual material was extracted with hot ethyl acetate. Addition of hexane to the extract yielded the white crystalline product, 221 mg. (50.4%), m.p. 39°. The product was recrystallized from ethyl acetate–hexane and gave the analytical sample; yield, 164 mg. (37.5%), m.p. 39°. ν in cm.$^{-1}$ (KBr): 3350 (OH); 3250 and 1620 (NH); 1580 and 1540, sh (C=C, C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): 0.1 *N* HCl–10% EtOH, 262 (1.48); H_2O–10% EtOH, 265 (1.40); 0.1 *N* NaOH–10% EtOH, 265 (1.40).

Anal.—Calcd. for $C_{10}H_{15}N_5O$: C, 54.27; H, 6.83; N, 31.66. Found: C, 54.19; H, 7.10; N, 31.45.

6-Dimethylamino-9-(2-hydroxybutyl)purine Hydrochloride (VIIa).—A mixture of 499 mg. (2.20 mmoles) of IV*a* and 5 ml. of ethanol and 25 ml. of 25% aqueous dimethylamine solution was heated at reflux for 4 hr. After the reaction mixture was evaporated *in vacuo*, the residual liquid was extracted with chloroform. The extract was cooled and hydrogen chloride gas was passed through the solution. The crude hydrochloride salt of the product which precipitated was collected by filtration; yield, 533 mg. (89.6%), m.p. 188–190.5°. Recrystallization of the crude product from isopropyl alcohol gave the pure material; yield, 316 mg. (53.0%), m.p. 189–192°. ν in cm.$^{-1}$ (KBr): 3300 (OH); 2700 (acidic hydrogen); 1650 (NH^+); 1590 and 1540 (C=C, C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): 0.1 *N* HCl, 268 (1.63); H_2O, 275 (1.65); 0.1 *N* NaOH, 275 (1.71).

Anal.—Calcd. for $C_{11}H_{18}ClN_5O$: C, 48.60; H, 6.68; Cl, 13.04; N, 25.77. Found: C, 48.88; H, 6.75; Cl, 13.23; N, 25.50.

6-Hydroxy-9-(2-hydroxybutyl)purine Hydrochlo-

[1] The infrared spectra were determined on a Perkin-Elmer model 137 spectrophotometer; the ultraviolet spectra were determined on a Perkin-Elmer model 202 spectrophotometer; the enzyme studies were done on a Gilford instrument model 2000 spectrophotometer. The melting points, unless otherwise noted, were taken in open capillary tubes on a Mel-Temp apparatus and are corrected.

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

ride (VIIIa).—A solution of 399 mg. (1.76 mmoles) of IV*a* in 10 ml. of 1 *N* hydrochloric acid and 5 ml. of water was heated at reflux for 30 min. Evaporation of the reaction mixture *in vacuo* gave a syrupy material which was recrystallized from ethanol–ether; yield, 339 mg. (78.7%), m.p. 188.5–193°. ν in cm.$^{-1}$ (KBr): 3450 (OH); 2800–2300 (acidic hydrogen); 1720 (C=O, enol); 1680 (C=N^{+}—H); 1560 and 1540 (C=C, C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): 0.1 *N* HCl, 250 (1.11); H_2O, 250 (1.14); 0.1 *N* NaOH, 256 (1.22).

Anal.—Calcd. for $C_9H_{13}ClN_4O_2$: C, 44.17; H, 5.36; Cl, 14.49; N, 22.90. Found: C, 44.17; H, 5.35; Cl, 14.54; N, 23.04.

6-Mercapto-9-(2-hydroxybutyl)purine (IXa).—A mixture of 402 mg. (1.77 mmoles) of IV*a* and 135 mg. (1.77 mmoles) of thiourea in 10 ml. of 1-propanol was heated at reflux for 15 min. Upon cooling the reaction mixture, the crystalline product precipitated and was collected by filtration, 281 mg. (70.8%), m.p. 277–280° Recrystallization of the product from methanol–propanol gave the analytical material, m.p. 276–280°. ν in cm.$^{-1}$ (KBr): 3450 (OH); 2700 (acidic hydrogen); 1590 and 1545 sh (C=C, C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): 10% EtOH–0.1 *N* HCl, 326 (1.90); 10% EtOH–H_2O, 323 (2.24); 10% EtOH–0.1 *N* NaOH, 313 (2.12).

Anal.—Calcd. for $C_9H_{12}N_4OS$: C, 48.19; H, 5.39; N, 24.98; S, 14.30. Found: C, 48.33; H, 5.38; N, 25.08; S, 14.29.

5 - Amino - 4 - chloro - 6 - (2 - hydroxyheptylamino)pyrimidine (IIIb).—A mixture of 283 mg. (1.72 mmoles) of I, 249 mg. (1.90 mmoles) of 1-amino-2-heptanol (6), and 253 mg. (2.50 mmoles) of triethylamine in 25 ml. of 1-butanol was refluxed for 17.5 hr. under a nitrogen atmosphere. Evaporation of the reaction mixture *in vacuo* gave a liquid which was crystallized from ethanol–water to give 328 mg. (73.5%), m.p. 120°. Further recrystallization from ethanol–water yielded 267 mg. (59.9%) of the analytical sample, m.p. 121°. ν in cm.$^{-1}$ (KBr): 3350 (OH); 3250 (NH); 1640 (NH); 1575 (C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): 0.1 *N* HCl, 305 (1.14); H_2O, 265 (0.821) and 293 (0.877); 0.1 *N* NaOH, 265 (0.809) and 293 (0.842).

Anal.—Calcd. for $C_{11}H_{19}ClN_4O$: C, 51.04; H, 7.36; Cl, 13.74; N, 21.65. Found: C, 51.14; H, 7.50; Cl, 13.91; N, 21.47.

6-Chloro-9-(2-hydroxyheptyl)purine (IVb).—A mixture of 638 mg. (2.46 mmoles) of III*b* and 0.4 ml. of concentrated hydrochloric acid (4.78 mmoles) was stirred at room temperature in 40 ml. of triethyl orthoformate for 9.5 hr. The reaction mixture was evaporated *in vacuo* to a syrupy residue which was extracted with hot hexane. Cooling the extract yielded 342 mg. (51.8%) of pure white solid product, m.p. 96°. Recrystallization of the material from chloroform–hexane yielded the analytical sample, m.p. 96°. ν in cm.$^{-1}$ (KBr): 3325 (OH); 1590 and 1560 (C=C, C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): 10% EtOH–0.1 *N* HCl, 266 (0.912); 10% EtOH–H_2O, 266 (0.929); 10% EtOH–0.1 *N* NaOH, 267 (0.956).

Anal.—Calcd. for $C_{12}H_{17}ClN_4O$: C, 53.63; H, 6.38; Cl, 13.19; N, 20.85. Found: C, 53.53; H, 6.30; Cl, 13.48; N, 20.63.

6-Amino-9-(2-hydroxyheptyl)purine (Vb).—A mixture of 402 mg. (1.49 mmoles) of IV*b* in 25 ml. of methanolic ammonia (20%) was heated at 80–84° for 20.5 hr. in a steel bomb. The reaction mixture was evaporated *in vacuo* to a residual solid, which was recrystallized from ethanol–water and gave 190 mg. (51.2%) of pure white crystalline solid, m.p. 190–193°. ν in cm.$^{-1}$ (KBr): 3400 (OH); 3350 and 1710 (NH); 1620 and 1570 (C=C, C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): 10% EtOH–0.1 *N* HCl, 260 (1.39); 10% EtOH–H_2O, 261 (1.41); 10% EtOH–0.1 *N* NaOH, 261 (1.39).

Anal.—Calcd. for $C_{12}H_{19}N_5O$: C, 57.81; H, 7.68; N, 28.09. Found: C, 57.78; H, 7.64; N, 27.90.

6 - Methylamino - 9 - (2 - hydroxyheptyl)purine (VIb).—A mixture of 498 mg. (1.85 mmoles) of IV*b* in 5 ml. of ethanol and 20 ml. of 40% aqueous methyl amine solution was heated at reflux for 3 hr. The reaction mixture was evaporated *in vacuo* to a residual glass, which was recrystallized from chloroform–hexane, giving 239 mg. (49.1%) of white crystalline product, m.p. 112–113°. ν in cm.$^{-1}$ (KBr): 3400 (OH); 3175 (NH); 1625 (NH); 1570 and 1530 (C=C, C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): 10% EtOH–0.1 *N* HCl, 261 (1.83); 10% EtOH–H_2O, 266 (1.58); 10% EtOH–0.1 *N* NaOH, 265 (1.78).

Anal.—Calcd. for $C_{13}H_{21}N_5O$: C, 59.30; H, 8.04; N, 26.60. Found: C, 59.35; H, 7.88; N, 26.41.

6 - Dimethylamino - 9 - (2 - hydroxyheptyl)purine (VIIb).—In a steel bomb was heated a mixture of 536 mg. (1.94 mmoles) of IV*b* in 5 ml. of methanol and 20 ml. of aqueous dimethylamine (25%) at 86° for 18.5 hr. After the reaction had been evaporated *in vacuo* to a liquid residue, the crude product was extracted from the residue with hot hexane. Cooling of the hexane extract yielded 356 mg. (62.2%) of a glassy material which upon recrystallization from hexane gave the white crystalline solid; yield, 280 mg. (49.0%), m.p. 55–59°. ν in cm.$^{-1}$ (KBr): 3475 (OH); 1610 and 1570 (C=C, C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): 5% EtOH–0.1 *N* HCl, 268 (1.57); 5% EtOH–H_2O, 274 (1.66); 5% EtOH–0.1 *N* NaOH 274 (1.66).

Anal.—Calcd. for $C_{14}H_{23}N_5O$: C, 60.61; H, 8.72; N, 25.25. Found: C, 60.90; H, 8.44; N, 25.33.

6-Hydroxy-9-(2-hydroxyheptyl)purine Hydrochloride (VIIIb).—A mixture of 317 mg. (1.18 mmoles) of IV*b*, 7.1 ml. of 1 *N* HCl, and 5 ml. of water was heated at reflux for 30 min. The reaction mixture was evaporated *in vacuo* and gave white solid material. The crude product was recrystallized from ethanol saturated with hydrogen chloride giving the pure product; yield, 185 mg. (54.7%), m.p. 197–201°. ν in cm.$^{-1}$ (KBr): 3475 (OH); 2700–2300 (acidic hydrogen); 1725 (C=O, enol); 1680 (C=N^{+}—H); 1570 and 1540 (C=C, C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): 10% EtOH–0.1 *N* HCl, 248 (1.14); 10% EtOH–H_2O, 248 (1.18); 10% EtOH–0.1 *N* NaOH, 253 (1.28).

Anal.—Calcd. for $C_{12}H_{19}ClN_4O_2$: C, 50.22; H, 6.68; Cl, 12.36; N, 19.54. Found: C, 50.46; H, 6.66; Cl, 12.31; N, 19.54.

6-Mercapto-9-(2-hydroxyheptyl)purine (IXb).—A mixture of 468 mg. (1.80 mmoles) of IV*b* and 137 mg. (1.80 mmoles) of thiourea in 10 ml. of 1-propanol was refluxed for 15 min. Upon cooling the reaction mixture, the crystalline material which precipitated was collected by filtration, 325 mg. (67.6%), m.p. 263–267°. The product was purified by recrystallization from ethanol; yield, 280 mg. (58.4%), m.p. 267–271°. ν in cm.$^{-1}$ (KBr): 3375 (OH); 2700

(acidic hydrogen); 1595 and 1560 (C=C, C=N). $\lambda_{max.}$ in mμ (ε × 10^{-4}): 10% EtOH–0.1 *N* HCl, 325 (1.93); 10% EtOH–H_2O, 322 (2.10); 10% EtOH–0.1 *N* NaOH, 313 (1.87).

Anal.—Calcd. for $C_{12}H_{18}N_4OS$: C, 54.10; H, 6.81; N, 21.03; S, 12.04. Found: C, 54.07; H, 6.63; N, 21.08; S, 12.09.

5 - Amino - 4 - chloro - 6 - (2 - hydroxyoctylamino)pyrimidine (IIIc).—A mixture of 482 mg. (2.94 mmoles) of I, 466 mg. (3.21 mmoles) of 1-amino-2-octanol (7), and 404 mg. (4.00 mmoles) of triethylamine in 25 ml. of 1-butanol was heated at reflux temperature for 24.5 hr. The reaction mixture was evaporated *in vacuo* to a liquid residue which after crystallization from methanol–water gave 273 mg. (76.3%), m.p. 111–114°. Further recrystallizations of the solid from ethanol–water gave the analytical product, m.p. 115–116.5°, 109 mg. (30.5%). ν in $cm.^{-1}$ (KBr): 3450 (OH); 3300, 3150, 1660 (NH); 1580 (C=N). $\lambda_{max.}$ in mμ (ε × 10^{-4}): 0.1 *N* HCl, 305 (1.15); H_2O, 265 (0.873) and 293 (0.891); 0.1 *N* NaOH, 265 (0.842) and 293 (0.865).

Anal.—Calcd. for $C_{12}H_{21}ClN_4O$: C, 52.83; H, 7.76; Cl, 13.00; N, 20.54. Found: C, 52.77; H, 7.74; Cl, 13.15; N, 20.64.

6-Chloro-9-(2-hydroxyoctyl)purine (IVc).—A mixture of 2.72 Gm. (9.96 mmoles) of IIIc and 667 mg. (6.01 mmoles) of ethanesulfonic acid in 120 ml. of triethyl orthoformate was stirred at room temperature for 30 min. After the reaction mixture was cooled, 1.62 Gm., m.p. 125°, of solid material was collected by filtration. The filtrate was evaporated *in vacuo* and gave a syrup which was extracted with hot hexane. The hexane extract was cooled and gave 1.18 Gm. of additional solid, m.p. 91°. The crude solid fractions were combined, 2.80 Gm. (99.2%), and recrystallized from chloroform–hexane giving the pure white crystalline product; yield, 1.36 Gm. (48.3%), m.p. 71–73°. ν in $cm.^{-1}$ (KBr): 3350 (OH); 1590 and 1560 (C=C, C=N). $\lambda_{max.}$ in mμ (ε × 10^{-4}): 10% EtOH–0.1 *N* HCl, 262 (0.735); 10% EtOH–H_2O, 263 (0.738); 10% EtOH–0.1 *N* NaOH, 263 (0.747).

Anal.—Calcd. for $C_{13}H_{19}ClN_4O$: C, 55.21; H, 6.77; Cl, 12.54; N, 19.81. Found: C, 55.27; H, 6.91; Cl, 12.82; N, 19.41.

6-Amino-9-(2-hydroxyoctyl)purine (Vc).—A mixture of 281 mg. (0.992 mmoles) of IVc in 20 ml. of methanolic ammonia was heated at 80° for 66 hr. in a steel bomb. The reaction mixture was evaporated to a residual solid which was recrystallized from ethanol–water giving 202 mg. (77.6%), m.p. 186–189°. Further recrystallizations from ethanol–water gave the analytical sample; yield, 148 mg. (56.7%), m.p. 186–189°. ν in $cm.^{-1}$ (KBr): 3300 (OH); 3250 (NH); 1700 (NH); 1610 and 1560 (C=C, C=N). $\lambda_{max.}$ in mμ (ε × 10^{-4}): 10% EtOH–0.1 *N* HCl, 258 (1.18); 10% EtOH–H_2O, 260 (1.26); 10% EtOH–0.1 *N* NaOH, 260 (1.26).

Anal.—Calcd. for $C_{13}H_{21}N_5O$: C, 59.30; H, 8.04; N, 26.60. Found: C, 59.08; H, 8.17; N, 26.29.

6-Mercapto-9-(2-hydroxyoctyl)purine (IXc).—A mixture of 279 mg. (0.985 mmole) of IVc and 83.7 mg. (1.10 mmoles) of thiourea in 15 ml. of 1-propanol was heated at reflux temperature for 15 min. Upon cooling the reaction mixture, a precipitate formed which was collected by filtration, 242 mg. (87.9%), m.p. 263–267°. The crude product was recrystallized from 1-propanol; yield, 98.4 mg. (35.7%), m.p. 264–267°. ν in $cm.^{-1}$ (KBr): 3450 (OH); 2650 (acidic hydrogen); 1600 and 1570 sh, (C=C, C=N). $\lambda_{max.}$ in mμ (ε × 10^{-4}): 10% EtOH–0.1 *N* HCl, 323 (1.78); 10% EtOH–H_2O, 322 (1.64); 10% EtOH–0.1 *N* NaOH, 310 (1.90).

Anal.—Calcd. for $C_{13}H_{20}N_4OS \cdot 1/2C_3H_8O$: C, 56.10; H, 7.80; N, 18.05; S, 10.32. Found: C, 56.00; H, 7.57; N, 17.90; S, 9.93.

The sample was dried at 100° *in vacuo* for 30 hr. and reanalyzed.

Anal.—Calcd. for $C_{13}H_{20}N_4OS$: C, 55.68; H, 7.22; N, 19.98; S, 11.44. Found: C, 55.83; H, 7.20; N, 19.62; S, 11.20.

Reagents and Assay Procedure.—Adenosine and adenosine deaminase (type I, calf intestinal mucosa) were purchased from the Sigma Chemical Co. The assay procedure for reversible inhibitors has previously been described (1, 2, 8) and is a modification of the general procedure described by Kaplan (9). The measurements of the initial rates of the enzymic reactions were performed at 25° in 0.05 *M* phosphate buffer at pH 7.6. The stock solutions of the enzyme, substrate, and inhibitors were prepared in 0.05 *M* phosphate buffer at pH 7.6. Those inhibitors which were only slightly soluble in phosphate buffer were dissolved in phosphate buffer containing 10% dimethylsulfoxide and evaluated as previously described (10). 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 enzymic reaction, V = initial velocity of the inhibited reaction at various concentrations of inhibitor, and I = the various inhibitor concentrations (8). Each plot was made with five different concentrations of inhibitor; each point for each plot is an average of at least three assays, and each reported value is an average of at least two different determinations of the concentration of inhibitor required for 50% inhibition of the enzyme. The concentration of adenosine was 0.066 m*M* in all experiments. The ratio of the m*M* concentration of the inhibitor for 50% inhibition to the m*M* concentration of the substrate $[I/S]_{0.5}$; *i.e.*, the inhibition index, was used to compare the inhibitory properties of the various compounds.

RESULTS AND DISCUSSION

As reported earlier, the authors believe that in the formation of a reversible enzyme–inhibitor complex with adenosine deaminase, the enzyme has a polar and a nonpolar region in the area where the 9-substituent of a 6-substituted purine is bound (1, 2). If the polar area (hydroxyl binding region) and the nonpolar area (hydrophobic region) of the enzyme are spatially closely related, it should be possible to synthesize inhibitors which take advantage of both the polar and nonpolar regions of adenosine deaminase. One way in which these two areas on the enzyme may be related to each other is in a "linear" manner; *i.e.*, in which the carbon chain at the 9-position of the purine nucleus is unbranched and has a hydroxyl group at the 2-position of the alkyl chain.

In agreement with previous findings, those 9-substituted compounds with a 6-chloro, a 6-dimethylamino, a 6-hydroxy, or a 6-mercapto group were either noninhibitory or only weakly inhibitory com-

TABLE I.—INHIBITION INDEX AND PARTIAL INHIBITION OF ADENOSINE DEAMINASE BY SOME 9-(HYDROXYALKYL)ADENINES

Compd.[a]	No.	mM Concn. for 50% Inhibition[b]	$[I/S]_{0.5}$[b]
Ad CH_2—CH—C_2H_5 OH	V*a*	0.078 ± 0.003[c]	1.2 ± 0.04[c]
Ad CH_2—CH—C_5H_{11} OH	V*b*	0.187 ± 0.001	2.8 ± 0.02
Ad CH_2—CH—C_8H_{13} OH	V*c*	0.209 ± 0.001	3.2 ± 0.02
Ad CH_2—CH_2 OH	XII	0.070 ± 0.004	1.1 ± 0.05[d]
Ad CH_2—CH—CH_3 OH	XIII	0.016 ± 0.002	0.25 ± 0.02[d]

[a] None of these compounds served as substrates of adenosine deaminase. [b] The concentration of adenosine in all experiments was 0.066 m*M*. In no experiment of reversible inhibition did the concentration of inhibitor exceed 0.12 m*M*. In those cases where a higher concentration is shown for 50% inhibition, the value was obtained by extrapolation of a plot of V_0/V *vs. I*. [c] Average deviation. [d] Data taken from *Reference 2*.

pared to the corresponding 6-amino derivative when evaluated against adenosine deaminase (2). However, an examination of Table I reveals some interesting features about the binding of some 9-substituted adenines to adenosine deaminase. Thus, as the 9-substituent of the adenine derivative is increased from a 2-hydroxybutyl group (V*a*) to a 2-hydroxyheptyl group (V*b*) to a 2-hydroxyoctyl group (V*c*), the compounds become progressively weaker as inhibitors. For comparison the $[I/S]_{0.5}$ of 9-(2-hydroxyethyl)adenine (XII) is 1.1, and the $[I/S]_{0.5}$ of 9-(2-hydroxypropyl)adenine (XIII) is 0.25. Similar results were obtained with the 6-methylamino derivatives (VI*a* and VI*b*) whose $[I/S]_{0.5}$ are 2.7 and 5.0, respectively.

From these results the conclusion is that the hydroxyl binding region and the principal hydrophobic binding region on the enzyme cannot be bridged by a straight chain compound with a 2-hydroxy group. However, there appears to be a specific binding region for a methyl group as noted by the increase in binding by XIII compared to XII. This methyl binding region has limited bulk tolerance[3] since the binding was decreased when the chain was lengthened from V*a* to V*b* to V*c*. Studies are continuing in an attempt to prepare 9-substituted adenines which can bind to both the hydroxyl and hydrophobic binding region of adenosine deaminase. Preliminary results with some hydroxylated branched chain compounds of the general structure of 9-(1-hydroxy-2-alkyl)-6-substituted purines indicated that these compounds are binding to both regions and will be the subject of a future paper.

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[3] It is also possible that the area on the enzyme beyond the methyl binding region is quite polar and thereby repels the longer chain compounds (V*a*, V*b*, and V*c*).