dose-related tachycardia in the dosage range of 1.7–15.0 mg. total dose. Efficiency of transfer from the small intestine to the heart by way of the circulation is low, probably less than 1.0%. The total response is proportionately greater as the dose is decreased. Rectal absorption of isoproterenol in dogs is more efficient than that following oral administration.

A method of determining the first-order absorption rate constant for drugs having rapid elimination rates relative to slow absorption rates is described. Application of this method to isoproterenol indicates that the apparent oral absorption rate constant is 0.735 hr.$^{-1}$, and the apparent rectal absorption rate constant is 0.40 hr.$^{-1}$. The threshold dose both rectally and orally is quite similar (0.8–0.9 mg.). Rectally administered isoproterenol is more efficiently utilized than the orally administered drug.

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Enzyme Inhibitors XI

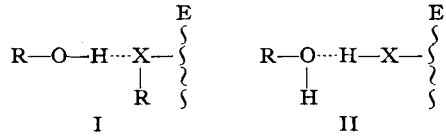
Mode of Binding of the Hydroxyl Group of Some 9-(Hydroxyalkyl)-adenines to Adenosine Deaminase

By HOWARD J. SCHAEFFER, CHARLES F. SCHWENDER, and R. N. JOHNSON

To study the mode of binding to adenosine deaminase by the hydroxyl group of 9-(2-hydroxyethyl)- and 9-(3-hydroxypropyl)adenines, a variety of 9-(2-methoxyethyl)-, 9-(3-methoxypropyl)-6-substituted purines and 9-(acetoxyalkyl)adenines were synthesized. Enzymatic evaluation of these compounds as inhibitors of adenosine deaminase revealed that they were less inhibitory than the corresponding compounds with a free hydroxyl group. These data indicate that the mode of binding of the hydroxyl group on the alkyl chain at the 9-position of adenine is by means of a hydrogen bond and that the structure of the hydrogen bond is from the hydrogen of the hydroxyl group on the inhibitor to an electronegative atom on the enzyme.

IN SEVERAL previous studies, the authors have been interested in determining which atoms and functional groups of adenosine are important for its binding to the enzyme, adenosine deaminase (1–3). In the ribofuranosyl portion of adenosine it appears that the 2′-hydroxyl group makes a contribution to binding since it has been found that in some 9-substituted adenines, those compounds with a hydroxyl group on the second or third carbon atom from the 9-position of adenine bind more tightly to the enzyme than do the corresponding nonhydroxylated compounds (4).

It is possible that the hydroxyl group of the adenine derivative exerts its influence on binding by means of a hydrogen bond.[1] One may postulate, therefore, that two different types of hydrogen bonds may be important. In the first case (I), the hydrogen of the hydroxyl group of the substrate or inhibitor forms a hydrogen bond



I II

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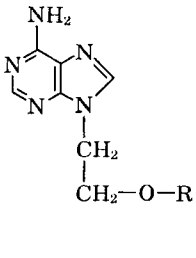
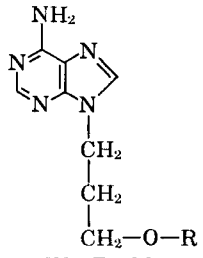
[1] For a discussion on the hydrogen bond, see Pimentel, J. C., and McClellan, A. L., "The Hydrogen Bond," W. H. Freeman and Co., San Francisco, Calif., 1960, Chap. 6.

with an electronegative atom of the enzyme, such as the oxygen atom of an alcohol or a carbonyl group, or the nitrogen atom of an amino group. In the second case (II), a hydrogen atom of, for example, an amino or a hydroxyl group on the enzyme forms a hydrogen bond with the oxygen atom of the hydroxyl group on the substrate or inhibitor. The authors believe that it should be possible to differentiate between these two types of binding (I and II) by synthesizing a potential inhibitor and blocking the hydroxyl group so that it cannot form a hydrogen bond with the enzyme of the type shown in I but which still could form a hydrogen bond of the type shown in II. Compounds which meet this requirement are generalized by III*a,b* and IV*a,b*. Thus, comparison of the inhibitory power of III*c* or IV*c* to III*a* or IV*a* and III*b* or IV*b* should yield information about the types of hydrogen bonds involved in the enzyme–inhibitor complex. The present paper describes the syntheses of some 6-substituted-9-(2-methoxyethyl)-and 9-(3-methoxypropyl)purines and the 9-(2-acetoxyethyl)-, 9-(3-acetoxypropyl)-, and 9-(4-acetoxybutyl)adenines and describes their evaluation as inhibitors of adenosine deaminase.



III*a*, R = Me
b, R = Ac
c, R = H

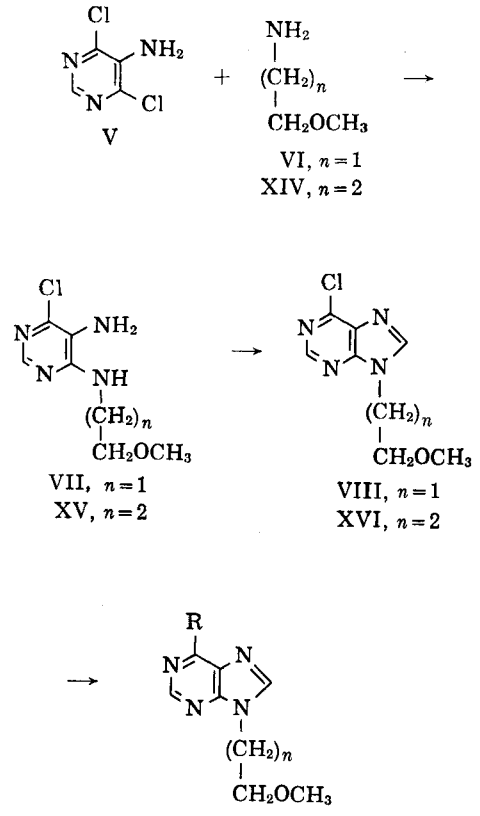


IV*a*, R = Me
b, R = Ac
c, R = H

DISCUSSION

The syntheses of the 9-(2-methoxyethyl)- and 9-(3-methoxypropyl)-6-substituted purines employ a modification of the procedure previously utilized to prepare 9-alkyl-6-chloropurines (5, 6). Condensation of 5-amino-4,6-dichloropyrimidine (V) with the appropriate amine (VI or XIV) gave the corresponding 4-(methoxyalkylamino)-5-amino-6-chloropyrimidine (VII or XV). Cyclization of the substituted pyrimidine (VII or XV) with triethyl orthoformate and hydrochloric acid gave the desired 9-(methoxyalkyl)-6-chloropurines (VIII or XVI) in good yields. When VIII or XVI were allowed to react with the appropriate nucleophilic reagents, the desired 6-substituted derivatives were obtained. (Scheme I.)

Several attempts were made to prepare the 9-(acetoxyalkyl)adenines by acetylation of 9-(hydroxyalkyl)adenines with acetyl chloride or acetic anhydride, but the yields of the desired products were extremely low. Finally, it was found that acid-catalyzed esterification of XXII, XXIV, or XXVI with glacial acetic acid gave the desired products (XXIII, XXV, or XXVII) in good yields. (Scheme II.)



V

VI, $n=1$
XIV, $n=2$

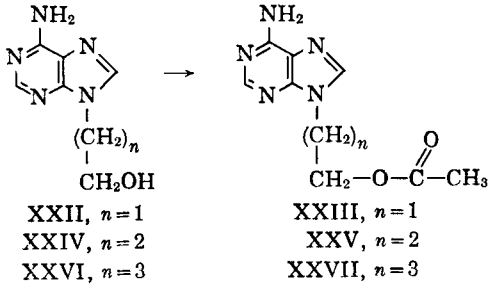
VII, $n=1$
XV, $n=2$

VIII, $n=1$
XVI, $n=2$

where

	$n=1$	$n=2$
	IX, R = NH_2	XVII, R = NH_2
	X, R = NHMe	XVIII, R = NHMe
	XI, R = NMe_2	XIX, R = NMe_2
	XII, R = OH	XX, R = OH
	XIII, R = SH	XXI, R = SH

Scheme I



XXII, $n=1$
XXIV, $n=2$
XXVI, $n=3$

XXIII, $n=1$
XXV, $n=2$
XXVII, $n=3$

Scheme II

EXPERIMENTAL[2]

9-(2-Methoxyethyl)-6-chloropurine (VIII).—A solution of 4.10 Gm. (25.0 mmoles) of V, 2.08 Gm. (27.6 mmoles) of 2-methoxyethylamine, and 2.78 Gm. (27.5 mmoles) of triethylamine in 100 ml. of 1-butanol was refluxed for 17 hr. The volatile materials were evaporated *in vacuo*, and the residue was extracted with hot benzene (2 × 50 ml.). The extract was evaporated *in vacuo* to a semisolid. Addition of 25 ml. of dry acetone precipitated triethylamine hydrochloride, which was removed by filtration. Addition of anhydrous ether to the acetone solution precipitated additional triethylamine hydrochloride. The mixture was filtered, and the filtrate was evaporated *in vacuo* to give the crude 4-chloro-5-amino-6-(2-methoxyethylamino)pyrimidine (VII). ν in cm.$^{-1}$ (film): 3400, 3270, and 1640 (NH); 1570 (C=N and C=C).

The crude pyrimidine was dissolved in 100 ml. of triethyl orthoformate, and to this solution was added 2.71 ml. (32.5 mmoles) of concentrated hydrochloric acid over a period of 10 min. This mixture was stirred at room temperature for 19 hr. The volatile materials were evaporated *in vacuo* to a brown oil, which was crystallized and recrystallized from benzene–hexane; yield (VIII), 3.37 Gm. (63.6%), m.p. 82–83°. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 265 (10.6); pH 7, 265 (10.6); pH 13, 265 (10.4); ν in cm.$^{-1}$ (KBr): 1600 and 1560 (C=N and C=C).

Anal.[3]—Calcd. for $C_8H_9ClN_4O$: C, 45.18; H, 4.27; N, 26.35. Found: C, 45.40; H, 4.15; N, 26.60.

9-(2-Methoxyethyl)-6-aminopurine (IX).—A solution of 315 mg. (1.49 mmoles) of VIII in 16 ml. of 20% methanolic ammonia was heated in a steel bomb at 92 ± 3° for 14 hr. The reaction mixture was filtered, and the crude product was washed with methanol; yield, 197 mg. (68.9%), m.p. 174–176°. One recrystallization of the crude product from ethanol gave 173 mg. (60.5%) of the analytical sample (IX), m.p. 175–177°. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): pH 1, 259 (1.38); pH 7, 261 (1.46); pH 13, 261 (1.43); ν in cm.$^{-1}$ (KBr): 3350, 3170, and 1670 (NH); 1600 and 1570 (C=N and C=C).

Anal.—Calcd. for $C_8H_{11}N_5O$: C, 49.73; H, 5.74; N, 36.25. Found: C, 49.58; H, 5.68; N, 36.39.

9-(2-Methoxyethyl)-6-methylaminopurine (X).—A solution of 265 mg. (1.25 mmoles) of VIII in 16 ml. of 40% aqueous methylamine was heated in a steel bomb at 79 ± 3° for 20 hr. After evaporation of the solution *in vacuo*, the residue was extracted with hot *n*-hexane (3 × 20 ml.). Upon cooling, the white product was collected by filtration; yield, 159 mg. (61.5%), m.p. 95–96°. An additional recrystallization from *n*-hexane gave the analytical sample (X) (132 mg., 51.0%), m.p. 96–97°. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): pH 1, 264 (1.66); pH 7, 267 (1.51); pH 13, 267 (1.58); ν in cm.$^{-1}$ (KBr): 3300 (NH); 1620 and 1580 (C=N and C=C).

Anal.—Calcd. for $C_9H_{13}N_5O$: C, 52.16; H, 6.32; N, 33.80. Found: C, 51.99; H, 6.43; N, 33.69.

9-(2-Methoxyethyl)-6-dimethylaminopurine (XI).—A solution of 303 mg. (1.46 mmoles) of VIII in 16 ml. of 25% aqueous dimethylamine was heated in a stainless steel bomb for 24 hr. at 100 ± 3°. The reaction mixture was filtered, and the filtrate was evaporated *in vacuo* to dryness. After extraction of the residue with acetone (2 × 10 ml.), the extract was evaporated *in vacuo* and gave 305 mg. (98.7%) of a yellow solid, m.p. 63–70°. One recrystallization of the crude product from *n*-hexane gave the pure product (XI); yield, 220 mg. (71.2%), m.p. 79–80°. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): pH 1, 269 (1.88); pH 7, 276 (1.86); pH 13, 276 (1.90); ν in cm.$^{-1}$ (KBr): 1590 and 1570 (sh) (C=N and C=C).

Anal.—Calcd. for $C_{10}H_{15}N_5O$: C, 54.28; H, 6.83; N, 31.65. Found: C, 54.40; H, 6.84; N, 31.41.

9-(2-Methoxyethyl)-6-hydroxypurine (XII).—A solution of 109 mg. (0.515 mmole) of VIII in 5 ml. of 88% aqueous formic acid was heated under reflux for 15 min. After the volatile materials were evaporated, the residue was recrystallized from ethanol–ethyl acetate to give 75 mg. of a white solid, m.p. 174–176°. Analysis of this material indicated that it was a hydrate. Drying the sample *in vacuo* for 18 hr. at 100° gave the analytical sample (XII), m.p. 223–225°; yield, 60.5%. $\lambda^{H_2O}_{max.}$ in mμ ($\epsilon \times 10^{-4}$): pH 1, 250 (1.12); pH 7, 250 (1.17); pH 13, 254 (1.34); ν in cm.$^{-1}$ (KBr): 1680 (C=O); 1590 and 1540 (C=N and C=C).

Anal.—Calcd. for $C_8H_{10}N_4O_2$: C, 49.48; H, 5.19; N, 28.86. Found: C, 49.17; H, 5.13; N, 28.63.

9-(2-Methoxyethyl)-6-mercaptopurine (XIII).—To a solution of 319 mg. (1.50 mmoles) of VIII in 10 ml. of 1-propanol was added 127 mg. (1.66 mmoles) of thiourea. After refluxing the solution for 1 hr., the mixture was cooled, and the product which precipitated was collected by filtration to give 287 mg. (91.1%) of the crude material, m.p. 273–278° dec. One recrystallization from ethanol gave the pure product (XIII); yield, 249 mg. (79.0%), m.p. 276–278° dec. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$); pH 1, 325 (2.08); pH 7, 323 (2.00); pH 13, 314 (1.92); ν in cm.$^{-1}$ (KBr): 2900–2300 (acidic hydrogen); 1600 and 1570 (sh) (C=N and C=C).

Anal.—Calcd. for $C_8H_{10}N_4OS$: C, 45.70; H, 4.79; N, 26.65. Found: C, 45.95; H, 4.95; N, 26.94.

9-(3-Methoxypropyl)-6-chloropurine (XVI).—A solution of 1.64 Gm. (10.0 mmoles) of V, 0.894 Gm. (10.0 mmoles) of 3-methoxypropylamine, and 1.13 Gm. (11.0 mmoles) of triethylamine in 20 ml. of 1-propanol was refluxed for 4 hr. The volatile materials were evaporated *in vacuo;* the residue was extracted with hot benzene (3 × 20 ml.) and the extract evaporated *in vacuo*. Since this residue still contained some triethylamine hydrochloride, the residual oil was re-extracted with acetone (3 × 20 ml.). Evaporation of the extract gave 1.88 Gm. (86.5%) of the crude product (XV). ν in cm.$^{-1}$ (film): 3330, 3250 (sh), and 1630 (NH); 1570 (C=N and C=C).

To a stirred solution of 1.88 Gm. of the crude pyrimidine in 20 ml. of triethyl orthoformate was

[2] 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, unless noted otherwise, were taken in capillary tubes on a Mel-Temp apparatus and are corrected.

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

added 1.1 ml. (13 mmoles) of concentrated hydrochloric acid over a period of 10 min. The reaction mixture was stirred at room temperature for 29 hr. The volatile materials were evaporated *in vacuo* and the residual oil purified by distillation onto a cold finger; yield (XVI); 1.50 Gm. (79.5%), m.p. 64–65°. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-3}$): pH 1, 266 (9.82); pH 7, 266 (9.82); pH 13, 266 (10.0); ν in cm.$^{-1}$ (KBr): 1590 and 1570 (C=N and C=C).

Anal.—Calcd. for $C_9H_{11}ClN_4O$: C, 47.69; H, 4.89; N, 24.72. Found: C, 47.80; H, 5.02; N, 24.72.

9-(3-Methoxypropyl)-6-aminopurine (XVII).—A solution of 229 mg. (1.01 mmoles) of XVI in 15 ml. of 20% methanolic ammonia was heated at 97 ± 3° in a steel bomb for 15.5 hr. The reaction mixture was evaporated to dryness *in vacuo*, and the purine was extracted with 25 ml. of dry acetone. Evaporation of the extract *in vacuo* gave 159 mg. (76.0%) of the crude product, m.p. 116–119°. One recrystallization of the crude material from benzene gave 133 mg. (63.5%) of the pure product (XVII), melting at 124–125°. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): pH 1, 260 (1.45); pH 7, 261 (1.48); pH 13, 261 (1.48); ν in cm.$^{-1}$ (KBr); 3330, 3150, and 1660 (NH); 1590 and 1570 (C=N and C=C).

Anal.—Calcd. for $C_9H_{13}N_5O$: C, 52.16; H, 6.32; N, 33.80. Found: C, 51.90; H, 6.27; N, 33.49.

9-(3-Methoxypropyl)-6-methylaminopurine (XVIII).—A solution of 340 mg. (1.50 mmoles) of XVI in 16 ml. of 40% aqueous methylamine was heated in a steel bomb at 79 ± 3° for 19.5 hr. Evaporation of the solution *in vacuo* gave a residue which was extracted with hot *n*-hexane (3 × 20 ml.). The extract, upon cooling, produced 260 mg. (78.1%) of the crude product which was collected by filtration, m.p. 50–62°. One recrystallization of the crude material from *n*-hexane gave the analytical sample (XVIII); yield, 171 mg. (51.5%), m.p. 69–70°. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): pH 1, 264 (1.95); pH 7, 267 (1.79); pH 13, 267 (1.70); ν in cm.$^{-1}$ (KBr): 3300 (NH); 1620 and 1570 (C=N and C=C).

Anal.—Calcd. for $C_{10}H_{15}N_5O$: C, 54.28; H, 6.83; N, 31.66. Found: C, 54.07; H, 6.81; N, 31.73.

9-(3-Methoxypropyl)-6-dimethylaminopurine (XIX).—A solution of 100 mg. (0.51 mmole) of XVI in 5 ml. of ethanol and 8 ml. of 25% aqueous dimethylamine was heated in a steel bomb at 90 ± 3° for 16 hr. The reaction mixture was evaporated *in vacuo*, and the purine was extracted from the residue with 10 ml. of benzene. The extract was filtered and evaporated *in vacuo* to an oil which solidified on cooling; yield, 0.12 Gm. (98%). Two recrystallizations of the crude product from *n*-hexane gave the analytical sample (XIX); yield, 47 mg. (39%), m.p. 57–58.5°. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): pH 1, 270 (1.95); pH 7, 277 (2.00); pH 13, 277 (1.98); ν in cm.$^{-1}$ (KBr): 1600 and 1570 (sh) (C=N and C=C).

Anal.—Calcd. for $C_{11}H_{17}N_5O$: C, 56.15; H, 7.28; N, 29.77. Found: C, 56.39; H, 7.50; N, 29.51.

9-(3-Methoxypropyl)-6-hydroxypurine (XX).—A solution of 115 mg. (0.510 mmole) of XVI in 5 ml. of 88% formic acid was refluxed for 15 min. Evaporation of the volatile materials *in vacuo* gave a colorless oil which was recrystallized from ethanol–ethyl acetate. The product was collected by filtration and dried overnight *in vacuo* at 100°; yield, 45 mg., m.p. 196–197°. An additional 16 mg. was obtained from the filtrate, m.p. 196–197°; total yield, 61 mg., 58%. One recrystallization of this material from ethyl acetate gave the analytical sample, m.p. 196–197°. $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): pH 1, 250 (1.05); pH 7, 250 (1.16); pH 13, 255 (1.18); ν in cm.$^{-1}$ (KBr): 2800–2400 (acidic hydrogen); 1680 (C=O); 1590 and 1540 (C=N and C=C).

Anal.—Calcd. for $C_9H_{12}N_4O_2$: C, 51.91; H, 5.81; N, 26.91. Found: C, 52.15; H, 5.93; N, 26.74.

9-(3-Methoxypropyl)-6-mercaptopurine (XXI).—To a solution of 110 mg. (0.50 mmole) of XVI in 5 ml. of 1-propanol was added 42 mg. (0.55 mmole) of thiourea. After the reaction mixture was heated under reflux for 0.75 hr., it was cooled, and the product which precipitated was collected by filtration; yield, 0.10 Gm. (93%). One recrystallization of the crude product from ethanol gave the analytical sample (XXI); yield, 81 mg. (72%), m.p. 278–280° (aluminum block). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$); pH 1, 322 (2.10); pH 7, 320 (2.12); pH 13, 311 (2.00); ν in cm.$^{-1}$ (KBr): 2800–2300 (acidic hydrogen); 1580 (C=N and C=C).

Anal.—Calcd. for $C_9H_{12}N_4OS$: C, 48.20; H, 5.39; N, 24.98. Found: C, 48.44; H, 5.33; N, 25.16.

9-(2-Acetoxyethyl)adenine (XXIII).—A mixture of 107 mg. (0.593 mmole) of XXII (2), 80.0 mg. (0.720 mmole) of ethanesulfonic acid in 7 ml. of glacial acetic acid was heated at reflux for 8.5 hr. The reaction mixture was evaporated *in vacuo*, and the residual solid was dissolved in 20 ml. of chloroform and washed with saturated sodium bicarbonate solution (3 × 1 ml.). After drying the chloroform layer with anhydrous magnesium sulfate, it was evaporated *in vacuo* to a crude solid product, 135 mg., m.p. 181°. The crude product was recrystallized from chloroform–hexane and gave 67.6 mg. (51.6%) of XXIII, m.p. 182°. ν in cm.$^{-1}$ (KBr): 3400 and 3190 (NH); 1740 (C=O); 1660 (NH); 1600 and 1575 (C=C and C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): pH 1, 260 (1.27); pH 7, 259 (1.30); pH 13, 261 (1.32).

Anal.—Calcd. for $C_9H_{11}N_5O_2$: C, 48.86; H, 5.01; N, 31.66. Found: C, 49.01; H, 5.07; N, 31.40.

9-(3-Acetoxypropyl)adenine (XXV).—This compound was prepared by a procedure similar to that used for XXIII. The crude product was recrystallized from ethyl acetate and hexane and gave 76.5 mg. (61.3%) of XXV, m.p. 162°. ν in cm.$^{-1}$ (KBr): 3375 and 3200 (NH); 1730 (C=O); 1670 (NH); 1595 and 1570 (C=C and C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): pH 1, 262 (1.27); pH 7, 262 (1.31); pH 13, 260 (1.30).

Anal.—Calcd. for $C_{10}H_{13}N_5O_2$: C, 51.04; H, 5.57; N, 29.68. Found: C, 51.19; H, 5.44; N, 29.90.

9-(4-Acetoxybutyl)adenine (XXVII).—This compound was prepared by a modification of the method used for XXIII. The crude product was recrystallized from chloroform and hexane and gave 160 mg. (63.8%) of XXVII, m.p. 161°. ν in cm.$^{-1}$ (KBr): 3350 and 3200 (NH); 1735 (C=O); 1680 (NH); 1605 and 1570 (C=C and C=N). $\lambda_{max.}$ in mμ ($\epsilon \times 10^{-4}$): pH 1, 260 (1.33); pH 7, 261 (1.35); pH 13, 260 (1.35).

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

Compd.[a]	No.	mM Concn. for 50% Inhibition[b]	$[I/S]_{0.5}$	% Inhibition by 0.12 mM Concn. of Inhibitor
Ad—$(CH_2)_2$—OMe	IX	...	...[c]	24
Ad—$(CH_2)_3$—OMe	XVII	0.38 ± 0.02	5.8 ± 0.3	24
Ad—$(CH_2)_2$—OAc	XXIII	0.41 ± 0.03	6.2 ± 0.4	24
Ad—$(CH_2)_3$—OAc	XXV	0.26 ± 0.01	3.9 ± 0.1	32
Ad—$(CH_2)_4$—OAc	XXVII	0.11 ± 0.01	1.7 ± 0.1	52
Ad—$(CH_2)_2$—OH	XXII[d]	0.070 ± 0.04	1.1 ± 0.05	65
Ad—$(CH_2)_3$—OH	XXIV[d]	0.046 ± 0.005	0.70 ± 0.08	74
Ad—$(CH_2)_4$—OH	XXVI[d]	0.128 ± 0.009	1.9 ± 0.14	49

[a] In each case Ad means an adenine group with the indicated group substituted at the 9-position. [b] The concentration of adenosine in all experiments was 0.066 mM. [c] In the determination of the I_{50} value, this compound gave a linear plot only to a concentration of 0.12 mM. Therefore, the $[I/S]_{0.5}$ is not reported. Instead, the percentage inhibition by a 0.12 mM solution of IX is given in column 5. [d] See *Reference 2* for the preparation of these compounds.

Anal.—Calcd. for $C_{11}H_{15}N_5O_2$: C, 52.98; H, 6.18; N, 28.09. Found: C, 52.73; H, 5.98; N, 27.80.

REAGENTS AND ASSAY PROCEDURE

Adenosine and adenosine deaminase (type I) were obtained from the Sigma Chemical Co. The assay procedure has been described (4) previously and is a modification of the general procedure of Kaplan (7). All enzymatic 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. For each assay the cell contained a total volume of 3.1 ml. which was 0.066 m*M* with respect to substrate. Sufficient amounts of enzyme were employed so that the initial rate of reaction gave a change in absorbance of approximately 0.8 units/min. The ratio of the millimolar concentration of inhibitor to the millimolar concentration of substrate for 50% inhibition $[I/S]_{0.5}$; *i.e.*, the inhibition index was used to compare the inhibitory properties of the various compounds. The method employed for the determination of the concentration of inhibitor for 50% inhibition has been described previously (8).

RESULTS AND DISCUSSION

Evaluation of this series of compounds revealed that those compounds with an amino group at the 6-position of the purine nucleus were inhibitors of adenosine deaminase. Those compounds with a 6-methylamino group were less inhibitory than those compounds with a 6-amino group, whereas those compounds with a chloro, dimethylamino, hydroxy, or mercapto group at the 6-position were essentially noninhibitory.

The present data give information concerning the mode of binding by a hydroxyl group on an alkyl chain which is attached to the 9-position of adenine. Examination of Table I reveals that when the hydroxyl group of 9-(2-hydroxyethyl)adenine (XXII) or 9-(3-hydroxypropyl)adenine (XXIV) is blocked by conversion to a methoxy (IX or XVII) or acetoxy group (XXIII or XXV), the degree of inhibition is significantly decreased. Although one cannot eliminate the possibility of this observation being the result of a steric effect, it appears reasonable to assume that an important factor for the formation of a hydrogen bond has been removed. This type of decrease in inhibitory ability would be predicted if the mode of binding by the hydroxyl group were by means of a hydrogen bond from the hydrogen of the hydroxyl group to an electronegative atom of the enzyme (I). This decrease in inhibition would not be predicted if the hydrogen bond were from a hydrogen atom of an amino or hydroxyl group of the enzyme to the oxygen atom of the hydroxyl group of the inhibitor (II). Consequently, the authors believe these data support the mode of binding as illustrated in I.

Finally, on the basis of this and other studies (4), it can be seen that the hydroxyl group of 9-(4-hydroxybutyl)adenine (XXVI) makes little, if any, contribution to the formation of an enzyme–inhibitor complex because the corresponding acetoxy derivative (XXVII) has essentially the same index of inhibition. Indeed, it appears likely that most of the contribution to binding by the 9-substituent of XXVI and XXVII is due to hydrophobic bonding of the butyl chain since the index of inhibition of XXVI and XXVII is nearly equal to that of 9-*n*-butyladenine (4).

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