Enzyme Inhibitors. 25. An Equation to Calculate the Unknown K_i from Two Known Values of K_i in an *R*, *S*, and *RS* Series. Stereoselectivity of Inhibition of Adenosine Deaminase by (*R*)-, (*S*)-, and (*RS*)-9-(1-Hydroxy-2-alkyl and -aralkyl)adenines†

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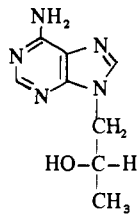
The synthesis of some (*R*)-, (*S*)-, and (*RS*)-9-(1-hydroxy-2-alkyl and -aralkyl)adenines is described. Evaluation of these compounds as inhibitors of adenosine deaminase revealed that those compounds with a chiral center of *R* configuration in the 9 substituent are more potent inhibitors than those with an *S* chiral center. In addition, an equation has been derived which allows one, for competitive inhibitors, to calculate K_i of *R*, or *S*, or *RS* if any 2 of the values are known.

9-(2-Hydroxypropyl)adenine (**1**) has been shown to exhibit stereoselectivity in the inhibition of adenosine deaminase in that the *S* isomer is about 10 times more potent an inhibitor than is the *R* isomer.[1] Because we have recently shown that 9-(1-hydroxy-2-alkyl)adenines (**2**) are potent inhibitors of adenosine deaminase,[2] it became of interest to determine if inhibitors of the general structure **2** would also exhibit a stereoselectivity in the formation of an enzyme-inhibitor complex. This paper describes the synthesis and enzymic evaluation of several racemic and resolved isomers of **2** and presents a derivation of an equation which allows one to calculate K_i of *R*, *S*, or *RS* if any 2 of these values are known.



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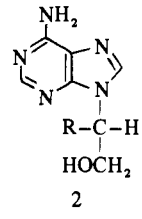

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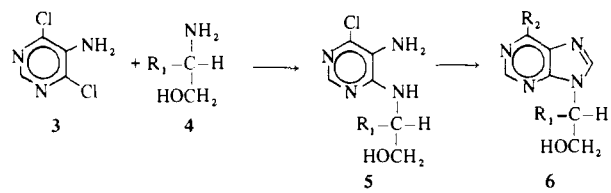
The compounds used in this study were prepared by the general procedure[1-4] of condensation of an appropriate amino alcohol (**4**) with 5-amino-4,6-dichloropyrimidine (**3**); the resultant substituted pyrimidine (**5**) was cyclized with triethyl orthoformate to give a 6-chloropurine intermediate (**6**, R_2 = Cl) which upon reaction with NH_3 produced the desired 9-substituted adenines (**6**, $R_2 = NH_2$).

Scheme I



3 4 5 6

$R_1 = CH_3, C_2H_5$, or C_6H_5 R_1 = (Cl or NH_2)

When these 9-substituted adenines were evaluated as inhibitors of adenosine deaminase, it was found that they were reversible, competitive inhibitors of this enzyme. Examination of the data in Table I reveals that in the cases of **7**, **8**, and **9**, those compounds with a chiral center of *R* configuration are more potent inhibitors of adenosine deaminase than their corresponding enantiomers. It was previously found that when the 9-substituent on adenine is a 2-hydroxypropyl group, the chiral compound with the *S* configuration exhibited the greatest amount of inhibition of adenosine deaminase when compared to its enantiomer[1] (Table I). With this insight into the stereoselectivity of inhibition of adenosine deaminase at 2 different chiral centers in the 9 substituent on several adenine derivatives, it should be possible to design other inhibitors with enhanced inhibitory properties.

Table I. Inhibition of Adenosine Deaminase by Some 9-Substituted Adenines

Compd. No.	9-Substituent	$K_i \times 10^5 M$ (found)[a]	$K_i \times 10^5 M$ (calcd)[b]
7-*RS*[c]	CH_3CHCH_2OH	4.7	5.4
7-*R*		3.2	2.7
7-*S*		17.7	8.8
8-*RS*[c]	$C_2H_5CHCH_2OH$	1.8	1.7
8-*R*		1.0	1.1
8-*S*		5.1	9.0
9-*RS*	$C_6H_5CHCH_2OH$	1.9	1.8
9-*R*		1.0	1.1
9-*S*		7.9	19.0
1-*RS*[d]	$CH_2CH(OH)CH_3$	1.0	1.1
1-*R*[d]		5.9	3.3
1-*S*[d]		0.59	0.55

[a] The compds were competitive inhibitors as detd by the method of Lineweaver and Burk.[5] [b] Calcd from eq 8, this paper. [c] Prepn described in ref 2. [d] Prepn described in ref 1.

Whenever a comparative study is made of the effect on enzyme inhibition by racemic and the corresponding resolved compounds, it would be most helpful if one could calculate the K_i of the third material once the K_i's of any 2 of the compounds are known. Toward this end, the following derivation is presented which appears to be generally useful for reversible, competitive enzyme inhibitors. If one studies the racemic compound, the standard rate expression[6] is shown in eq 1.

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}(S)}\left[1 + \frac{(DL)}{K_{DL}}\right] \quad (1)$$

where v is the initial velocity, V_{max} is the maximum velocity, K_m is the Michaelis constant for the substrate, (S) is the

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concentration of the substrate, (DL)‡ is the concentration of the racemic compound, and K_{DL} is the dissociation constant of the racemic compound. If, however, a mixture of the resolved enantiomers is employed in the enzymatic study, the rate expression can be treated in a way which is analogous to the use of two different competitive inhibitors. For this case, the rate equation[6] takes the form

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}(S)}\left[1 + \frac{(D)}{K_D} + \frac{(L)}{K_L}\right] \quad (2)$$

where v, V_{max}, K_m, and (S) have the definitions given above, (D) and (L) are the concentrations of the two inhibitors, respectively, and K_D and K_L are the dissociation constants of the two enantiomers. Plots of $1/v$ *vs.* 1/(S) of eq 1 and 2 yield relationships for the slopes of the lines which can be rearranged into eq 3 and 4, respectively

$$\frac{(\text{Slope})(V_{max})}{K_m} - 1 = \frac{(DL)}{K_{DL}} \quad (3)$$

$$\frac{(\text{Slope})(V_{max})}{K_m} - 1 = \frac{(D)}{K_D} + \frac{(L)}{K_L} \quad (4)$$

Since the terms on the left of eq 3 and 4 are equal, one obtains eq 5 from eq 3 and 4.

$$\frac{(DL)}{K_{DL}} = \frac{(D)}{K_D} + \frac{(L)}{K_L} \quad (5)$$

For the racemic compound, the following relationship exists

$$(D) = \frac{(DL)}{2} = (L) \quad (6)$$

Substitution of 6 into 5 gives eq 7

$$\frac{(DL)}{K_{DL}} = \frac{(DL)}{2K_D} + \frac{(DL)}{2K_L} \quad (7)$$

Solving eq 7 for K_{DL} gives eq 8 which describes the relationship of the dissociation constant of the racemic compound to its enantiomers for competitive enzyme inhibitors.§

$$K_{DL} = \frac{2K_DK_L}{K_D + K_L} \quad (8)$$

The dissociation constants for the racemic compounds were calculated from eq 8 using the experimental data presented in Table I. In all cases, the calcd K_i's for the racemic compound agree well with the found values (see Table I). Similarly, if one uses the K_i's of the racemic compound and the less active enantiomer to calculate the K_i of the more active enantiomer, good agreement is obtained between the calculated and found values of K_i. However, if one uses the K_i's of the racemic compound and the more active enantiomer to calculate the K_i of the less active enantiomer, moderate agreement is found between the calcd and found values of K_i. The reason for the greater discrepancy between the calcd and found values of K_i for the weaker inhibitor becomes apparent when eq 8 is rearranged to eq 9:

$$K_L = \frac{K_{DL}K_D}{2K_D - K_{DL}} \quad (9)$$

If K_L represents the dissociation constant of the weaker inhibitor, the expression in the denominator will approach 0 because K_{DL} will approach $2K_D$, particularly in those cases where K_L is a much larger number than K_D. In such circumstances, small experimental errors in K_D or K_{DL} will result in relatively larger errors in the calculation of K_L. However, in the present examples (Table I), the calculated K_i's of the weaker inhibitors differ only by a factor of about 2 from the observed values. In the calculation of the K_i of the racemic compound or the more active enantiomer, the calcd value agrees within about 10% with the observed value of K_i.

Experimental Section#

Method A. (*S*)-(+)-5-Amino-4-chloro-6-(1-hydroxy-2-propylamino)pyrimidine (5-*S*, R_1 = Me). A mixt of 5.00 g (66.6 mmoles) of (*S*)-(+)-2-amino-1-propanol,[7] 9.94 g (60.6 mmoles) of **3**, and 7.07 g (70.0 mmoles) of $(Et)_3N$ was heated at reflux for 23.5 hr under N_2 in 100 ml of *n*-BuOH. The reaction mixt was evapd *in vacuo* and gave a liquid residue which crystd upon trituration with 1 ml of H_2O. The crude solid material was recrystd from H_2O and gave a white cryst product; yield, 8.05 g (65.3%), mp 155°. A portion of the material was further recrystd from H_2O and the analytical material was obtained; mp 155°, $[\alpha]^{25}D$ +14.8° (*c* 1.7, EtOH). *Anal.* ($C_7H_{11}ClN_4O$) C, H, Cl, N.

Method B. (*S*)-(−)-6-Chloro-9-(1-hydroxy-2-propyl)purine (6-*S*, R_1 = Me, R_2 = Cl). A mixt of 563 mg (2.70 mmoles) of **5**-*S* (R_1 = Me) and 37.4 mg (0.339 mmole) of $EtSO_3H$ in 10 ml of triethyl orthoformate was stirred at room temp for 8 hr. The reaction mixt was evapd *in vacuo* to a liquid residue which after dissn in $CHCl_3$ followed by the addn of hexane, gave a crystn product; 486 mg, (85.2%), mp 194–196° dec. Further recrystn from $CHCl_3$ gave the analytical material; yield, 212 mg (37.2%), mp 199–202° dec, $[\alpha]^{22}D$ −4.58° (*c* 1.2, 66% EtOH). *Anal.* ($C_8H_9ClN_4O$) C, H, Cl, N.

Method C. (*S*)-(−)-9-(1-Hydroxy-2-propyl)adenine (7-*S*). A mixt of 525 mg (2.42 mmoles) of **6**-*S* (R_1 = Me, R_2 = Cl) and 25 ml of methanolic NH_3 was heated at 90° for 19 hr in a steel bomb. The reaction mixt was evapd *in vacuo* and gave crude solid material which was extd with hot EtOH. Upon cooling the EtOH ext, 418 mg (89.5%) of cryst product was obtd, mp 209–216°. Further recrystn from EtOH gave analytical material; yield, 189 mg (40.4%), mp 216–219°, $[\alpha]^{22}D$ −9.20° (*c* 1.3, 1 *N* HCl). *Anal.* ($C_8H_{11}N_5O$) C, H, N

(*R*)-(−)-5-Amino-4-chloro-6-(1-hydroxy-2-propylaminopyrimidine (5-*R*, R_1 = Me). Prepd by method A from **3** and (*R*)-(−)-2-amino-1-propanol;** yield, 61%, mp 151–153° (H_2O), $[\alpha]^{25}D$ −15.4° (*c* 2.1, EtOH). *Anal.* ($C_7H_{11}ClN_4O$) C, H, Cl, N.

(*R*)-(+)-6-Chloro-9-(1-hydroxy-2-propyl)purine (6-*R*, R_1 = Me; R_2 = Cl). Prepd by method B from **5**-*R* (R_1 = Me); yield, 31%, mp 202–203.5° dec ($CHCl_3$), $[\alpha]^{25}D$ +4.68° (*c* 1.8, 67% EtOH). *Anal.* ($C_8H_9ClN_4O$) C, H, Cl, N.

(*R*)-(+)-9-(1-Hydroxy-2-propyl)adenine (7-*R*). Prepd by method C from **6**-*S* (R_1 = Me, R_2 = Cl); yield, 74%, mp 215–219° (EtOH), $[\alpha]^{22}D$ +9.50° (*c* 1.6, 1 *N* HCl). *Anal.* ($C_8H_{11}N_5O$) C, H, N.

Method D. (*S*)-(−)-6-Chloro-9-(1-hydroxy-2-butyl)purine (6-*S*, R_1 = Et, R_2 = Cl). A mixt of 4.77 g (29.0 mmoles) of **3**, 2.72 g (30.5 mmoles) of (*S*)-(+)-2-amino-1-butanol,†† 5.55 ml (40.0 mmoles) of $(Et)_3N$ in 50 ml of *n*-PrOH was heated at reflux under N_2 for 19 hr. After the reaction mixt had been evapd *in vacuo* and had given a syrupy product, 4.40 g of the crude material was dissolved in $CHCl_3$ and placed on a neutral alumina column (29.5 ×

‡In the derivation of the equation, we elect to use DL, D, and L to represent the racemic compound and its enantiomers rather than *RS*, *S*, and *R* in order to avoid confusion with the symbol for the substrate, *S*.

§In theory, eq 8 also applies to noncompetitive inhibitors but examples of racemic and resolved noncompetitive enzyme inhibitors could not be found in the literature so that the calculations could not be verified.

#The melting points, unless noted otherwise were taken in open capillary tubes on a Mel-Temp block and are uncorrected. All analytical samples had ir and uv spectra compatible with their assigned structures and moved as single spots on tlc on Brinkman silica gel. Optical rotations were determined on a Perkin-Elmer Model 141 polarimeter. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. The analyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn.

**Prepared from (*R*)-alanine by the method of ref 7.

††Prepared by the procedure of Radke, *et al.*,[8] from the appropriate α-amino acid.

400 mm, 200 g) and eluted with a MeOH–$CHCl_3$ mixture. Fractions of 100 ml were collected. Chromatog pure product was obtd from fractions 24–47 (2% MeOH in $CHCl_3$), fractions 48–66 (5% MeOH in $CHCl_3$), and from fractions 67–72 (20% MeOH in $CHCl_3$). The eluate was combined and evapd *in vacuo* giving a glass; 2.21 g (50.2%) which would not cryst.

A mixt containing 1.95 g (9.01 mmoles) of the chromatog homogeneous (*S*)-4-chloro-5-amino-6-(1-hydroxy-2-butylamino)-pyrimidine, 150 mg (1.36 mmoles) of $EtSO_3H$, and 10 ml of triethyl orthoformate in 50 ml of $CHCl_3$ was stirred at room temp for 1 hr. The mixt was evapt *in vacuo* and gave a liquid residue which when dissolved in $CHCl_3$ and hexane yielded the crystn product; 1.28 g (62.3%), mp 125–133°. Recrystn from $CHCl_3$–hexane gave the analytical material; mp 139.5–142.5°, yield, 532 mg (25.6%), $[\alpha]^{25}D$ −19.0° (*c* 2.1, 50% EtOH). *Anal.* ($C_9H_{11}ClN_4O$) C, H, Cl, N.

(*S*)-(−)-9-(1-Hydroxy-2-butyl)adenine (8-*S*). Prepd by method C from 6-*S* (R_1 = Et, R_2 = Cl); yield, 59%, mp 160–163° (Me_2CO–C_6H_{14}), $[\alpha]^{25}D$ −24.0° (*c* 1.7, EtOH). *Anal.* ($C_9H_{13}N_5O$) C, H, N.

(*R*)-(+)-6-Chloro-9-(1-hydroxy-2-butyl)purine (6-*R*, R_1 = Et, R_2 = Cl). Prepd by method D from 3 and (*R*)-(−)-2-amino-1-butanol;†† yield, 41%, mp 140–144° ($CHCl_3$–C_6H_{14}), $[\alpha]^{25}D$ +19.2° (*c* 2.1, 50% EtOH). *Anal.* ($C_9H_{11}ClN_4O$) C, H, Cl, N.

(*R*)-(+)-9-(1-Hydroxy-2-butyl)adenine (8-*R*) was prepd from 6-*R* (R_1 = Et, R_2 = Cl) by method C; yield, 53%, mp 160–163° (*i*-PrOH–C_6H_{14}), $[\alpha]^{25}D$ +24.2° (*c* 1.6, EtOH). *Anal.* ($C_9H_{13}N_5O$) C, H, N.

(*RS*)-, (*S*)-, and (*R*)-5-Amino-4-chloro-6-(α-hydroxymethylbenzyl)pyrimidines (5-*RS*, 5-*S*, and 5-*R* where R_1 = C_6H_5) were prepd by the general method A from 3 and (*RS*)-,[9] (*S*)-,[10] and (*R*)-[10,11]1-phenyl-2-hydroxyethylamine; yield of 5-*RS*, 67%, mp, softens at *ca.* 80°, resolidifies and melts at 150–151° (MeOH); yield of 5-*S*, 44%, mp 140–141° (toluene), $[\alpha]^{26}D$ −86.5° (*c* 0.92, 2.5% HCl); yield of 5-*R*, 38%, mp 140–141° (toluene), $[\alpha]^{23}D$ +86.8 (*c* 0.93, 2.5% HCl). *Anal.* for 5-*RS*, 5-*S*, and 5-*R* ($C_{12}H_{13}ClN_4O$) C, H, Cl, N.

(*RS*)-, (*S*)-, and (*R*)-6-Chloro-9-(α-hydroxymethylbenzyl)purine (6-*RS*, 6-*S*, and 6-*R* where R_1 = C_6H_5 and R_2 = Cl). Prepd by general method B; yield of 6-*RS*, 75%, mp 129–130° (toluene); yield of 6-*S*, 78%, mp 114–116° (EtOH, $[\alpha]^{26}D$ −5.15° (*c* 11.1, MeOH); yield of 6-*R*, 74%, mp 114–116°, $[\alpha]^{24}D$ +5.46° (*c* 10.6, MeOH). *Anal.* for 6-*RS*, 6-*S*, and 6-*R* ($C_{13}H_{11}ClN_4O$) C, H, Cl, N.

(*RS*)-, (*S*)-, and (*R*)-9-(α-Hydroxymethylbenzyl)adenine (9-*RS*, 9-*S*, and 9-*R*) were prepd by the general procedure of method C; yield of 9-*RS*, 64%, mp 200–201° (*i*-PrOH); yield of 9-*S*, 57%, mp 211–213°(MeOH), $[\alpha]^{25}D$ −6.55° (*c* 1.8, 2.5% HCl); yield of 9-*R*, 55%, mp 213–214° (*i*-PrOH), $[\alpha]^{23}D$ +6.69° (*c* 1.7, 2.5% HCl). *Anal.* for 9-*RS*, 9-*S*, and 9-*R* ($C_{13}H_{13}N_5O$) C, H, N.

Reagents and Assay Procedures. Adenosine deaminase (Type I, calf intestinal mucosa) was purchased from the Sigma Chemical Co. The procedure for the assay of reversible inhibitors has previously been described[1,2] and is a modification of the method of Kaplan[12] based on the work of Kalckar.[13]

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Accumulation of Cyclic Adenosine Monophosphate in Incubated Slices of Brain Tissue. 1. Structure–Activity Relationships of Agonists and Antagonists of Biogenic Amines and of Tricyclic Tranquilizers and Antidepressants

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A radiometric technique, involving the use of brain slices prelabeled by incubation with adenine-^{14}C, provides a simple method to assess the effects of a variety of compounds on the accumulation of cyclic AMP-^{14}C in brain tissue. In guinea pig cerebral cortical slices only catecholamines containing a β-OH group such as norepinephrine, α-methylnorepinephrine, and isoproterenol are active. Dopamine, adrenalone, and 6-hydroxydopamine are inactive, as are most phenolic amines such as tyramine, normetanephrine, and octopamine. Both α and β receptors appear to be involved in the enhanced accumulation of cyclic AMP-^{14}C evoked by catecholamines. Serotonin, α-methylserotonin, and 4-hydroxytryptamine stimulate accumulations of cyclic AMP-^{14}C, while other isomeric hydroxytryptamines are inactive. The effect of serotonin is blocked by methsergide. Histamine and related compounds stimulate accumulation of cyclic AMP-^{14}C. α-Methylhistamine is inactive. The effect of histamine is antagonized by antihistaminics. Accumulation of cyclic AMP-^{14}C is evoked by certain tricyclic tranquilizers and antidepressants, such as chlorpromazine and imipramine. The stimulatory effect of these psychotropic agents is blocked by theophylline.

A variety of substances including norepinephrine, serotonin, histamine, ouabain, veratridine, batrachotoxin, adenosine, and tricyclic antidepressants stimulate the accumulation of cyclic AMP-^{14}C in slices of brain tissue that have been prelabeled by incubation with adenine-^{14}C.[1-8] The present paper provides structure-activity correlations for the effects of catecholamines, serotonins, histamines, antidepressants, tranquilizers, and various biogenic amine antagonists on the cyclic-AMP-^{14}C generating system present in cerebral cortical slices from guinea pig. In the accompanying paper,[9] depolarizing agents, membrane stabilizers, phosphodiesterase inhibitors, adenosine analogs, and their interactions with this system have been investigated.

Results and Discussion

General. Various biogenic amines such as norepinephrine, histamine, and serotonin cause accumulation of cyclic AMP-^{14}C in brain slices preincubated with adenine-^{14}C.[1,5,10-13] Often the effect of the biogenic amine is potentiated under conditions of membrane depolarization; *i.e.*, 43 m*M* K^+.[5] Indeed with guinea pig cerebral cortical