

The preparation of polydecarboxypeptides IXb-g, XI, and XIII is described in Table I.

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The Role of the 5'-Hydroxyl Group of Adenosine in Determining Substrate Specificity for Adenosine Deaminase

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The relationship between structural alterations in the carbohydrate moiety of adenosine and the resulting changes in substrate activity was examined with adenosine deaminase. Of the 43 analogs studied, 16 were deaminated, all of them at slower rates than the natural substrate. With the exception of adenosine 2'- or 3'-monophosphate, modifications at the 2' or 3' positions, including the simultaneous removal of the 2'- and 3'-hydroxyl groups or changes in their steric configuration, did not abolish substrate activity. Replacement of the bridge oxygen with a sulfur atom allowed deamination, but modifications at the 1' position prevented it. Replacement or substitution of the 5'-hydroxyl group with a variety of other groups, or removal of the 4'-hydroxymethyl group, invariably led to loss of substrate activity. Very low activity was retained when an amino group replaced the 5'-hydroxyl group, or when, in the absence of the 5'-hydroxyl, a hydroxyl group was present at carbon 3' in a configuration *cis* to the base moiety. These data show that the 2'- or 3'-hydroxyl groups of adenosine are not required for substrate activity, but that the 5'-hydroxyl group is essential for binding to the enzyme unless its function can be assumed to a very limited extent by an amino or possibly other hydrogen bonding groups, or by a hydroxyl group at the 3' position *cis* to the base. The implication of these observations for the design of adenosine analogs of interest in chemotherapy is discussed.

Nucleoside analogs, particularly of the adenosine variety, have long been of interest in chemotherapy. One of the factors which severely limits the usefulness of many of these analogs is their ready degradation by adenosine deaminase. It is of interest, therefore, to determine which modifications in the nucleoside molecule decrease or abolish the susceptibility of an analog to deamination. Numerous investigations of this nature have been carried out in recent years, utilizing structural analogs of adenosine modified in the base or in the sugar moiety or in both.¹ In particular, the contribution to binding made by the 2'- and 3'-hydroxyl groups of the carbohydrate moiety has received close attention, and the conclusions reached have varied, depending upon the types of analogs available for testing.²

The availability of new adenosine analogs, all modified in the sugar moiety and particularly in the 5' position, prompted us to examine further the relationship between structural alterations in the carbohydrate portion of adenosine and the ensuing changes in susceptibility to deamination.

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(2) (a) H. J. Schaeffer, K. K. Kaistha, and S. K. Chakraborti, *J. Pharm. Sci.*, **53**, 1371 (1964); (b) H. J. Schaeffer, D. D. Godse, and G. Liu, *ibid.*, **53**, 1510 (1964); (c) J. E. Cory and R. J. Snehadolink, *Biochemistry*, **4**, 1729 (1965); (d) H. J. Schaeffer and P. S. Bhargava, *ibid.*, **4**, 71 (1965); (e) H. J. Schaeffer and E. Odin, *J. Pharm. Sci.*, **54**, 421 (1965); (f) S. Frederikson, *Arch. Biochem. Biophys.*, **113**, 383 (1966); (g) J. I. York and G. A. LePage, *Can. J. Biochem.*, **44**, 331 (1966).

Materials and Methods

The deamination of adenosine, 2'-deoxyadenosine, and of their structural analogs was followed spectrophotometrically at 265 μ .³ The measurements were carried out at 25° with a Gilford absorbance recorder (Model 2000) and a Beckman monochromator.

Adenosine deaminase, type I, from intestinal mucosa (Lot 95B-9022) was purchased from Sigma Chemical Co. As specified by the supplier, this preparation deaminates adenosine 5'-monophosphate (5'-AMP) at less than 0.01% the rate at which adenosine is deaminated. This activity is held to be due to the presence of phosphatase. Nucleoside phosphorylase, myokinase, xanthine oxidase, and guanase activity is also below 0.01% of the activity found in the crude extract. The enzyme was diluted in 0.05 *M* phosphate buffer, pH 7.5, to a concentration of 0.280 unit/ml (1 unit being defined as that amount of enzyme which causes the deamination of 1 μ mole of adenosine to inosine/min at pH 7.5 and 25°). The assays were carried out by adding 0.1 ml of this solution to 2.9 ml each of 5×10^{-5} *M*, 1×10^{-4} *M*, and 2×10^{-4} *M* substrate in 0.05 *M* phosphate buffer, pH 7.5. Since the rates of deamination at the two highest substrate concentrations differed little, the enzyme appears to be essentially saturated at these levels. Progress of the deamination was recorded until the reaction was virtually completed. The rates of deamination of those analogs which were deaminated very slowly were determined by increasing the amount of enzyme from 5 to 100 times (see Table I). All of the compounds which were apparently not deaminated by the initial low enzyme concentrations were re-examined in the presence of 30 times the amount of enzyme. Since the activity of the enzyme in the buffer solution changes markedly upon standing (in the cold), a new solution was prepared after every 4 hr, and the rate of deamination of adenosine was determined alongside each analog determination.

The buffer-insoluble compound X was first dissolved in 0.02 ml of dimethyl sulfoxide (DMSO),⁴ and its rate of deamination was compared to the rate of deamination of adenosine in a mixture containing an equal amount of DMSO.

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(4) H. J. Schaeffer and E. Odin, *J. Med. Chem.*, **9**, 576 (1966).

TABLE I
ADENOSINE ANALOGS WHICH ARE SUBSTRATES FOR
ADENOSINE DEAMINASE

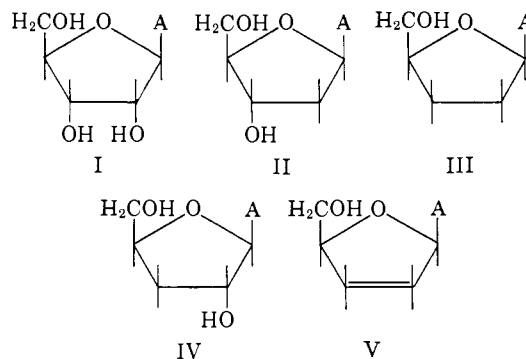
No.	Compound	Initial velocity/unit of enzyme relative to adenosine ^a	Ref
I	Adenosine	1.00	<i>b</i>
II	2'-Deoxyadenosine	1.15	<i>b</i>
III	2',3'-Dideoxyadenosine	0.60	<i>c</i>
IV	3'-Deoxyadenosine (synthetic)	0.71	<i>d</i>
V	2',3'-Didehydro-2',3'-dideoxyadenosine	0.10	<i>e</i>
VI	2',3'- <i>O</i> -Isopropylideneadenosine	0.43	<i>b</i>
VII	3'-Amino-3'-deoxyadenosine	0.45	<i>f</i>
VIII	2'- <i>O</i> -Methyladenosine	0.67	<i>g</i>
IX	3'- <i>O</i> -Acetyl-2'-deoxyadenosine	0.21	<i>c</i>
X	3'- <i>O</i> -Toluenesulfonyl-2'-deoxyadenosine	0.04	<i>c</i>
XI	Arabinofuranosyladenine	0.20	<i>h</i>
XII	Xylofuranosyladenine	0.35	<i>i</i>
XIII	Lyxofuranosyl adenine	0.015	<i>j</i>
XIV	4'-Thio-4'-deoxyadenosine	0.52	<i>k</i>
XV	5'-Deoxyxylofuranosyladenine	0.04	9
XVI	9- α -L-Threofuranosyladenine	0.01	<i>l</i>
XVII	5'-Amino-5'-deoxyadenosine	0.004	<i>m</i>
XVIII	5'-Amino-2',5'-dideoxyadenosine	0.008	<i>n</i>

^a The rates of deamination were determined with 0.0093 unit of enzyme/ml of assay mixture. Compound V was assayed with five times, compounds X, XIII, XV, XVI with 20 times, and compounds XVII and XVIII with 100 times this amount of enzyme. ^b Calbiochem. ^c M. J. Robins, J. R. McCarthy, Jr., and R. K. Robins, *Biochemistry*, **5**, 224 (1966). ^d D. H. Murray and J. Prokop, *J. Pharm. Sci.*, **54**, 1468 (1965). ^e J. R. McCarthy, Jr., M. J. Robins, L. Townsend, and R. K. Robins, *J. Am. Chem. Soc.*, **88**, 1549 (1966). ^f B. R. Baker, R. E. Schaub, and H. M. Kissman, *ibid.*, **77**, 5911 (1955). ^g A. D. Broom and R. K. Robins, *ibid.*, **87**, 1145 (1965). ^h E. J. Reist, A. Benitez, L. Goodman, B. R. Baker, and W. W. Lee, *J. Org. Chem.*, **27**, 3274 (1962). ⁱ B. R. Baker and K. Hewson, *ibid.*, **22**, 966 (1957). ^j E. J. Reist, D. F. Calkins, and L. Goodman, *ibid.*, **32**, 169 (1967). ^k E. J. Reist, D. E. Gueffroy, and L. Goodman, *Chem. Ind. (London)*, 1364 (1964). ^l J. Prokop and D. H. Murray, *J. Pharm. Sci.*, in press. ^m W. John, *Chem. Ber.*, **98**, 1705 (1965). ⁿ R. K. Robins, unpublished.

5'-Deoxyarabinosyladenine was examined with both the normal and the 100-fold enzyme complement. An optical density change of 0.01 was seen during the first 5 min in the presence of the larger amount of enzyme, but no further change was subsequently observed.

Results and Discussion

Of the 43 analogs of adenosine or 2'-deoxyadenosine modified in the carbohydrate moiety, only 16 were subject to deamination, and all of these were deaminated at slower rates than the natural substrates (Tables I and II). Some of the analogs shown in these tables have been examined in the past,² but have been included in this study for comparative purposes. Modifications at the 2' or 3' position, even if extensive, did not abolish substrate activity, indicating that neither the hydroxyl group in the 2' position nor that in the 3' position are required for deamination. This fact is illustrated particularly well by 2',3'-dideoxyadenosine (III), a compound which is deaminated at approximately two-thirds the rate of adenosine (I). Nevertheless, the 2',3'-hydroxyl groups, when present, do affect the extent of substrate activity. Thus, while 2'-deoxyadenosine (II) is deaminated somewhat more

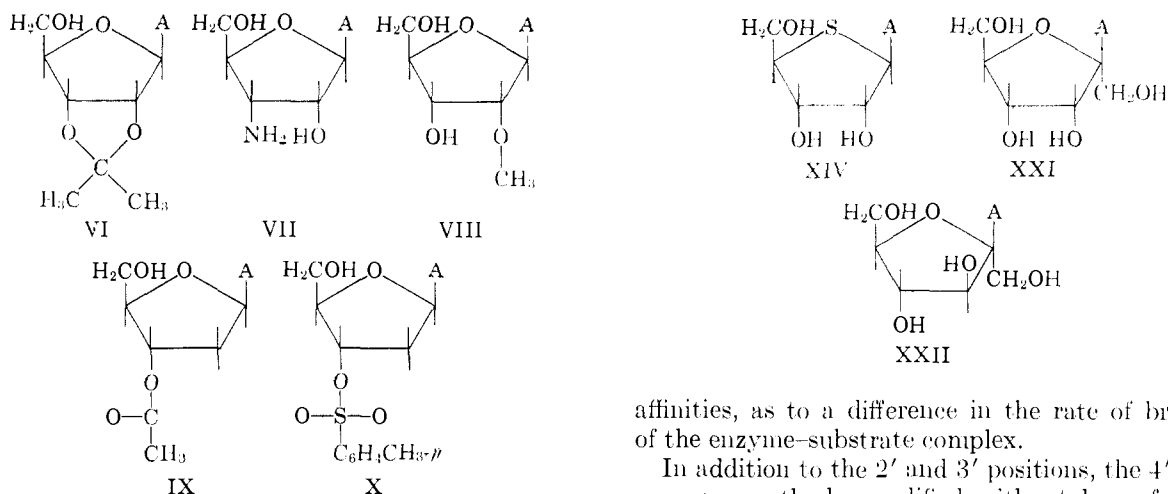


rapidly than is adenosine itself, the markedly decreased rate of deamination of 2',3'-dideoxyadenosine is a consequence of the removal of the 3'-hydroxy group. On the other hand, 3'-deoxyadenosine (IV) is deaminated more rapidly than is 2',3'-dideoxyadenosine, indicating that, in the absence of the 3'-hydroxy group, the 2'-hydroxy group affects the substrate activity of these analogs. Indeed, York and LePage^{2a} have calculated a K_m of $7.7 \times 10^{-5} M$ for 2',3'-dideoxyadenosine and of $5.2 \times 10^{-5} M$ for 3'-deoxyadenosine. 2',3'-Didehydro-2',3'-dideoxyadenosine (V) is a poorer substrate than is 2',3'-dideoxyadenosine. Not only the absence of the two hydrogen atoms, but also the increased rigidity of the furanose ring may decrease the extent of binding of the molecule to the enzyme.

That the free hydroxy groups are not required for substrate activity is also shown by the example of 2',3'-*O*-isopropylideneadenosine (VI), in which both hydroxy groups are blocked, but which, nevertheless, is subject to deamination. Similarly, replacement of the 3'-hydroxy group of adenosine with an amino

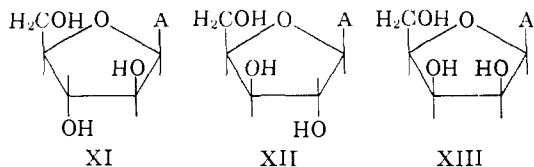
TABLE II
ADENOSINE ANALOGS WHICH ARE NOT SUBSTRATES FOR
ADENOSINE DEAMINASE

No.	Compound
XIX	Adenosine 2'-monophosphate
XX	Adenosine 3'-monophosphate
XXI	Psicofuranosyladenine
XXII	Fructofuranosyladenine
XXIII	5'-Deoxyadenosine
XXIV	2',5'-Dideoxyadenosine
XXV	2',3',5'-Trideoxyadenosine
XXVI	5'- <i>O</i> -Acetyladenosine
XXVII	5'- <i>O</i> -Benzyladenosine
XXVIII	3',5'-Di- <i>O</i> -acetyl-2'-deoxyadenosine
XXIX	5'-Methylthio-5'-deoxyadenosine
XXX	Adenosine 5'-monophosphate
XXXI	5'-Deoxyarabinofuranosyladenine
XXXII	9- β -D-Threofuranosyladenine
XXXIII	9- α -L-Erythrofuranosyladenine
XXXIV	9- β -D-Erythrofuranosyladenine
XXXV	5'-Deoxy-2',3'-anhydrolyxofuranosyladenine
XXXVI	9-(2'-Deoxy- β -D-ribofuranosyl)adenine
XXXVII	9-(2'-Deoxy- α -D-ribofuranosyl)adenine
XXXVIII	9-(β -D-Xylopyranosyl)adenine
XXXIX	9-(β -L-Xylopyranosyl)adenine
XL	9-(α -D-Arabinopyranosyl)adenine
XLI	9-(α -L-Arabinopyranosyl)adenine
XLII	9-(3'-Deoxy- β -D-glucopyranosyl)adenine
XLIII	9-(3'-Deoxy- β -D-galactopyranosyl)adenine
XLIV	9-(2'-Deoxy- α -L-ribofuranosyl)adenine
XLV	9-(2'-Deoxy- β -L-ribofuranosyl)adenine



group (VII) yields a compound which serves as a substrate. Substrate activity has also been reported with 2'-amino-2',3'-dideoxyadenosine and with 3'-amino-2',3'-dideoxyadenosine.^{2f} With increasing bulk of the substituents, the rate of deamination apparently decreases. When a methoxy group replaces the 2'-hydroxy group (VIII), the rate of deamination of adenosine is reduced by about one-third. This rate is decreased further when an isopropylidene group is attached to the 2',3' position (VI) and falls to about one-fifth its original value when an acetyl group is introduced at the 3' position of 2'-deoxyadenosine (IX). The presence of the very bulky tosyl group at the 3' position of 2'-deoxyadenosine (X) reduces the rate of deamination to approximately one-twentieth the rate at which deoxyadenosine is deaminated. While the sheer physical size of these substituents may be a primary factor affecting the rate of deamination, the possibility that the substituents bind to the enzyme in place of the hydroxy group or even at a different site cannot be excluded. The only substituent which abolished substrate activity when present at the 2' or 3' position of adenosine was the phosphate group (XIX, XX). This group being ionized at the test pH may alter the net charge of the molecule. As shown by Cory and Suhadolnik,^{2e} these two compounds are not inhibitors of the enzyme, suggesting that the charge and/or size of these groups interfere with binding to the enzyme. It should be added, however, that a nonspecific adenosine deaminase from takadiastase is capable of deaminating 3'-AMP.⁵

Changes in the steric configuration of the hydroxyl groups around carbon 2' or 3' do not affect the substrate nature of the compounds. Arabino- (XI), xylo- (XII), and lyxofuranosyladenine (XIII) are all deaminated, although at different rates. This variation



in rate has been suggested by York and LePage^{2g} to be due not so much to a difference in the enzyme-substrate

affinities, as to a difference in the rate of breakdown of the enzyme-substrate complex.

In addition to the 2' and 3' positions, the 4' position can apparently be modified without loss of activity. Replacement of the bridge oxygen with a sulfur atom results in an analog which is deaminated at approximately one-half the rate of adenosine.^{2f,6} Considering the greater physical size of the sulfur atom as compared to that of oxygen, this decrease in activity may be due to steric alterations. Other effects, such as may result from the greater polarizability of sulfur, cannot, however, be excluded.

Only two compounds modified in the 1' position of adenosine were available for testing. Neither psicofuranine nor fructofuranosyladenine (XXI, XXII) are substrates for the deaminase,^{2e-27} and it appears that the intact 1' position may be necessary for substrate activity indicating a lack of bulk tolerance in this region.^{2e} However, no definite conclusions can be reached until further analogs, modified in this position, have been examined.

The contribution to binding made by the 5' position can be evaluated with greater certainty. When the 5'-hydroxy group is replaced with a hydrogen atom, such as in 5'-deoxy- (XXIII), 2',5'-dideoxy- (XXIV), or 2',3',5'-trideoxyadenosine (XXV), activity is lost. Since 2'-deoxy- and 2',3'-dideoxyadenosine are substrates for the deaminase, loss of the 5'-hydroxyl group is clearly responsible for this change in substrate activity. Acetylation (XXVI) or benzylation (XXVII) of the 5' position also abolishes the substrate nature of adenosine, indicating either that a free hydroxyl group is essential for activity or that the enzyme-substrate complex cannot tolerate a large group at the 5' position, analogous to the loss in binding capacity of thymidine to thymidine kinase which results in the presence of large groups at the 5'-hydroxyl of thymidine.⁸ Similarly, replacement of the 5'-hydroxyl group of adenosine with a methylthio (XXIX) group results in loss of substrate activity. Phosphorylation of the 5' position also abolishes the substrate nature of adenosine, although, as has been suggested for the 2'- and 3'-monophosphate derivatives, this loss could be attributed to a change in the net charge of the molecule. Replacement of the 5'-hydroxyl group of adenosine or 2'-deoxyadenosine with an amino group (XVII) permits deamination, at an extremely slow rate, although with

(6) A. Bloch, *Proc. Am. Assoc. Cancer Res.*, **6**, 6 (1965).

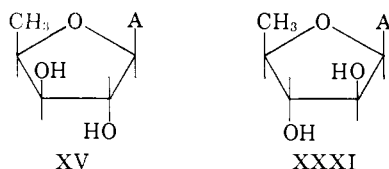
(7) A. Bloch and C. A. Nichol, *Biochem. Biophys. Res. Commun.*, **16**, 400 (1964).

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a relatively large amount of enzyme (100 times) qualitative conversion was obtained.

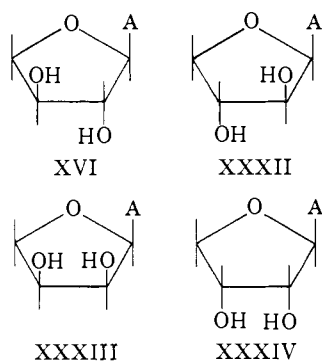
The function of the 5'-hydroxyl group can be assumed more effectively by a hydroxyl group on C-3 in a configuration *cis* to the adenine moiety. Thus, 5-deoxyxylosyladenine (XV) is deaminated, although much more slowly than adenosine, but more rapidly



than 5'-amino-5'-deoxyadenosine. A reason for this activity was given by Shah, *et al.*,⁹ who stated that the hydrogen of the C-3 hydroxyl group of 5'-deoxyxylosyladenine can occupy an almost identical position as does the hydrogen of the C-5' hydroxy in one of the conformations of adenosine. The observation by York and LePage^{2*} that the K_m for 5'-deoxyxylosyladenine ($8.0 \times 10^{-5} M$) approximates that of xylosyladenine ($10.0 \times 10^{-5} M$) could serve as an indication of the extent to which the 3'-hydroxyl substitutes for the 5'-hydroxyl group in binding of the substrate. The tenfold difference in the rate of breakdown of the enzyme-substrate complex ($V_{max} = 0.03$ for 5'-deoxyxylosyladenine *vs.* 0.29 for xylosyladenine) would, however, reflect the difference in the catalytic effectiveness of the complex formed.

The suggestion that the 3'-hydroxyl of 5'-deoxyxylosyladenine binds in place of the 5'-hydroxy group is supported by the observation that 5'-deoxyarabino-furanosyladenine (XXXI) is not deaminated, the 2'-hydroxy apparently being unable to take the place of the 5'-hydroxyl group.

In analogy with 5'-deoxyxylosyladenine, 9- α -L-threofuranosyladenine (XVI) is a substrate, whereas its stereoisomers in both the threose (XXXII) and erythrose (XXXIII, XXXIV) series are not.



The inability of 9- α -L-erythro-furanosyladenine (XXXIII) to act as a substrate may be explained by the formation of a hydrogen bond between the two neighboring hydroxy groups, preventing the 3'-hydroxy from attaching itself to the enzyme. Alternately, molecular models show that the introduction of a hydroxy group into the 2' position requires a spatial accommodation which affects the position of the

3'-hydroxy groups and, as a result, this group may be incapable of binding to the proper enzyme site. The fact that 9- β -D-erythro-furanosyladenine (XXXIV) is not a substrate further demonstrates the requirement for the intact 4'-D-hydroxymethyl group for substrate activity.

That the enzyme is rather specific for the furanose ring and for the D isomer of a pentose is shown by the observations that the pyranosides (XXXVI–XLII) and the L-enantiomer (XLIV) of 2'-deoxyadenosine were not substrates for the deaminase.

Taken together, these data suggest that the 1' and the 5' positions of adenine are of importance for substrate activity. The failure of 9- β -D-psicofuranosyl- and fructofuranosyladenine to act as substrates and the lack of activity of the anomer of the D-ribofuranose derivative of adenine suggest that a close fit exists at the anomeric position. The fact that 2',3'-dideoxyadenosine is a good substrate demonstrates that the two hydroxy groups at these positions are not required for substrate activity. The absence of substrate activity of the compounds lacking the 5'-hydroxy group indicates that this group is important for binding, unless its function can be assumed to a very limited extent by an amino or possibly other hydrogen bonding groups or by a 3'-hydroxyl in a position above the plane of the ring such as in 5'-deoxyxylofuranosyl- or in threopentofuranosyladenine.

From the point of view of chemotherapy, these observations relating structure and substrate activity can aid in the preparation of potentially useful adenosine analogs. The pharmacological effectiveness of a compound is in large measure a result of the relative rates of its conversion to the active form and its degradation or excretion. In general, activation of a nucleoside occurs through phosphorylation at the 5' position and removal of the 5'-hydroxyl group prevents this step. The advantages gained by eliminating deamination may thus become offset by the loss of the capacity for phosphorylation. However, such a loss does not invariably lead to the abolishment of all biological activity. Both 9- α -L- and 9- β -D-threofuranosyladenine (XVI, XXXII) for instance inhibit the growth of *Streptococcus faecalis* (ATCC 8043) by 50% at $2 \times 10^{-5} M$. This relatively moderate activity contrasts with the potent inhibitory effects of a number of adenosine analogs modified in the base, which are readily phosphorylated but are not deaminated.^{1d}

While the modifications at the 2' or 3' position permit deamination to varying degrees, they also allow for possible phosphorylation. The extent of conversion to the nucleotide would then be a result of the relative rates at which deamination and phosphorylation occur. Barring any interference with phosphorylation, those modifications which result in a marked decrease in the rate of deamination may be capable of significantly enhancing the effectiveness of a compound.

Since alterations at the 1' position of adenosine prevent deamination while permitting good growth inhibitory activity,⁷ modifications at this position, although chemically difficult, appear to be desirable.

Acknowledgment.—The authors appreciate the excellent technical assistance rendered by Mr. Robert Maue. They are grateful to the following donors of the analogs

(9) R. H. Shab, H. J. Schaeffer, and D. H. Murray, *J. Pharm. Sci.*, **54**, 15 (1965).

used in this study: Dr. Roland K. Robins for compounds III, V, VI, VIII-X, XVII, XVIII, XXIII-XXVI, XXVIII, XXIX, XXXVI, XXXVII, XLIV, and XLV; Drs. Daniel H. Murray and John Prokop for compounds IV, XV, XVI, XXVII, XXXII-XXXIV, and XXXVIII-XLIII; Drs. Leon Goodman

and Elmer J. Reist for compounds XI-XIV, XXXI, and XXXV; Dr. Charles G. Smith for compound XXI; Dr. Robert E. Handschumacher for compound VII; and the CCNSC for compound XXII. It is also a pleasure to acknowledge Dr. Charles A. Nichol for his interest and encouragement of this work.

Differential Binding to the Hydrophobic Bonding Region of T_2 Phage Induced, *Escherichia coli* B, and Pigeon Liver Dihydrofolic Reductases^{1,2}

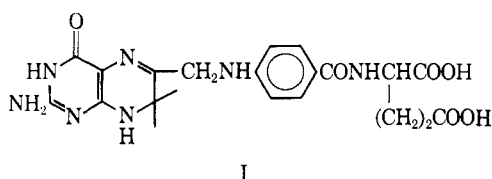
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Forty-nine selected 2,4-diamino heterocycles have been compared for their relative inhibition of the dihydrofolic reductases from pigeon liver, *Escherichia coli* B, and T_2 phage induction; 44 of these compounds were 1-substituted 4,6-diamino-1,2-dihydro-s-triazines. The results clearly showed that parts of the hydrophobic bonding region on dihydrofolic reductase were distinctly different in the enzymes from the three species; some areas in the hydrophobic region were quite similar on the enzyme from pigeon liver and T_2 phage induction, but other areas showed a closer similarity in the enzymes from *E. coli* B and T_2 phage induction. The hydrophobic bonding region of the dihydrofolic reductases was sufficiently different from *E. coli* B and T_2 phage induction that similar studies on tumors induced by viruses may reveal differences in binding to the hydrophobic region; these may be anticipated to be chemotherapeutically exploitable, particularly with active-site-directed irreversible inhibitors of dihydrofolic reductase. The antibacterial agent trimethoprim (L), which complexes 40,000-70,000 times stronger to bacterial enzymes than vertebrate enzymes, complexed to the T_2 phage induced enzyme in the poor manner observed with vertebrate enzymes; this observation along with comparative "inhibitor profiles" with the other compounds gives support to the possibility that the T_2 phage DNA particle diverged from a higher form of life than bacteria.

Dihydrofolic reductase is one of the key enzymes in cellular reproduction; the enzyme reduces dihydrofolic acid (I) and usually folic acid to tetrahydrofolic acid, the cofactor form of the vitamin which is then in-



involved in a spate of enzymatic reactions including purine and pyrimidine biosynthesis.³ The discovery of a hydrophobic bonding region on dihydrofolic reductase⁶ led to an intense study of the conformational aspects of binding to this region⁷⁻⁹ and its relative location on the enzyme. Evidence has been found¹⁰⁻¹² that this hydrophobic bonding region is located near the 4 or 8 position of dihydrofolate (I) when it is com-

plexed to the enzyme;¹³ since the hydrophobic bonding region is outside of the active-site, evolutionary changes¹⁴ of amino acids in this region might be expected to occur more easily, without destroying the function of the enzyme, than in the active site. That binding to the hydrophobic bonding region could differ among species was demonstrated with dihydrofolic reductases isolated from *Escherichia coli* B and pigeon liver,^{15,16} furthermore, these differences in hydrophobic bonding could be considerably amplified³ by use of active-site-directed irreversible inhibitors.^{13,17}

When *E. coli* is infected by T_{even} bacteriophages, the creation or stimulation of at least 12 enzymatic activities occurs,¹⁸ including dihydrofolic reductase;¹⁹ the dihydrofolic reductase induced by the phage has been established to be a new enzyme genetically controlled by the viral DNA and not just higher production of enzyme coded by the bacterial DNA.^{18,19} Furthermore, when mammalian cells in tissue culture are infected by such tumorigenic viruses as polyoma virus or SV40, the levels of some of the enzymes involved in DNA synthesis are increased, including dihydrofolic re-

(1) This work was generously supported by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) Paper XCVII in the series on Irreversible Enzyme Inhibitors³ and Paper X on Hydrophobic Bonding to Dihydrofolic Reductase.⁴

(3) For the previous paper of this series see B. R. Baker, *J. Chem. Edw.*, in press.

(4) For the previous paper of this series see B. R. Baker and G. J. Lourens, *J. Pharm. Sci.*, in press.

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(7) B. R. Baker and J. H. Jordaan, *ibid.*, **54**, 1740 (1965).

(8) B. R. Baker and B.-T. Ho, *J. Heterocyclic Chem.*, **2**, 335 (1965).

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