in vacuo. The free bases were employed for the reduction to amidines after recrystallization from xylene or toluene.

Amidines.—In a typical experiment 18.5 g. (0.1 mole) of hexahydro-1-azepinylpropionamidoxime was dissolved in 100 ml. of anhydrous ethanol, charged with 5 g. of 5% rhodium-on-alumina catalyst and shaken in a Parr hydrogenator under about 3.1 kg./cm.^{2} of hydrogen pressure until the theoretical amount of hydrogen was absorbed. Filtration directly into cold ethanol previously saturated with hydrogen bromide gave the crystalline hexahydro-1-azepinylpropionamidine dihydrobromide. Recrystallization from ethanol–hexane gave 13.2 g. (40%) of product, m. p. 164–166°. The other amidines listed in Table I were likewise obtained in yields of 40–45%.

COMMUNICATIONS TO THE EDITOR

Non-classical Antimetabolites. VII.[1,2] The Bridge Principle of Specificity with Exo-alkylating Irreversible Inhibitors

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Recently, we presented[3] strong experimental evidence to support the concept[4] of a new class of irreversible inhibitors that operate by exo-alkylation. A properly designed compound, such as I, can complex reversibly with an enzyme, then become irreversibly bound within the complex adjacent to the active site. In the detailed version of this experimental evidence,[2] the bridge hypothesis of specificity was proposed. Compared to a reversible inhibitor, the exo-alkylating type of irreversible inhibitor can have an extra dimension of specificity; this extra specificity is dependent upon the ability of the reversibly-bound inhibitor to bridge to and alkylate a nucleophilic group on the enzyme surface and upon the nucleophilicity of the enzymic group being alkylated.

This paper presents experimental evidence for the bridge hypothesis of specificity that warrants raising its status from hypothesis to principle.

It is not surprising that enzymes performing similar reactions—such as dehydrogenation of anionic substrates—would be reversibly

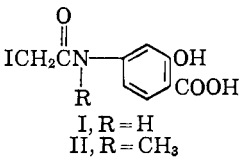
(1) This work was generously supported by Grant CY-5869 of the National Cancer Institute, U. S. Public Health Service.

(2) B. R. Baker, W. W. Lee and E. Tong, Paper VI of this series in press, *J. Theor. Biol.*

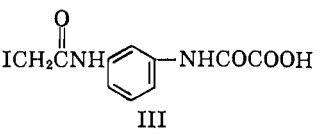
(3) B. R. Baker, W. W. Lee, E. Tong, and L. O. Ross, *J. Am. Chem. Soc.*, **83**, 3713 (1961).

(4) B. R. Baker, Cancer Chemotherapy Reports, No. 4, 1 (1959), published by the National Cancer Institute, Paper I of this series.

inhibited by similar compounds since these sites are by mechanistic necessity closely related. In contrast, there is reason to expect that the nucleophilic sites involved in exo-alkylation *should be dissimilar*, since these nucleophilic sites probably have no function in the mechanistic operation of the enzyme, but are merely part of the secondary and tertiary structure of the protein that at best can only have limited effects on reversible specificity. Thus, it could be anticipated that relatively minor changes in structure of an exo-alkylating irreversible inhibitor could greatly influence its irreversible specificity, but have much less influence on its reversible specificity.



I, R = H
II, R = CH_3



III

TABLE I

IRREVERSIBLE INHIBITION OF LDH AND GDH

Compound	$S_2O_3^=$ activity: Compound/I	Ratio of I_{50} of I to I_{50} of Compound: GDH[b]	LDH[c]	Ratio of rate of inactivation: Compound/I: GDH[d]	LDH[d]
II	1.64[a]	0.26	0.51	0	1.1
III	1.20[a]	0.37	13	0.75	0

[a] Thiosulfate activity determined according to ref. 2 and 3. [b] I_{50} determined for α-oxoglutarate → D-glutamate as described for pyruvate → lactate.[7,8] [c] Pyruvate → lactate.[7,8] [d] Rate of irreversible inhibition determined according to ref. 2 and 3.

4-(Iodoacetamidosalicylic acid (I) has been shown[2,3] to be an irreversible inhibitor of both LDH and GDH.[5] Two related compounds[6,7] (II, III) have now been investigated for their irreversible effect on LDH and GDH as shown in Table I. The data presented are relative to I which was used as a standard in simultaneous incubation runs. Both II and III were reversible inhibitors of both LDH and GDH, but their irreversible effects were direct antitheses of each other. Compound (II) was just as effective as I as an irreversible inhibitor of LDH, *but showed no measurable irreversible inhibition of GDH*. A cross-over specificity was shown by III; III was a more effective irreversible inhibitor of GDH than I, but *showed no measur-*

(5) Abbreviations used: LDH, lactic dehydrogenase; GDH, glutamic dehydrogenase.

(6) B. R. Baker, W. W. Lee, A. P. Martinez, and L. O. Ross, Paper IV of this series in press, *J. Org. Chem.*

(7) B. R. Baker, W. W. Lee, E. Tong, L. O. Ross, and A. P. Martinez, Paper V of this series, in press, *J. Theor. Biol.*

able irreversible inhibition of LDH even though III was bound reversibly to LDH 13 times more strongly than was I.

In II, the N-methyl and the carbonyl carbon of the iodoacetyl group necessarily have a fixed distance between them since they are bound to the same nitrogen. Thus when the iodoacetyl group of II approaches the enzymic nucleophilic group within the reversible enzyme complex there must be space available in the LDH-II complex for the counterbalancing N-methyl group, but *there is no space for this N-methyl group in the GDH-II complex as the iodoacetyl group approaches the nucleophilic site for bridging.*

It was previously noted[5] that the oxanilic acid-LDH complex does not have sufficient space for complete free rotation of the phenyl group; also, it can be clearly demonstrated with molecular models that the iodomethylene group of III can approach any point in space that the iodomethylene group of I can approach when the respective reversible binding points of both compounds are kept fixed. Therefore it follows that the LDH-III complex cannot tolerate the rotation of the benzene ring to a conformation which will allow the iodomethylene group of III to bridge to the nucleophilic site.

In the inhibitor–enzyme complex, the approach of the alkylating group to the enzymic nucleophilic site is dependent upon the combined environments of both the active site and nucleophilic site as well as the conformational and steric requirements between the two sites. It should be possible to bring into play other factors in order to exploit the bridge principle such as the space and conformational requirements for the transition state during nucleophilic attack of the inhibitor by the enzyme within the inhibitor–enzyme complex. These dual site requirements are obviously far more restrictive than the mere additive requirements of each site alone. Therefore the use of the bridge principle should make it possible to obtain highly selective irreversible inhibitors within any group of enzymes that are closely related by the nature of their substrates. Continued research in design of such highly specific inhibitors is warranted, particularly since they could have considerable utility in chemotherapy and protein structure studies.[9]

(8) B. R. Baker, W. W. Lee, W. A. Skinner, A. P. Martinez, and E. Tong, *J. Med. Pharm. Chem.*, **2**, 633 (1960); paper II of this series.

(9) The excellent technical assistance of Mrs. Geraldine Chirayath is gratefully acknowledged.