Halide–Silver Salt Method—*β,β'-Dichloro-di(n-propyl)amine Hydrochloride*—Thionyl chloride (12 ml.) was slowly added to 1,1'-iminodi-2-propanol (2 Gm.) with stirring and cooling until a homogeneous liquid was obtained. Then the excess thionyl chloride was distilled *in vacuo* and the residue was washed several times with chloroform and acetone. The salt that was obtained in 86% yield as white microcrystals melted at 197–198°.

Anal.—Calcd. for $C_6H_{14}Cl_3N$: C, 35.12; H, 6.83; Cl, 51.22; N, 6.83. Found: C, 34.77; H, 6.73; Cl, 51.52; N, 6.73.

The aqueous solution of the salt was rendered alkaline with cold saturated sodium hydrogen carbonate solution and the freed base rapidly extracted with ether. Then the extract was dried over anhydrous sodium sulfate for 24 hr. before the ether was evaporated *in vacuo*. The base thus obtained was immediately used in the condensation reaction.

Condensation with the Appropriate Silver Salt—The silver salt (3 Gm.) was intimately mixed with the halogeno base (0.9 Gm.) in a 100-ml. round-bottom flask. A condenser was fitted to the flask and the whole setup was dipped into a preheated oil bath at 130–140° for a few minutes. The mixture was then allowed to cool, then benzene (40 ml.) was added and the whole was refluxed for 48 hr. after which it was filtered while hot. Light feathery crystals cropped out on cooling and concentrating the benzene solution.

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Keyphrases

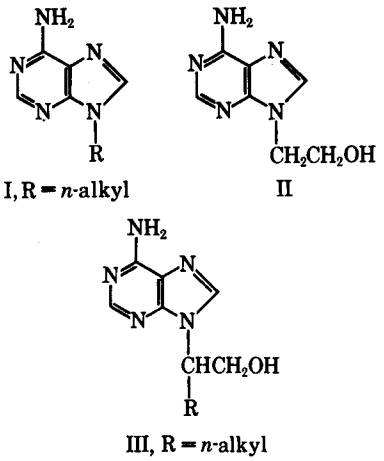
Alkanolamine esters
Monoester, 1,1'-iminodi-2-propanol—synthesis
Diester, 1,1-iminodi-2-propanol—synthesis
p-Nitrobenzoyl Cl—acylating agent

Communications

Enzyme Inhibitors XXII. Identity of Inhibitor Binding Site on Adenosine Deaminase

Sir:

Investigators concerned with active sites or receptor sites in macromolecules have interest in the number and types of binding sites that exist in the biological sample. In enzyme systems, whenever competitive inhibitors are studied, it is possible that the inhbitor is complexed at the active site of the enzyme. However, it is also possible that the inhibitor is complexed to an entirely different site. When two or more inhibitors of an enzyme have been prepared and evaluated as enzyme inhibitors, it is possible that they complex to the same site on the enzyme or to different sites. It occurred to us that it might be possible to obtain information concerning the identity or nonidentity of inhibitor binding sites by combining in one inhibitor the moieties which make a contribution to binding in two different inhibitors. For example, in a variety of 9-substituted adenines, it has been found that the adenine moiety of these inhibitors makes a contribution to binding to the enzyme, adenosine deaminase (1). Furthermore, it has been observed (2) that adenosine deaminase possesses a hydrophobic area which is important in the formation of a complex with the 9-substituent of some 9-*n*-alkyladenines (I). In addition, adenosine deaminase has a specific hydroxyl binding site (2, 3) which makes a contribution to complex formation of the hydroxyl group in a compound such as 9-(2-hydroxyethyl)adenine. Therefore, if the adenine moiety of the 9-*n*-alkyladenines and the 9-(2-hydroxyethyl)adenine (II) is complexed to the same site on the enzyme, it should be possible to prepare very potent inhibitors by combining in one molecule the alkyl chain and the 2-hydroxyethyl group at the 9 position of the adenine nucleus. Such a class of compounds would be the 9-(1-hydroxy-2-alkyl)adenines (III). These compounds were prepared by a modification of a general procedure (4) which involves the condensation of the appropriate amino alcohol with 5-amino-4,6-dichloropyrimidine. The resulting 5-amino-6-chloro-4-(1-hydroxy-2-alkylamino)pyrimidine was cyclized with triethyl

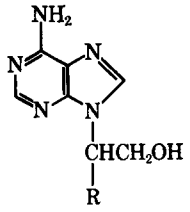


orthoformate to the 6-chloropurine intermediate, which on treatment with ammonia, gave the 9-(1-hydroxy-2-alkyl)adenines shown in Table I.[1]

When the series of compounds listed in Table I was evaluated as inhibitors of adenosine deaminase, it was found that as the alkyl group was lengthened, the compounds consistently became better inhibitors. Previously, on the basis of a study of the change in free energy of binding/methylene group with a series of 9-*n*-alkyladenines, it was found (2) that the $\Delta F/CH_2$ was greatest in those compounds bearing the 9-*n*-propyl through the 9-*n*-heptyl groups; *i.e.*, the main hydrophobic region of adenosine deaminase appears to extend from C_3 through C_7 in the conformation in which the 9-substituent is complexed to the enzyme. The average $\Delta F/CH_2$ group in proceeding from 9-*n*-propyl- through 9-*n*-heptyladenine was −350 cal. (2). An inspection of the $\Delta F/CH_2$ for the 9-(1-hydroxy-2-alkyl)adenines in Table I reveals that the main hydrophobic area also appears to terminate at approximately seven carbons in length[2] since the $\Delta F/CH_2$ for IX is −521 cal., whereas further increases in the carbon chain (X, XI, and XII) gave near minimum changes in free energy. However, note the unusually large change in free energy for the additional methylene unit when comparing V to VI. The magnitude of this change is clearly beyond simple hydrophobic transfer forces (5, 6) and might reflect a conformational change in the enzyme.

Finally, it should be noted that the 9-(1-hydroxy-2-alkyl)adenines are considerably more effective inhibitors than the corresponding parent

TABLE I—INHIBITION OF ADENOSINE DEAMINASE BY



Compd.[a]	R	$(I/S)_{0.5}$[b]	$\Delta F/CH_2$ (cal.)
II	H	1.1 ± 0.05[c]	—
IV	CH_3	1.2 ± 0.03	—
V	C_2H_5	0.49 ± 0.02	−521
VI	C_3H_7	0.071 ± 0.004	−1140
VII	C_4H_9	0.033 ± 0.006	−451
VIII	C_5H_{11}	0.015 ± 0.001	−467
IX	C_6H_{13}	0.0062 ± 0.0002	−521
X	C_7H_{15}	0.0047 ± 0.00003	−163
XI	C_8H_{17}	0.0039 ± 0.00002	−110
XII	C_9H_{19}	0.0030 ± 0.00006	−155

[a] None of these compounds served as substrates of adenosine deaminase. The enzyme (Type I, calf intestinal mucosa) was purchased from the Sigma Chemical Company. [b] The inhibition index $(I/S)_{0.5}$ is the ratio of the m*M* concentration of the inhibitor for 50% inhibition to the m*M* concentration of the substrate. The concentration of adenosine in all experiments was 0.066 m*M*. [c] Data taken from *Reference 2*.

inhibitors. For example, the $(I/S)_{0.5}$ for 9-*n*-heptyladenine is 0.32 (2) and for II is 1.1 (1), whereas the $(I/S)_{0.5}$ for IX is 0.0062. We interpret the enhanced inhibitory properties of the 9-(1-hydroxy-2-alkyl)adenines to be a result (*a*) of the identity of the adenine binding site utilized by I, II, and III and (*b*) of the close spatial relationship of the hydrophobic region and the hydroxyl binding region to the site to which adenine is bound on the enzyme.[3] Thus, the combination of a hydrophobic group and a properly positioned hydroxyl group at the 9 position of the adenine nucleus has resulted in the formation of compounds with greatly enhanced inhibitory properties.

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[1] The details of the synthesis of these compounds as well as the intermediates will be published in the full paper. All compounds gave acceptable combustion analyses for carbon, hydrogen, and nitrogen.

[2] For the purpose of comparing the chain length of the 9-(1-hydroxy-2-alkyl)adenines to the 9-*n*-alkyladenines, we have ignored the hydroxymethyl group; *i.e.*, V has an alkyl chain length of three.

[3] The close spatial relationship of the hydrophobic region and the hydroxyl binding region may be native or may be the result of a conformational change in the enzyme induced by the formation of the E···I complex.

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Keyphrases

Enzyme inhibitors
9-(1-Hydroxy-2-alkyl)adenines—synthesis
Adenosine deaminase—inhibitor binding site identified

Apparent Nonsteady-State Dissolution Phenomenon in Solutions of Colloidal Solubilizers

Sir:

It has been demonstrated in recent reports (1–3) that the dissolution rate of solids in solutions of surfactant above the critical micelle concentration cannot be predicted by the Noyes-Whitney equation (4). Higuchi (1) has suggested that the dissolution process in solutions of colloidal solubilizers may be quantitated by the diffusion layer theory. In a previous report (3) we have found reasonable agreement of diffusion layer theory with initial dissolution rate data obtained under well-stirred conditions. We have recently observed, however, that the initial dissolution rates of salicylic acid in micellar solutions of a nonionic polyoxyethylene surfactant represent presteady-state values. Accordingly, the enhancement of apparent steady-state dissolution rate by the colloidal solubilizer is significantly greater than predicted by diffusion layer theory.

Details of the experimental procedure will be presented in a subsequent report. Briefly, in each dissolution experiment nondisintegrating disks of salicylic acid were used which provided a constant surface area over the entire experimental time period. Dissolution was followed at 37° at a 50 r.p.m. agitation rate (provided by an overhead blade) in either 0.1 *N* HCl or in various concentrations of polyoxyethylene (23) lauryl ether[1] dissolved in 0.1 *N* HCl. The amount of salicylic acid in solution at various intervals of time was determined spectrophotometrically at 304 mμ.

A kinetic method has been devised to ascertain steady-state dissolution rates which introduces the concept of an *excess dissolution rate, i.e.,* $(DR_c - DR_w)$ where DR_c is the dissolution rate per unit area in the colloid system and DR_w is the dissolution rate per unit area in an aqueous system under equivalent hydrodynamic conditions. Both the Dankwerts theory (1, 5) and the diffusion layer theory (1) predict a first-order dependence of excess dissolution rate on $(C_M - C')$, the micellar phase concentration gradient.

According to Gibaldi and Feldman (6), dissolution in a simple aqueous system under constant-surface and nonsink conditions follows first-order kinetics and a plot of log $(C_S - C)$ *versus* time is linear (where C_S is the solubility of the drug in the dissolution medium and C is the concentration of drug in solution at time t). Since the excess dissolution rate is proportional to a concentration gradient term, it follows that a plot of log $(DR_c - DR_w)$ *versus* time should be linear.

A representative plot of the log of excess dissolution rate as a function of time is shown in the figure. As noted in the plot a significant time lag occurs before apparent linearity (attainment of steady state) is observed. A similar plot of log dissolution rate in 0.1 *N* HCl *versus* time shows

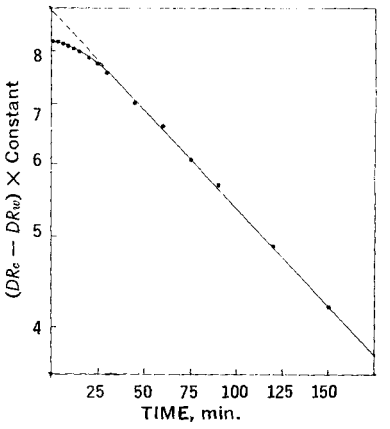

Fig. 1—Plot of the difference in dissolution rates of salicylic acid in 3% polyoxyethylene (23) lauryl ether solution, DR_c, *and 0.1 N HCl,* DR_w, *(on log scale)* versus *time under stirred conditions.*

[1] Brij 35 SP, Atlas Chemical, Wilmington, Del.