bromite[3] within a solvent cage. As this involves the reaction of two high-energy species in close proximity and with presumably a low activation energy, the relative lack of selectivity between *endo* and *exo* attack in the Hunsdiecker reaction, compared to the usual tendency for *exo* attack on such ring systems, is understandable.[8]

While the evidence in this system appears consistent only with the radical geminate recombination mechanism, we are continuing our investigation of the Hunsdiecker reaction to determine whether other mechanisms are available to it in other systems or under other experimental conditions.

(8) G. S. Hammond, THIS JOURNAL, **77**, 334 (1955).

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AN INVERSION OF THE USUAL ANTIPODAL SPECIFICITY OBSERVED IN α-CHYMOTRYPSIN CATALYZED REACTIONS[1]

Sir:

It is known that the antipodal specificity encountered in α-chymotrypsin catalyzed reactions is relative rather than absolute.[2,3] However, whenever antipodal specificity has been evident it has been only the L-enantiomorph that has been observed to react at the more rapid rate. Thus, it is generally believed that α-chymotrypsin exhibits a predominant antipodal specificity for substrates possessing the L-configuration although it is recognized that such antipodal specificity may be lost either wholly or in part with certain types of substrates.

In 1957 it was suggested[4] that one of the enantiomorphs of 1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline might be found to be a substrate of α-chymotrypsin. The DL-ester was first synthesized by condensation of *o*-cyanobenzyl bromide with diethyl acetamidomalonate to give ethyl DL-α-carbethoxy - α - acetamido - β - (*o* - cyanophenyl)-propionate, m.p. 104–105°, which was saponified, decarboxylated and cyclized to DL-1-keto-3-carboxy-1,2,3,4-tetrahydroisoquinoline, m.p. 235–237° (dec.), and the DL-acid esterified to give the desired DL-methyl ester, m.p. 114–115°. When this DL-ester was allowed to react with α-chymotrypsin, in aqueous solutions at 25° and *p*H 7.0 to 8.0, a rapid hydrolysis was observed until approximately 50% of the DL-ester had reacted. With this demonstration of not only substrate activity, but also of marked antipodal specificity, a stereospecific synthesis of D- and L-1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline, from D- and L-phenylalanine, was undertaken after it had been determined that the reaction sequence DL-phenylalanine $\xrightarrow[HCl]{CH_2O}$ DL-3-carboxy-1,2,3,4-tetrahydroisoquinoline (I), m.p. 313–316° (dec.), $\xrightarrow[NaOH]{C_6H_5COCl}$ DL-N-benzoyl-3-carboxy-1,2,3,4-tetrahydroisoquinoline (II), m.p. 170–172°, neut. equiv., 280 $\xrightarrow[K_2CO_3]{KMnO_4}$ DL-α-benzamido-β-(*o*-carboxyphenyl)-propionic acid (III), m.p. 210–211°, neut. equiv., 158 $\xrightarrow[H_2O]{HCl}$ DL-1-keto-3-carboxy-1,2,3,4-tetrahydroisoquinoline (IV), m.p. 234–235° (dec.), neut. equiv., 191 $\xrightarrow[SOCl_2]{CH_3OH}$ DL-1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline (V), m.p. 114–115°, gave an acid (IV) and methyl ester (V) that were identical with those obtained by the first method of synthesis. With D- and L-phenylalanine the above sequence of reactions gave D-I, m.p. 280° (dec.); L-I, m.p. 274° (dec.); D-II, m.p. 156–158°, $[\alpha]^{25}D$ 34°; L-II, m.p. 164°, $[\alpha]^{25}D$ −34°; D-III, m.p. *ca.* 181–184.5°, $[\alpha]^{25}D$ 119°; L-III, m.p. 180–182°, $[\alpha]^{25}D$ −105°; D-IV, m.p. 234–236.5°, $[\alpha]^{25}D$ −44°; L-IV, m.p. 236–238°, $[\alpha]^{25}D$ 41°; D-V, m.p. 87–89°, $[\alpha]^{25}D$ −79° and L-V, m.p. 88–90°, $[\alpha]^{25}D$ 76°. All rotations were determined in methanol with a solute concentration of *ca.* 2%.

When the D- and DL-1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinolines were allowed to react with α-chymotrypsin, in aqueous solutions at 25° and *p*H 7.9 and at substrate concentrations of approximately $10^{-3}M$ and an enzyme concentration of approximately $10^{-6}M$, it was observed that whereas the DL-mixture was hydrolyzed rapidly to the corresponding acid and methanol only to an extent of 50% the D-enantiomorph was hydrolyzed completely and at a more rapid rate. A preliminary analysis of the kinetics of hydrolysis of the DL-mixture indicated that under the above conditions the L-enantiomorph present in the DL-mixture participated in the reaction primarily as a competitive inhibitor rather than as a substrate.

When it is appreciated that D-1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline is hydrolyzed in the presence of α-chymotrypsin at a rate comparable in magnitude to the rates of hydrolysis of several acylated-L-phenylalanine methyl esters under similar experimental conditions and that L - 1 - keto - 3 - carbomethoxy - 1,2,3,4 - tetrahydroisoquinoline, in common with a number of acylated-D-phenylalanine methyl esters, is hydrolyzed at a far slower rate and can function as a competitive inhibitor in the above reaction it becomes evident that α-chymotrypsin and not a hitherto undisclosed enzyme, present as an impurity in the α-chymotrypsin preparation, is responsible for the observed preferential hydrolysis of the above D-enantiomorph.

In our observation of an inversion rather than of an abolition of the traditional antipodal specificity of systems involving α-chymotrypsin we have demonstrated that the predominant antipodal specificity of such systems can be determined by the structure of the substrate and that it is not an invariant property of the enzyme. The significance of this conclusion with respect to the mechanism of enzyme action is obvious. For one, it suggests that if the conformation of the active site of the enzyme is essentially invariant then combination of the substrate with the active site must involve interactions

(1) Supported in part by a grant from the National Institutes of Health, Public Health Service.

(2) H. Neurath and G. W. Schwert, *Chem. Revs.*, **46**, 69 (1950).

(3) R. M. Bock, unpublished experiments conducted in these laboratories.

(4) R. L. Bixler, Ph.D. Thesis, Calif. Inst. Tech., Pasadena, 1957.

beyond those contemplated in a so-called "three point interaction" with the result that the configuration about the asymmetric carbon atom of the combining molecule is but one of several structural factors determining antipodal specificity. Secondly, it is possible that enzyme-substrate complexes, although formed at only one active site of essentially invariant conformation, can decompose to give products through the intermediacy of more than one type of complex, and that in the case at hand, because of the particular structure of the substrate, hydrolysis proceeds *via* a previously unobserved path which favors the D-enantiomorph. Thirdly, it is not excluded that antipodal specificity may arise during the act of combination of enzyme and substrate by a change in the conformation of the active site of the enzyme that is mediated by the structure of the substrate. At present it is not possible to decide which of the above alternatives is the more acceptable.

The above inversion of antipodal specificity was achieved by constraining the structure of both an optical and an operational antipode of a substrate, by a bridge linking the α-acyl moiety and the side chain of the α-amino acid moiety contributing the carboxylic acid derivative involved in the solvolytic reaction, *i.e.*, D-1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline may be viewed as benzoyl-D-alanine methyl ester, or as formyl-D-phenylalanine methyl ester, cyclized through the loss of two hydrogen atoms, one from the side chain and the other from the α-acyl moiety, or alternatively as benzoyl-D-phenylalanine methyl ester cyclized by being deprived of one of its two benzene nuclei. These considerations suggest that this as well as other modes of constraint may provide not only additional examples of inversion of the traditional antipodal specificity of α-chymotrypsin, but also families of compounds, which because of their more precisely defined steric characteristics, will be more useful than the more conventional substrates of the L-configuration in attaining the goal of definition of the nature of the active site of this enzyme.

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COMPARISON OF RATE OF AMMONIUM ION-AMMONIA HYDROGEN EXCHANGE WITH AMMONIUM ION-ELECTRON REACTION[1]

Sir:

Reported herein are data which bear on (a) the rate of labile hydrogen isotope exchange in solution, (b) the chemical nature of the electron in liquid ammonia solutions of alkali metals, and (c) the comparison of rates of very fast reactions.

We wished to determine whether ammonium ions were attacked so rapidly by electrons in alkali metal solution (reaction I)[2] that they had insufficient time to reach isotopic equilibrium with the solvent molecules in the alkali metal solution (reaction II). Reaction II (*sans* tritium) has been described

$$NH_4^+ + e^-(NH_2T)_x \longrightarrow NH_3 + xNH_2T + {}^1/_2H_2 \quad (I)$$

$$NH_4^+ + NH_2T \rightleftharpoons NH_3 + NH_3T^+ \quad (II)$$

as an ultrafast reaction by Ogg,[3] who studied the nuclear magnetic resonance of 0.1 *M* ammonium bromide solutions in liquid ammonia. From Ogg's data it can be estimated that the mean lifetime of a proton on any given nitrogen is substantially less than 0.007 sec.[4]

We determine the relative rates of reaction of alkali metal solutions with various proton donors in liquid ammonia labeled with tritium by measuring the volumes and radioactivities of hydrogen produced at time intervals. These experiments are carried out in an apparatus[5] which provides for the preparation of two separated reactant solutions at $-33°$, jet-mixing of these solutions, and quantitative collection of hydrogen produced. In experiments with reactant solutions of sodium and of ethanol, respectively, it was found that the radioactivity of the hydrogen liberated after mixing was the same when the initial tritium-labeling was restricted to the sodium solution as when the initial labeling was restricted to the ethanol solution, *i.e.*, the exchange of alcohol (or whatever proton donating species it gives rise to) with ammonia was essentially complete before the hydrogen liberation reaction took place.

However, the rates of reaction of alkali metal with alcohols are slow compared to reaction I. To study the relative rates of reactions I and II, three experiments were carried out.

Run 1.—A solution of 0.145 g. NH_4Br in 25 ml. of NH_2T[6] was mixed with 0.588 g. of Li in 25 ml. NH_2T; the radioactivity of the 16.0 ml. of hydrogen evolved was 0.115 μc./ml.

Run 2.—A solution of 0.194 g. of NH_4Br in 25 ml. of NH_2T was mixed with 0.515 g. of Li in 25 ml. of NH_3; activity of 16.5 ml. of evolved hydrogen, 0.098 μc./ml. Comparing run 2 with 1, note that the use of unlabeled NH_3 in the metal solution reduces the activity of evolved hydrogen but little.

Run 3.—A solution of 0.176 g. of NH_4Br in 25 ml. of NH_3 was mixed with 0.514 g. of Li in 25 ml. of NH_2T; activity of 19.8 ml. of evolved hydrogen, 0.007 μc./ml. Comparing run 3 with 1, note that the use of unlabeled NH_3 in the ammonium bromide solution reduces the activity of evolved hydrogen greatly.

Considering the fact that in the metal solutions the ammonia molecules are present in swamping concentration compared to the electron concentration, it appears that the rate of electron attack (I) is

(1) This work was performed under Contract No. AT-(40-1)-1983 between the University of Tennessee and the U. S. Atomic Energy Commission.

(2) The symbol NH_2T designates tritium-labeled ammonia. Use of the symbol $e^-(NH_2T)_x$ implies no conclusion about the nature of the reacting entity.

(3) R. A. Ogg, *Faraday Soc. Disc.*, **17**, 215 (1954).

(4) J. D. Roberts, "Nuclear Magnetic Resonance," McGraw-Hill Book Company, Inc., New York, N. Y., 1959, pp. 77–78.

(5) E. J. Kelly, Ph. D. Thesis, University of Tennessee, March, 1959. A simplified version of the apparatus is described by J. F. Eastham and D. L. Larkin, THIS JOURNAL, **81**, 3652 (1959).

(6) In each experiment the initial activity of NH_2T was 0.338 μc./ml. of gas. Before use, the solutions of NH_4Br in NH_2T were allowed to stand one hour to assure isotopic equilibration; *cf.* C. J. Wyman, Si-Chang Fung and H. W. Dodgen, THIS JOURNAL, **72**, 1033 (1950). Delivery of solutions to the reaction chamber was about 97% complete.