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.

an order of magnitude faster than the ultrafast reaction of $NH_4^+:NH_3$ hydrogen exchange (II).[7] We infer that the chemical mobility of the electrons in these solutions matches their physical mobility (*e.g.*, electrical conductivity), and that this may be correlated with the physical models being proposed[8] to describe metal–ammonia solutions. In these solutions, the electron attack on an electron-attracting entity appears to be *via* a mechanism quite different from that of ordinary ionic transport.

(7) Because the reactant ratio of $Li:NH_4^+$ is high, there is the possibility that only the ammonia associated with one equivalent of lithium should be considered as exchanging with the ammonia associated with ammonium ion. This seems improbable, since it would involve the mixing of all the NH_4Br solution with only a part of the Li solution. It is more straight-forward to assume that the mixing involves approximately equal amounts of both solutions at all times, that both solutions mix fairly uniformly in order for all the NH_4^+ to react, that both reactions I and II are diffusion controlled, and that the rate of attack of NH_4^+ ions by electrons competes effectively with the rate of attack (exchange) of NH_4^+ ions by NH_3 molecules.

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THE SYNTHESIS OF 2-DEOXY-D-RIBOFURANOSE 1-PHOSPHATE

Sir:

Cleavage of a ribonucleoside with a nucleoside phosphorylase was reported by Kalckar[1] to give a ribose 1-phosphate. Subsequently, this product was shown to be α-D-ribofuranose 1-phosphate on the basis of certain chemical tests, as well as by chemical synthesis of both anomers.[2] In a presumably similar fashion, the action of the appropriate nucleoside phosphorylases on deoxyribonucleosides leads to the formation of an extremely acid-labile 2-deoxy-D-ribofuranose 1-phosphate,[3-5] which has been isolated as a crystalline di-(cyclohexylammonium) salt.[4,5] The optical rotation of this material, $[\alpha]_D$ +38.8°,[5] and the manner in which it is enzymatically synthesized, suggest[5] that, like its D-ribose analog, it possesses the α-configuration. This conclusion is supported by the present work, in which we describe the chemical synthesis of a 2-deoxy-D-ribofuranose 1-phosphate of high biological activity.

Crystalline 3,5-di-*O*-*p*-toluoyl-2-deoxy-D-ribofuranosyl chloride[6] was condensed in benzene solution with disilver phosphate[7] (previous workers who have used this material for the synthesis of phosphates[8] have termed it "monosilver phosphate"). The product of the condensation was saponified in aqueous alcohol with lithium hydroxide, the lithium replaced by cyclohexylamine using an ion exchange resin and the crude dry salts extracted with *n*-propyl alcohol to remove cyclohexylammonium *p*-toluoate. Inorganic phosphate then was removed as magnesium ammonium phosphate and the resulting material crystallized as the di-(cyclohexylammonium) salt from methanol–ether or from aqueous acetone. The product, which was obtained in a yield of *ca.* 30%, based on the chloride, showed $[\alpha]^{20}D$ of about +22° in water. *Anal.* Calcd. for $C_{17}H_{37}N_2O_7P$ (412.47): C, 49.49; H, 9.04; N, 6.79; P, 7.51. Found: C, 49.33; H, 9.32; N, 6.63; P, 7.53.

In *n*-propyl alcohol–ammonia–water and in isopropyl alcohol–ammonia–water the material is chromatographically homogeneous, and possesses the same R_f as authentic, enzymatically prepared 2-deoxy-D-ribofuranose 1-phosphate. Fractional crystallization of the synthetic salt has not been entirely successful; however, using methanol–ether, fractions were obtained with $[\alpha]^{20}D$ as high as +30° (C, 49.44; H, 8.76; N, 6.87; P, 7.48). One of these fractions, when assayed enzymatically using fish nucleoside phosphorylase,[5] had about 80% of the activity of natural 2-deoxy-D-ribofuranose 1-phosphate.

Since crystalline acylated 2-deoxy-D-ribofuranosyl halides have been shown[6] to give rise to anomeric mixtures of 2-deoxy-D-ribofuranosides, it seems probable that the phosphate obtained here is a mixture of anomers. A comparison of our product and the natural material on the basis of optical rotation and biological activity supports the assumption[5] that the natural substance of $[\alpha]_D$ +38.8° is the α-anomer and, furthermore, one may calculate that the β-anomer would have an $[\alpha]_D$ of approximately −10°.

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THE PHOTOREDUCTION OF PORPHYRINS AND THE OXIDATION OF AMINES BY PHOTO-EXCITED DYES

Sir:

Two distinct series of hydro-porphyrins are known. The hydrogens may be located on the peripheral "pyrrole" ring carbons (chlorophyll and bacteriochlorophyll), or they may be on the methine carbons (porphomethenes,[1] I and II, and porphyrinogens). The photoreduction of uroporphyrin produces, in addition to a transitory compound absorbing at 440 and 735 mμ, a more stable substance absorbing at 500 mμ which is related to the second series of hydroporphyrins, the porphomethenes.

Porphyrins may be photoreduced by a wide variety of compounds, *e.g.*, ascorbic acid, glutathione and tertiary amines. The reduction of uroporphyrin with ethylenediaminetetraacetic acid

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