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1-Benzyldihydronicotinamide—A Model for Reduced DPN

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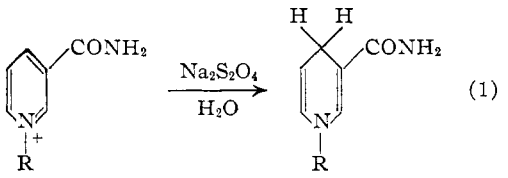
1-Benzyldihydronicotinamide will reduce malachite green to its leuco base. The reduction product of nicotinamide-1-benzylochloride in D_2O transfers deuterium to malachite green, as does 1-benzyl-4-deuterodihydronicotinamide; but neither the 2-deutero nor the 6-deutero isomer transfers deuterium to the dye. 1-Benzyldihydronicotinamide also reduces diphenylpicryl hydrazyl, but does not reduce acetone or pyruvic acid. The chemical reductions are compared to the enzyme-promoted reactions of diphosphopyridine nucleotide.

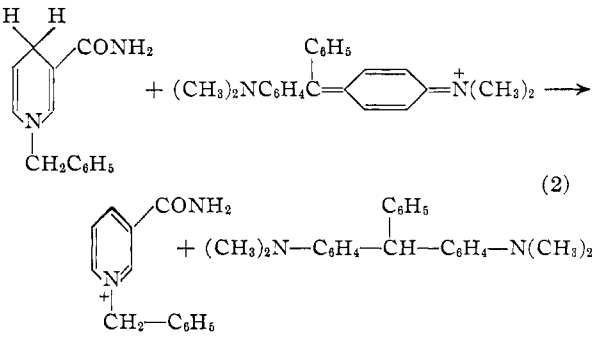
Alcohol dehydrogenase[1] and lactic dehydrogenase[2] promote the direct transfer of hydrogen from their substrates to the coenzyme, diphosphopyridine nucleotide (hereafter called DPN). Karrer and his collaborators[3] demonstrated that this reduction takes place in the pyridine ring, and Pullman, San Pietro and Colowick[4] made use of the direct enzymatic transfer of deuterium[1,2] from substrate to coenzyme to demonstrate that reduced DPN (formula II, where R = ribose-pyrophosphate-adenosine) is a derivative of 1,4-dihydropyridine. Recently Rafter and Colowick[5] have shown that the chemical reduction of nicotinamide-1-methochloride (I, R = CH_3) also takes place in the 4-position of the pyridine ring.



(1)

I II

Karrer and his collaborators[6,7] reduced several N-substituted quaternary salts I of nicotinamide to the corresponding dihydro compounds II, and demonstrated that these dihydro compounds, in turn, reduce methylene blue to its colorless leuco base. However, the hydrogen attached to nitrogen in leuco methylene blue probably exchanges too rapidly with the solvent to permit a determination of whether or not reduction of the dye is accompanied by direct hydrogen transfer. In the present investigation, 1-benzyldihydronicotinamide (II, R = $CH_2C_6H_5$) was prepared; and this compound was shown to reduce malachite green, in excellent yield, according to equation 2



(2)

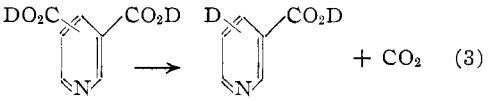
The pathway for this reaction was investigated with the aid of deuterium as a tracer. Several related reactions also were examined.

Experimental

Materials

Dihydropyridine Derivatives.—1-Methyldihydronicotinamide (yellow oil), 1-propyldihydronicotinamide (m.p. 91–92° dec.) and 1-tetraacetylglucosyldihydronicotinamide (m.p. 154° dec.) were prepared according to Karrer.[6] 1-Benzyldihydronicotinamide[7] was prepared by adding 1.00 g. of nicotinamide-1-benzylochloride (I, R = $C_6H_5CH_2$) to a solution of 1.38 g. of anhydrous sodium carbonate and 2.57 g. of Mallinckrodt sodium dithionite (83% pure by ferricyanide titration[8]) in 10–100 cc. of H_2O or D_2O at 45–50°. The reaction mixture was shaken for ten minutes, the yellow solid collected by filtration, and recrystallized several times from ethanol–water. The product (yield 0.70 g.) melted with decomposition at 120–122°; log ϵ_{max} (355 mμ) 3.86. 2,6-Dimethyl-3,5-dicarbethoxydihydrolutidine was prepared according to Meyer and Tropsch.[9]

Deuterium Compounds.—Deuteroacetic acid, CH_3CO_2D, was prepared by refluxing equivalent quantities of D_2O and acetic anhydride for 3 or 4 hours in a well dried apparatus. In order to prepare the required deuterated nicotinic acids, the corresponding dibasic acids were decarboxylated, according to equation 3



(3)

These syntheses went smoothly with quinolinic and isocinchomeronic acids. However, decarboxylation of cinchomeronic acid yields a mixture of nicotinic and isonicotinic acids from which the less abundant[10] and lower melting nicotinic acid can be isolated only with difficulty.

Nicotinic acid-2-*d*, prepared by the decarboxylation of quinolinic acid in deuteroacetic acid, was generously contributed by Professor D. Harris and Dr. F. A. Loewus of the University of Chicago.

Nicotinic acid-6-*d* was prepared from isocinchomeronic acid provided by Professor Harris and Dr. Loewus. Four grams of recrystallized acid in a Soxhlet thimble were extracted with 100 cc. of recovered (isotopically impure) deuteroacetic

(1) F. H. Westheimer, H. F. Fisher, E. E. Conn and B. Vennesland, THIS JOURNAL, **73**, 2403 (1951); H. F. Fisher, E. E. Conn, B. Vennesland and F. H. Westheimer, *J. Biol. Chem.*, **202**, 687 (1953); F. A. Loewus, F. H. Westheimer and B. Vennesland, THIS JOURNAL, **75**, 5018 (1953).

(2) F. A. Loewus, P. Offner, H. F. Fisher, F. H. Westheimer and B. Vennesland, *J. Biol. Chem.*, **202**, 699 (1953); B. Vennesland and F. H. Westheimer, McElroy and Glass, in "The Mechanism of Enzyme Action," the Johns Hopkins Press, Baltimore, Md., 1954, p. 357.

(3) P. Karrer, G. Schwartzenbach, F. Benz and U. Solmssen, *Helv. Chim. Acta*, **19**, 811 (1936); P. Karrer and O. Warburg, *Biochem. Z.*, **285**, 297 (1936).

(4) M. Pullman, A. San Pietro and S. P. Colowick, *J. Biol. Chem.*, **206**, 129 (1954).

(5) G. W. Rafter and S. P. Colowick, *ibid.*, **209**, 773 (1954).

(6) P. Karrer, *et al.*, *Helv. Chim. Acta*, **19**, 1028 (1936); **20**, 55, 72, 622 (1937); **21**, 223, 1174 (1938); **29**, 1152 (1946); **32**, 960 (1949).

(7) P. Karrer and F. J. Stare, *ibid.*, **20**, 418 (1937).

(8) W. Christiansen and A. Norton, *Ind. Eng. Chem.*, **14**, 1126 (1922).

(9) H. Meyer and H. Tropsch, *Monatsh.*, **35**, 207 (1914).

(10) S. Hoogewerff and W. A. van Dorp, *Ann.*, **204**, 113 (1880).

acid. The solution was evaporated *in vacuo*, and the residue extracted with 150 cc. of isotopically pure deuteroacetic acid. The solution was again vacuum evaporated, and the solid deuterated acid quickly transferred to 100 cc. of purified dry nitrobenzene. The decarboxylation required 15 min. at the boiling point of nitrobenzene. 6-Deuteronicotinic acid (1.96 g.) was recovered from the nitrobenzene by addition of ligroin, and was then recrystallized from 95% ethanol.

Nicotinic Acid-4-*d*.—Cinchomeronic acid anhydride[11] was powdered in a dry-box, and 23 g. was added to 10 cc. of pure D_2O in a 100-cc. flask. The flask was connected to a reflux condenser protected by a P_2O_5 tube, and the mixture heated for four hours on the steam-bath. The excess D_2O was removed by vacuum evaporation. The resulting deuterated cinchomeronic acid melted at 267–268° with decomposition. It was decarboxylated in boiling nitrobenzene, and the mixture of nicotinic acid-4-*d* and isonicotinic acid-3-*d* recovered from the nitrobenzene by the addition of ligroin.

The mixture was separated by the method of Hoogewerff.[12] Repeated recrystallization of the mixture of acids from methanol yielded about 7 g. of impure isonicotinic acid and about 7 g. of material melting around 215–230°, enriched in nicotinic acid. The latter was dissolved in absolute ethanol (30 cc./g. of mixture) and saturated with dry HCl. The temperature rose to 70°; as soon as all the solid dissolved, the mixture was cooled, whereupon a white solid (principally nicotinic acid hydrochloride) separated. Repetition of this process five times gave 2.8 g. of nicotinic acid hydrochloride, m.p. 268–270°; its melting point was not depressed by admixture with an authentic sample.

Deuterated Nicotinamides.—Two grams of a deuteronicotinic acid (or of its hydrochloride) was dissolved in 40 cc. of absolute methanol, and the solution saturated at 0° with dry HCl. The mixture was allowed to warm to 25° overnight, refluxed for three hours, and the solvent evaporated under vacuum. The residue was neutralized at 0° with saturated sodium carbonate solution, quickly extracted with seven 50-cc. portions of ether. The ether extracts were dried with anhydrous Na_2SO_4, and the ether removed by distillation. The residue of crude methyl nicotinate was converted directly to the nicotinamide except in the case of the 4-deutero derivatives, where the ester was first recrystallized from ligroin. The methyl nicotinate was dissolved in 10 cc. of methanol and added to 20 cc. of liquid ammonia in a small Dewar. After 36 hours, the solvent was vacuum evaporated, and the solid first extracted with ligroin, then recrystallized from acetone. The yield was about 1 g. of deuterated nicotinamide melting at 132–133°.

Other Materials.—A sample of α,α-diphenyl-β-picryl hydrazyl was generously provided by Dr. A. Kowalsky.[13] Other materials were of reagent grade.

Reductions

Reduction of Malachite Green. First Procedure.—A solution of 100 mg. of 1-benzyl-1,4-dihydronicotinamide (with or without deuterium) in 5 cc. of 95% ethanol was added to 194 mg. of malachite green oxalate in 25 cc. of 95% ethanol under a stream of Linde nitrogen. The green color of the dye was slowly discharged; after 15 hours at 25–30° the light greenish-yellow solution was vacuum evaporated, and the brown residue dissolved in 10 cc. of 0.2 *M* hydrochloric acid. The solution was centrifuged, cooled to 0°, and the *p*H adjusted to 7 with 2 *M* NaOH. The solid (crude leucomalachite green) was centrifuged from the solution(S), and recrystallized from ethanol–water. Leucomalachite green is known in three crystalline modifications, one melting at 102°, one at 93–94°, and one of still lower melting point.[14]

The material from the reduction here cited usually melted at 96°, but occasionaly at 102°; in any case its melting point was not depressed by admixture with an authentic sample of m.p. 102°. The leuco compound was further identified by analysis, by reoxidation to malachite green, and by its infrared spectrum, which was identical with that of the authentic sample. Since the material obtained in the reduction process had no infrared absorption above 3070 cm.$^{-1}$, little or no carbinol was present. *Anal.* Calcd. for $C_{23}H_{26}N_2$: C, 83.59; H, 7.93. Found: C, 83.36; H, 8.08.

The solution (S) was freed of oxalate with calcium chloride solution, and then vacuum evaporated. The residue was extracted with 25 cc. of boiling ethanol, and the filtered extracts evaporated to about 5 cc. Nicotinamide 1-benzylochloride separated from the cooled solution; after two recrystallizations from ethanol, it melted at 235–239° dec., and did not depress the melting point of an authentic sample of the salt. The yields of leuco malachite green averaged about 55%; the yields of recovered salt varied between 25 and 75% of theory.

Second Procedure.—Although the isolation procedure given above is the most direct, it led to some difficulty when nicotinamide 1-benzylochloride was analyzed for deuterium, since the chloride poisoned the zinc converter. The following modified and indirect procedure was therefore preferred. After the reduction, and evaporation of the alcoholic solution, the solid residue was mixed with 20 cc. of water. Extraction with three 15-cc. portions of chloroform followed by evaporation yielded leuco malachite green. The aqueous solution was aerated (to free it from chloroform) centrifuged, and treated at 45° with 0.4 g. of Na_2CO_3 and 0.8 g. of $Na_2S_2O_4$. The substituted nicotinamide was then isolated as the dihydro compound (see Materials). This second method gave about 80% yields of leuco malachite green, and an average yield of 45% (maximum yield 70%) of 1-benzyl-1,4-dihydronicotinamide.

Reduction of Pyruvic Acid.—Three cc. of freshly distilled pyruvic acid and 10.0 g. of dihydro-3,5-dicarbethoxy-2,6-lutidine were heated under nitrogen on the steam-bath for 18 hours. The mixture was dissolved in 50 cc. of dilute hydrochloric acid, made alkaline with 20% NaOH, and extracted with chloroform. 3,5-Dicarbethoxy-2,6-lutidine was isolated from the chloroform extract, and identified by its melting point and mixed melting point with an authentic sample. The aqueous solution was acidified, clarified by extraction with chloroform, saturated with sodium sulfate, and continuously extracted with ether for 24 hours. After removal of the ether, 3.9 g. of an oil remained. This oil was chromatographed on silica gel[15] to obtain lactic acid. The lactic acid fraction was extracted again into ether, rechromatographed,[15] and then converted to phenacyl lactate,[16] m.p. 115°. The sample was identified by mixed melting point and by comparison of its infrared spectrum with that of an authentic sample. The yield of lactic acid, determined by titration after the second chromatographing, was 7%.

Other Reductions.—α,α-Diphenyl-β-picryl hydrazyl reacts with various 1-substituted dihydronicotinamides to discharge the color of the free radical and to reduce the intensity of the absorption band (340–360 mμ) characteristic of the 1,4-dihydropyridine derivatives. In particular, the reaction was carried out at room temperature in ethanol with 1-propyl- and with 1-tetraacetylglucosyl-1,4-dihydronicotinamides, and in benzene with the 1-propyl and 1-benzyl compounds. Alloxan is reduced rapidly at 0° in water, *p*H 6–11, by either the methyl or propyl derivative, slowly in water at room temperature, *p*H 6–11, by the tetraacetyl derivative. The reaction was characterized by the disappearance of the 340–360 mμ absorption band of the dihydropyridine and by the positive color test for dialuric acid.[17] Spectrophotometric evidence was also obtained for the reduction of quinone and phenanthraquinone by 1-methyl-1,4-dihydronicotinamide in neutral aqueous solution.

Attempted Reductions.—Attempts were made to reduce chloral, acetone, biacetyl and pyruvamide with 1-propyl-, 1-methyl- and 1-tetraacetylglucosyl-1,4-dihydronicotinamides, in aqueous solutions, *p*H 6–10. The course of the reaction was followed by observing the ultraviolet absorption spectrum of the dihydropyridine compounds in the region around 360 mμ. In control experiments, this ultraviolet band slowly disappears because of an acid-catalyzed decomposition of the dihydropyridine derivative; addition of the carbonyl compounds listed above did not appreciably increase the rate of spectral change.

Deuterium analyses were carried out by burning the samples to water, converting the water to a mixture of H_2

(11) B. Fels, *Ber.*, **37**, 2137 (1904).

(12) S. Hoogewerff and W. A. van Dorp, *Ann.*, **207**, 219 (1881).

(13) A. Kowalsky, Dissertation, University of Chicago, 1954.

(14) E. Fischer and O. Fischer, *Ber.*, **12**, 796 (1879)

(15) W. A. Bullen, J. E. Varner and R. C. Burrell, *Anal. Chem.*, **24**, 187 (1952).

(16) W. L. Judefind and E. E. Reid, THIS JOURNAL, **42**, 1043 (1920).

(17) D. Davidson and E. Epstein, *J. Org. Chem.*, **1**, 305 (1936).

and HD, and analyzing the gas for deuterium with a Consolidated-Nier Isotope Ratio mass spectrometer.[1,2,18]

The results of the analyses are given in Table I. Readings for duplicate analyses on the same sample agreed to within ±2%, but repetition of experiments showed maximum spread as high as 10%. The percentage error (but not the absolute error) in atoms D per molecule increases rapidly as the reading approaches the background value for the natural abundance of deuterium in hydrogen. The background value of this isotope ratio was 0.00045, and this value was always used in the calculations, although occasional measurements gave values as high as 0.00055; the difference is presumably due to "memory" effects.[18] The difference would affect the calculated results in Table II only slightly except for the very low values, which should perhaps be closer to zero.

TABLE I

DEUTERIUM ANALYSES

Compound	Diluted with	Dilution factor	Isotope ratio,[a] R
Dihydro, x-d[b]	Dihydro[c]	9.43	0.01113
Leuco MG[d]	...	1.00	.01069
Salt[e]	Salt	11.30	.00836
Dihydro, 2-d[f]	Dihydro	8.58	.00948
Leuco MG	...	1.00	.00056
Salt	Nicotinamide	27.5[g]	.00307
Dihydro, 6-d[h]	Dihydro	10.30	.01165
Leuco MG	...	1.00	.00066
Salt	Benzoic acid	11.13[g]	.00834
Dihydro, 4-d[i]	Dihydro	21.00	.00753
Leuco MG	Leuco MG	2.99	.00545
Salt (as dihydro)[j]	Dihydro	16.20	.00748
Dihydro, 4-d_2[k]	Dihydro	41.00	.00736
Leuco MG	Leuco MG	15.15	.00462[l]

[a] Actual mass-spectrometer reading, uncorrected for background; see text. [b] Product obtained by reducing nicotinamide 1-benzylochloride (I, R = $CH_2C_6H_5$) in D_2O; 1-benzyl-4-deutero-1,4-dihydronicotinamide. [c] 1-Benzyl-1,4-dihydronicotinamide. [d] Leucomalachite green. [e] Nicotinamide 1-benzylochloride (I, R = $CH_2C_6H_5$). [f] 1-Benzyl-2-deutero-1,4-dihydronicotinamide. [g] The dilution factor was calculated on the basis of the total hydrogen present. [h] 1-Benzyl-6-deutero-1,4-dihydronicotinamide. [i] 1-Benzyl-4-deutero-1,4-dihydronicotinamide. [j] The second isolation procedure was used; see text. [k] 1-Benzyl-4,4-dideutero-1,4-dihydronicotinamide. [l] This ratio was increased by 4%, to bring the analysis into agreement with those for empirical standards prepared from D_2O.

TABLE II

DEUTERIUM CONTENT OF REACTANTS AND PRODUCTS OF REACTION (2)

No.	Nicotinamide 1-benzylochloride reduced	Solvent for reduction	Atoms D/molecule: Dihydro compound	Leuco malachite green	Nicotinamide 1-benzylochloride
1	Undeut.	D_2O	0.70 ± 0.04	0.13 ± 0.007	0.58 ± 0.1
2	2-d	H_2O	0.54 ± .03	0.001 ± .001	.47 ± .1
3	6-d	H_2O	0.80 ± .04	.003 ± .002	.57 ± .1
4	4-d	H_2O	1.04 ± .05	.19 ± .01	.79 ± .04[a]
5	4-d	D_2O	1.98 ± .1	.86 ± .04	

[a] As 1-benzyl-1,4-dihydronicotinamide; see text.

The atoms of deuterium per molecule, A, in any given compound was calculated from the isotope ratios, R, given in Table I, by equations 4

$$A = \frac{R'}{R' + 2} DN \text{ and } R' = R - R(\text{background}) \quad (4)$$

where D is the dilution factor and N the number of hydrogen plus deuterium atoms per molecule in the compound under discussion.

(18) R. B. Alfin-Slater, S. M. Rock and M. Swislocki, *Anal. Chem.*, **22**, 421 (1950); J. Graff and D. Rittenberg, *ibid.*, **24**, 878 (1952).

Results

The deuterium contents, calculated as atoms of deuterium per molecule, for the reactants and products of equation 2 are shown in Table II.

The second column of this table lists the salts (formula I, R = $CH_2C_6H_5$) which were reduced with dithionite according to equation 1. The third column shows the solvent in which this reduction was carried out. The fourth column gives the deuterium content of the 1-benzyl-1,4-dihydronicotinamide obtained from the dithionite reduction. The fifth and sixth columns give the deuterium contents of the products obtained when the dihydro compounds (listed in column four) reacted in alcoholic solution with malachite green oxalate. Despite the fact that the deuterium contents of some of the dihydro compounds (column 4) fall below one atom per molecule, the results of these investigations are unambiguous.

The reduction (line 1) of nicotinamide 1-benzylochloride gives a dihydro compound which transfers about a fifth of its deuterium to malachite green. The reduction (equation 2) therefore occurs by a direct hydrogen transfer from reductant to oxidant; furthermore, the selection factor in favor of hydrogen (*i.e.*, the ratio, k_H/k_D, of the rate constants for hydrogen and for deuterium) is around 4.5 (see Appendix).

Reduction of 2-deutero and of 6-deuteronicotinamide 1-benzylochloride in H_2O led to products which do not transfer deuterium to malachite green; whereas reduction of the 4-deutero compound in H_2O led to a reduction product which transferred about a fifth of an atom of deuterium to the dye. The k_H/k_D ratio for 1-benzyl-4-deutero-1,4-dihydronicotinamide is therefore about the same as that for the product listed in line 1 of Table II. When 4-deuteronicotinamide-1-benzylochloride is reduced in D_2O, the product transfers almost one atom of deuterium to malachite green. These data, obtained by a quite different method from that of Colowick and his collaborators,[4,5] confirm their demonstration that the dithionite reduction takes place in the 4-position of the pyridine ring. This fact has also been demonstrated by Talalay, Loewus and Vennesland,[19] who used DPNase to exchange some of our 4-deuteronicotinamide into DPN, and demonstrated that the deuterium is in the enzymatically active site.

Discussion

The reduction of malachite green (equation 2) by 1-benzyl-1,4-dihydronicotinamide is not a good model for the reduction of carbonyl compounds, but resembles more closely the DPN–DPNH transhydrogenase system[20]; like the latter, the model system involves direct transfer of hydrogen from reductant to oxidant.[21]

The detailed mechanism for the model reaction is not yet known, but the ready reduction by 1-benzyl-

(19) P. Talalay, F. A. Loewus and B. Vennesland, THIS JOURNAL, **76**, June (1954).

(20) N. O. Kaplan, S. P. Colowick and E. Neufield, *J. Biol. Chem.*, **195**, 107 (1952).

(21) A. San Pietro, in "The Mechanism of Enzyme Action," McElroy and Glass, The Johns Hopkins Press, Baltimore, Md., 1954, p. 382.

1,4-dihydronicotinamide of α,α-diphenyl-β-picryl hydrazyl suggests that a free radical mechanism may obtain. This impression is strengthened by the ready reduction of quinone and of malachite green, both of which may form free radicals.[22] The reduction of pyruvic acid by 2,6-dimethyl-3,5-dicarboethoxydihydrolutidine was scarcely conducted under "physiological conditions"; further the nitrogen atom of the dihydropyridine ring had not been alkylated. The reduction of a simple ketone by a 1-alkyl-1,4-dihydronicotinamide has not yet been achieved.

Appendix

In the reduction of malachite green by deuterated 1-benzyl-1,4-dihydronicotinamide, the reducing agent was present only in slight excess over the dye. Since k_H/k_D is significantly greater than unity, the concentration of deuterium in the reducing agent changes as the reaction proceeds, and the calculation of the k_H/k_D ratio from the deuterium content of the product is not obvious. However, the evaluation can be made as follows: Assume the reducing agent consists of a mixture of dihydro compound (XH_2) and monodeutero compound (XHD), with the latter at concentration A. This mixture reacts with malachite green, present at a concentration B.

$$XH_2 + \underset{(B)}{M} \xrightarrow{2k_H} MH + XH \qquad (5)$$

$$\underset{(A)}{XHD} + \underset{(B)}{M} \xrightarrow{k_H} MH + \underset{(K)}{XD} \qquad (6)$$

$$\xrightarrow[k_D]{} \underset{(J)}{MD} + XH \qquad (7)$$

(22) L. Michaelis, *Chem. Revs.*, **16**, 243 (1935); J. B. Conant and N. M. Bigelow, THIS JOURNAL, **53**, 676 (1931); E. Weitz, L. Müller and K. Dinges, *Ber.*, **85**, 878 (1952).

Here MH is leucomalachite green, MD is deuterated leuco malachite green at a concentration (J), XH is I (R = $CH_2C_6H_5$) and XD is deutero I (R = $CH_2C_6H_5$), the latter at a concentration K. Now

$$d(K)/dt = k_H(A)(B) \text{ and } d(J)/dt = k_D(A)(B) \qquad (8)$$

Therefore, regardless of the stage of the reaction, or of the k_H/k_D ratio

$$d(K)/d(J) = k_H/k_D, \text{ and } (K)/(J) = k_H/k_D \qquad (9)$$

Line 4, Table II, corresponds to an experiment with isotopically pure XHD, and therefore the data give directly (J) and (K) for completion of the reaction; k_H/k_D can be calculated from eq. 9. With isotopically impure material (line 1) (K) is not known accurately; it can, however, be estimated at the end of the reaction by assuming the stoichiometry of eq. 2. Then

$$(K)_{final} = (A)_{initial} - (J)_{final} \qquad (10)$$

Combining equations 9 and 10

$$k_H/k_D + 1 = (A)_{initial}/(J)_{final} \qquad (11)$$

A parallel development to that for equation 11 yields an equation suitable for calculating k_H/k_D when a mixture of mono and dideutero compounds is used as reducing agent (as in line 5, Table II). From this equation, from equations 9 and 11, and from the data of Table II, the average value of 4.5 $\pm$ 0.5 for k_H/k_D was found.

Acknowledgment.—The authors wish to thank Dr. F. A. Loewus for his generous assistance with the deuterium analyses here reported. The funds for the purchase of the mass-spectrometer used in this research were supplied by the Atomic Energy Commission under contract No. At(11-1)-92. One of us (D. M.) gratefully acknowledges support of a N. S. F. Fellowship.

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[CONTRIBUTION FROM THE CHEMISTRY DIVISION, RESEARCH DEPARTMENT, U. S. NAVAL ORDNANCE TEST STATION]

Kinetics of the Isomerization of Substituted 5-Aminotetrazoles

BY RONALD A. HENRY, WILLIAM G. FINNEGAN AND EUGENE LIEBER

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The kinetics of the equilibrium reaction shown in Table I, where R is 4-ClC_6H_4, C_6H_5, 4-$CH_3C_6H_4$, 4-$CH_3OC_6H_4$, or C_2H_5, were studied in the range 116–137°. In the initial stages of the isomerization, the first-order rate law was obeyed. For the forward reaction, the rates (k_1) decreased along the indicated series, the energies of activation increased from about 32,500 to 37,000 cal. per mole and the heats of reaction varied from 4300 cal. per mole (evolved) to 1350 cal. per mole (absorbed). For the aryl substituted aminotetrazoles there was a good correlation between the logarithm of the rates of isomerization and Hammett's sigma values for groups. There was a reasonable agreement both in magnitude and sign between heats of reaction calculated from the temperature coefficients of the equilibrium constants, and the differences in the heats of combustion of isomeric pairs. The kinetics of the cyclizations of guanyl and nitroguanyl azides to the respective tetrazoles were also investigated in a preliminary manner. When 1-phenyl-5-aminotetrazole was heated in aqueous alkali, a rapid, hydrolytic decomposition occurred to yield aniline, ammonia, carbonate and azide ion.

1-Substituted-5-aminotetrazoles and 5-substituted aminotetrazoles are thermally unstable and can be isomerized without appreciable decomposition in ethylene glycol or undisturbed melts at 180–200° to equilibrium mixtures of both isomers.[1,2] The present work is concerned with the kinetics of this isomerization. Since one of the steps in the proposed mechanism involves the cyclization of a guanyl azide, this reaction was also examined kinetically.

(1) W. G. Finnegan, R. A. Henry and Eugene Lieber, *J. Org. Chem.*, **18**, 779 (1953).

(2) R. A. Henry, W. G. Finnegan and Eugene Lieber, THIS JOURNAL, **76**, 88 (1954).

Experimental

Materials.—The 1-substituted 5-aminotetrazoles were prepared by the diazotization of substituted aminoguanidines and cyclization of the resulting guanyl azides in aqueous basic solution. The 5-arylaminotetrazoles were made by isomerizing 1-aryl-5-aminotetrazoles under non-equilib-