on whether tritium or deuterium was used as the labeling device.

Thus it may be noted from the last column of Table I that the deuterium and tritium were not equally diluted in the biological reactions but that relatively more deuterium was lost than tritium by the factors given in the table. Although certain interpretations of the results can be made, the mechanisms of the reactions involved are not understood. It is therefore impossible to account quantitatively for the results in Table I. However, if it were assumed that the three isotopes were indistinguishable completely in these reactions, then the results in the last column would be unity. Since this is not the case, we may conclude that selection is involved. It is planned to carry out similar experiments with methanol in which the deuterium-containing group is CH_2D, similar to the tritium-containing group CH_2T.

There are at least two important aspects of the problem of selection. On the one hand, there is the case in which the different isotopes of hydrogen are actually attached to the same carbon, and selection may occur in the cleavage of the carbon–hydrogen bond. On the other hand, there is the case in which one molecule contains deuterium or tritium and the other only protium. A particularly interesting case would be the one in which all the hydrogen in one molecule is replaced by deuterium and in the other is entirely protium. There may be selection *metabolically* between these isotopically different *molecules*. In experiments in which a change in isotopic ratio occurs, as in the case of methanol, selection of both these types must be considered. It should, however, be pointed out that when no change in ratio is obtained, as in the earlier experiments in which the doubly labeled methyl group of methionine served as the source of the methyl group of choline, one may conclude that the cleavage of the carbon–hydrogen bond followed by reduction is not a significant pathway and that no appreciable selection between the molecular species $-S-C^{14}H_3$ and $-S-C^{12}D_3$ has occurred, unless the possibility obtains of fortuitous compensation.

It is of interest in connection with the work reported herein that Thorn[8] found that tetradeuteriosuccinic acid and α,α'-dideuteriosuccinic acid were oxidized by the succinic acid oxidase system at only 40 and 70%, respectively, of the rate at which ordinary succinic acid was oxidized.

NEW YORK, N. Y.

The Reaction of Ethylamine-1-C^{14} with Nitrous Acid[1,2]

BY JOHN D. ROBERTS AND JOEL A. YANCEY

Ethylamine-1-C^{14} on treatment with perchloric acid and sodium nitrite in aqueous solution gave ethylene and a 38% yield of C^{14}-labeled ethanol which was shown by degradation and C^{14}-analysis to contain 1.5% of the rearrangement product, ethanol-2-C^{14}. The rearrangement product was demonstrated not to be formed in the degradation procedure or by hydration of the ethylene produced in the amine–nitrous acid reaction. It is concluded that the ethyl cation is not converted to ethyleneprotonium ion (II) at a rate which is comparable to that of its reaction with water. The reaction of ethylamine with perchloric acid and sodium nitrite in 99.8% deuterium oxide gave ethanol which contained only 1.1 atom % of deuterium attached to carbon. This result indicates that less than 10% of the ethanol could have been formed *via* diazoethane as an intermediate.

Recent interest in the structures of carbonium ions[3] has led to speculation as to whether the ethyl cation is most appropriately formulated as a simple solvated electron-deficient entity (I), a "non-classical" bridged ethyleneprotonium ion (II) or possibly as an equilibrium mixture of the two forms.[3a,4]

$$\overset{\oplus}{CH_3}-CH_2 \qquad \overset{\overset{H}{\diagup\ \overset{\oplus}{\ }\ \diagdown}}{CH_2 \!=\!\!=\! CH_2}$$
$$\text{I} \qquad\qquad \text{II}$$
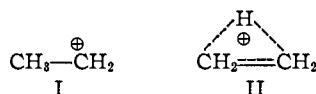
(1) Supported in part by the program of research of the Office of Naval Research and the U. S. Atomic Energy Commission.

(2) Presented at the Symposium on Reaction Mechanisms at the 75th Anniversary Meeting of the American Chemical Society, September 7, 1951.

(3) (a) J. D. Roberts, R. E. McMahon, W. Bennett and E. W. Holroyd, Jr.. THIS JOURNAL, **74**, 4283 (1952); (b) *cf.* S. Winstein and co-workers, *ibid.*, **74**, 1113, 1120, 1127. 1133, 1140, 1147, 1154 (1952), for other references.

(4) M. J. S. Dewar, "The Electronic Theory of Organic Chemistry," The Oxford University Press, London, 1949, pp. 211-213. See D. J. Cram, THIS JOURNAL. **74**, 2137 (1952), for an exceptionally thorough discussion of hydrogen-bridged cations and elimination reactions in the 3-phenyl-2-butanol system.

Isotopic tracer techniques for use in problems of this type have been developed[3a,5] and the only important difficulty was a method for irreversible generation of the desired cation in as "free" a state as possible. The reaction of ethylamine with nitrous acid was chosen for this purpose since the corresponding reactions of a number of primary alkylamines such as *n*-propylamine,[6] *n*-butylamine,[7] isobutylamine,[8] neopentylamine[9] and cyclopropylcarbinylamine[5b,10] lead to rearrangement products which are characteristic of carbonium ion processes.

With ethylamine-1-C^{14} and nitrous acid, decom-

(5) (a) J. D. Roberts, R. E. McMahon and J. S. Hine, THIS JOURNAL. **72**, 4237 (1950); (b) J. D. Roberts and R. H. Mazur. *ibid.*, **73**, 3542 (1951); (c) J. D. Roberts and C. C. Lee, *ibid.*, **73**, 5009 (1951).

(6) A. Siersch, *Ann.*, **144**, 137 (1867).

(7) F. C. Whitmore and D. P. Langlois, THIS JOURNAL, **54**, 3441 (1932).

(8) E. Linnemann, *Ann.*, **162**, 12 (1872).
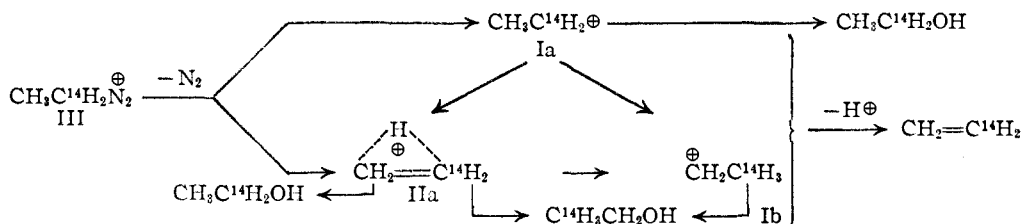
(9) M. Freund and F. Lenze, *Ber.*, **24**, 2150 (1891).

(10) J. D. Roberts and R. H. Mazur, THIS JOURNAL, **73**, 2509 (1951).

<center>TABLE I</center>
<center>RADIOACTIVITY ANALYSES</center>

Reaction sequence	Compound	Measured activity. counts/min.[a]	Activity/labeled C-atom[b]	% rearrangement[c]
$CH_3C^{14}H_2NH_2$ + HONO	$C_2H_5ODNB^d$	379.1 ± 3.4	1180 ± 11	
	CHI_3	51 ± 2.6	18 ± 1	1.5 ± 0.1
$CH_2C^{14}H_2$ + HONO	C_2H_4	......	(1180)[e]	
	$C_2H_5ODNB^{d,f}$	0.6 ± 1.5	1.8 ± 5	(0.0 ± 0.4)[g]
	$C_2H_5ODNB^{d,h}$	1.7 ± 1.2	5.3 ± 4	
	(carrier)	3.2 ± 3.8	10.0 ± 11	
		0.5 ± 1.2	1.6 ± 4	
$CH_3C^{14}H_2OH^i$	C_2H_5ODNB	696.9 ± 12	2165 ± 37	
	CHI_3	11.5 ± 2	4.0 ± 1	0.18 ± 0.04

[a] Activity (corrected for background) with standard deviations of "infinitely thick" barium carbonate samples having a cross-sectional area of 2.90 cm.[2] measured with a methane-filled windowless proportional counter, cf. J. D. Roberts, W. Bennett, E. W. Holroyd and C. H. Fugitt, Anal. Chem., 20, 904 (1948). [b] Activities in dis./min. corrected for self absorption and dilution by the carbon atoms at the unlabeled positions. [c] % rearrangement = activity of iodoform/activity of ethanol derivative × 100. [d] C_2H_5ODNB = ethyl 3,5-dinitrobenzoate. [e] Assumed to be the same as the activity of the ethanol. [f] Activity given for derivative prepared from ethanol isolated from the nitrous acid reaction mixture by carrier technique. [g] Calculated amount of ethylene hydration. [h] Activity of carrier ethanol before use in nitrous acid reaction. [i] Prepared by lithium aluminum hydride reduction of $CH_3C^{14}O_2H$.

position of the intermediate diazonium ion (III) could lead directly either to the classical ion (Ia) or the bridged cation (IIa).[3a] The isomeric ion (Ib) might be formed from Ia or IIa. Loss of a proton from any of these entities would yield ethylene-C^{14} while reaction with water would yield ethanol-1-C^{14} from Ia, ethanol-2-C^{14} from Ib and equal amounts of the two isomers from IIa (neglecting the isotope effect on the reactivity of C-1 and C-2). The tracer technique cannot, of course, distinguish between formation of IIa and just half as much Ib but can give information as to how readily IIa and Ib are obtained from Ia relative to its rate of reaction with water to yield ethanol.

or Ib. Although the formation of IIa is expected to proceed from Ia with a low activation energy,[4,11] the activation energy for the reaction of a primary cation with water should be essentially negligible. In any event, IIa is certainly not formed directly in the decomposition of III in the manner familiar in some analogous reactions.[3,5] The second possibility is that the extent of rearrangement is small because III reacts directly with water by the $SN2(N)$[12] type mechanism without yielding any free carbonium ions at all. While not excluded by the available evidence for III itself, this hypothesis is made rather unlikely by the results obtained with other primary alkylamines as mentioned earlier.

Treatment of ethylammonium-1-C^{14} perchlorate with sodium nitrite in aqueous solution gave ethylene and a 38% yield of C^{14}-labeled ethanol. Degradation of the ethanol with sodium hypoiodite to iodoform (C-2 of the ethanol) and radioactivity analysis (cf. Table I) indicated the presence of 1.5 ± 0.1% of ethanol-2-C^{14} in the reaction product. The amount of rearrangement in the reaction was small but nonetheless almost certainly real since degradation of authentic ethanol-1-C^{14} prepared by reduction of acetic-1-C^{14} acid gave iodoform containing but 0.18 ± 0.04% of the activity of the ethanol. The observed rearrangement was not apparently due to hydration of the ethylene formed in the decomposition of the diazonium salt since ten passes of a sample of C^{14}-labeled ethylene through a simulated reaction mixture gave no detectable amount of C^{14}-labeled ethanol.

There are a number of ways in which the results may be explained. First, and to us most likely, is the possibility that the ethyl cation Ia when formed reacts with the solvent to give ethylene or ethanol considerably more rapidly than it rearranges to IIa

A third formulation of the reaction which would not lead to extensive rearrangement is conversion of III by loss of a proton to diazoethane which conceivably could go directly to ethanol by reaction with acidulated water in a manner analogous to certain diphenyldiazomethane reactions.[13] This route is substantially eliminated by the finding that ethylamine with nitrous acid in 99.8% deuterium oxide solution yields ethanol containing only 1.1 atom % of deuterium bound to carbon. If all of the ethanol were formed by way of diazoethane, the product would be expected to contain about 12.5 atom % of deuterium and therefore less than 10% of the reaction could have proceeded by this path.

Acknowledgment.—We are indebted to Mrs. C. M. Regan for the C^{14}-analyses and to Mr. D. B. Kellom for the deuterium analysis.

(11) H. Eyring, H. M. Hulburt and R. A. Harman, Ind. Eng. Chem., 35, 511 (1943).

(12) S. Winstein, E. Grunwald and H. W. Jones, THIS JOURNAL, 73, 2700 (1951).

(13) J. D. Roberts and W. Watanabe, ibid., 72, 4869 (1950); J. D. Roberts, W. Watanabe and R. E. McMahon, ibid., 73, 760, 2521 (1951).

Experimental

Reaction of Ethylamine-1-C¹⁴ with Nitrous Acid.—An aqueous solution of ethylamine-1-C¹⁴ was prepared from sodium cyanide-C¹⁴ (100 μcuries of C¹⁴) by the procedure of Kilmer and du Vigneaud[14] on a 0.23-mole scale. About 40% of the reduction mixture was acidified with hydrochloric acid, diluted with 6.1 g. of ethylamine hydrochloride and evaporated almost to dryness under reduced pressure. The residue was treated with excess 20% sodium hydroxide solution and steam distilled until all of the ethylamine was removed. The steam distillate was titrated with dilute perchloric acid (0.112 eq., 68%) to pH 4. The solution was cooled to 3° and 23 g. (0.34 mole) of sodium nitrite in 50 ml. added rapidly. After one-half hour, the mixture was heated at 50° for an hour and then distilled slowly for 2.5 hours. The ethylene which was evolved during the reaction was absorbed in a gas washing bottle equipped with a fritted disk and containing liquid bromine at 10°. During the distillation, the last of the ethylene was swept out of the system into the trap with a stream of nitrogen. The recovery of ethylene as the dibromide was 1.1 g. after purification by distillation.

The aqueous distillate was neutralized and steam distilled. The distillate was saturated with potassium carbonate and continuously extracted with ethanol-free diethyl ether for three days. The extract was dried over magnesium sulfate and fractionated. The yield of ethanol, b.p. 77–78°, was 1.94 g. (38%). Part of the material was converted to the 3,5-dinitrobenzoate, m.p. 92.5–92.8°, for C¹⁴-assay.

The labeled ethanol was degraded as follows. A solution of 0.5 g. of ethanol-C¹⁴ in 30 ml. of water was treated simultaneously with 10% sodium hydroxide and iodine-potassium iodide solutions (100 g. of potassium iodide, 50 g. of iodine in 400 ml. of water) at 50° until a persistent iodine color was obtained. The mixture was poured into ice-water, the iodoform collected and crystallized twice from ethanol-water, m.p. 118° (dec.).

A check degradation was performed on ethanol-1-C¹⁴ obtained by lithium aluminum hydride reduction of acetic-1-C¹⁴ acid prepared as described earlier.[15]

The radioactivity analyses are given in Table I.

Action of Nitrous Acid on Ethylene-C¹⁴.—The ethylene dibromide obtained above was converted back to ethylene by the action of zinc powder in boiling ethanol then mixed with nitrogen and passed ten times through a fritted disk immersed in a solution at 60° containing 1.2 g. (0.017 mole) of sodium nitrite, 11.2 ml. of 1.57 N perchloric acid (0.018 mole) and 1.23 g. of ordinary ethanol over a period of an hour. The ethanol in the reaction mixture was isolated as described above and converted to the 3,5-dinitrobenzoate for C¹⁴-assay. For comparison, the ethanol used as a carrier was also assayed (Table I). The ethylene used amounted to 0.0049 mole (activity of 1180 dis./min./labeled C-atom) and if it were all hydrated the recovered ethanol would be expected to have an activity of 180 dis./min./labeled C-atom. Since the recovered ethanol did not have an activity significantly different from the carrier ethanol it is unlikely that any hydration occurred under the reaction conditions.

Reaction of Ethylamine with Nitrous Acid in Deuterium Oxide.—Ethylamine hydrochloride (9.3 g., 0.11 mole) was dissolved in 120 ml. of water and treated with 30 ml. of 20% sodium hydroxide solution. The liberated ethylamine was steam distilled, the distillate neutralized with perchloric acid and evaporated to dryness under reduced pressure. After one hour at room temperature at 1 mm., 2 ml. of 99.8% deuterium oxide[16] was added and the mixture allowed to stand for 0.5 hour. The water was removed under reduced pressure as before and the residue dissolved in 40 ml. of 99.8% deuterium oxide. Sodium nitrite (23 g., 0.34 mole) was added and the balance of the reaction and isolation of the products were carried out as described above. The yield of ethanol was 1.2 g. (45%). The product was converted to the 3,5-dinitrobenzoate for deuterium analysis which was carried out by combustion and assay of the resulting water by the "falling-drop" procedure. The ester contained 0.70 atom % deuterium which after correction for the hydrogens of benzene ring gave 1.1 atom % deuterium for the hydrogens bound to carbon in the ethanol.

(14) G. W. Kilmer and V. du Vigneaud. J. Biol. Chem., 154, 247 (1944).

(15) J. D. Roberts, D. R. Smith and C. C. Lee. THIS JOURNAL, 73, 618 (1951).

(16) Obtained on allocation by the U. S. Atomic Energy Commission.

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[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Malonyl-α-aminopyridine. II. Hydrolysis of its Diazonium Coupling Products and Related Compounds[1]

BY H. R. SNYDER AND MICHAEL M. ROBISON[2]

Diazonium coupling products (II) derived from malonylaminopyridine decompose in the presence of dilute aqueous base, even at room temperature, in an unexpected fashion. The primary products are carbonic and hydrocyanic acids, α-pyridyl isocyanate and aromatic amines. The acids mentioned are produced as salts, at least in part; the pyridyl isocyanate undergoes further hydrolysis to α-aminopyridine, which reacts with the isocyanate to give the dipyridylurea; the aromatic amines formed also react with the pyridyl isocyanate to produce unsymmetrical ureas. Certain other compounds that are closely related to the coupling products decompose in the same fashion. The limitations of the reaction are explored and mechanisms are discussed.

In attempts to effect the purification of the product (IIa) obtained[1] by the Japp–Klingemann reaction of benzenediazonium chloride with malonylaminopyridine (I), water was employed as a recrystallization solvent. A white crystalline solid, rather than the colored coupling product, was obtained, indicating that hydrolysis had occurred. The analysis of the hydrolysis product corresponded to the empirical formula $C_{12}H_{11}N_3O$. By analogy to the path[1] taken in the reaction of al-

cohols with molecules of type II, i.e., the cleavage of the central amide linkage to yield esters of type III (vide infra), it seemed not unlikely that a parallel hydrolysis might have taken place, with subsequent decarboxylation of the acid to yield the phenylhydrazone of N-(α-pyridyl)-glyoxylamide (IV). This compound, however, is of the empirical composition $C_{13}H_{12}N_4O$, which differs from that of the actual hydrolysis product by the elements of hydrogen cyanide. A test on the gases evolved from the hydrolysis mixture showed that hydrogen cyanide was formed, and an independent synthesis proved that the white product was N-phenyl-N'-

(1) For preceding paper in this series see H. R. Snyder and Michael M. Robison, THIS JOURNAL, 74, 4910 (1952).

(2) Allied Chemical and Dye Corporation Fellow, 1951–1952.