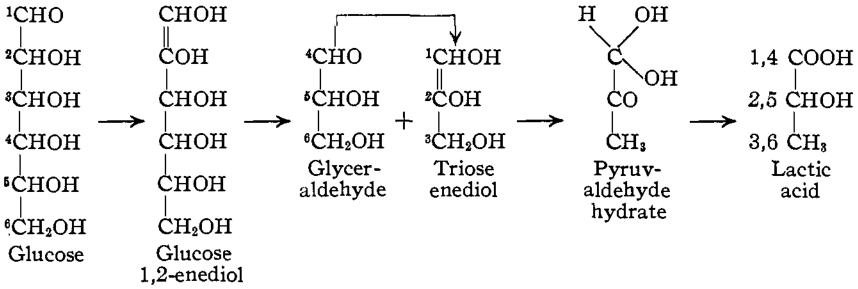
[CONTRIBUTION FROM BROOKHAVEN NATIONAL LABORATORY UNDER THE AUSPICES OF THE ATOMIC ENERGY COMMISSION]

On the Mechanism of the Chemical Formation of Lactic Acid from Glucose Studied with C^{14} Labeled Glucose

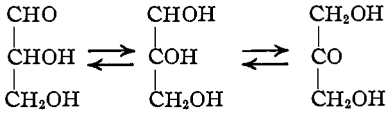
BY MARTIN GIBBS

The conversion of sugar in alkaline solution to lactic acid was first studied systematically by Nef[1,2] and more recently by Evans and coworkers.[3] To explain the presence of the compounds he isolated during the action of alkali on sugar, Nef proposed that sugars first undergo enediol formation. Evans accepted this proposal and studied the intermediates between the enediols and lactic acid.

Recently Evans[3] reviewed his work concerned with the intermediates formed during the action of alkali on carbohydrates. The conversion of glucose into lactic acid is assumed to proceed according to the reaction



It was also assumed by Evans[4] that alkaline solutions of glyceraldehyde could undergo a Lobry de Bruyn and van Ekenstein reaction; so that glyceraldehyde could rearrange into dihydroxyacetone.



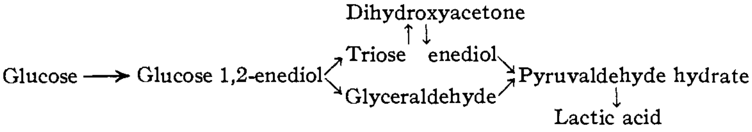
In the present investigation the mechanism of reaction proposed by Evans was tested with the aid of C^{14}-labeled glucose.

According to this proposed mechanism, the aldehyde carbon of glucose would become the carboxyl and methyl carbon of lactic acid due to a randomization of this carbon atom as it passed through symmetrical dihydroxyacetone to lactic acid. This assumption has now been substantiated by degrading the lactic acid formed by the action of alkali on 1-C^{14}-D-glucose. An equal amount of the label was located in the carboxyl carbon and in the beta carbon of the lactic acid. On comparing the radioactivity of the 1-C^{14} glucose with the amount of label found in the lactic acid, it can be calculated that about 42% of carbon atoms 1, 2 and 3 of glucose become lactic acid while 58% of carbon atoms 4, 5 and 6 are converted to lactic acid.

The reaction mechanism proposed by Evans should give the same distribution of label when 3,4-C^{14}-glucose is treated in the same manner. This did not occur. More activity (2.4 times) was found in the carboxyl carbon than in the beta position of the lactic acid.

Due to the low yield of lactic acid and due to the many unknown products which complicate the reaction, one can only speculate as to the meaning of this 2.4 ratio. If no randomization occurred between carbon atoms 4 and 6 and if each half of glucose was converted to lactic acid in equal amounts, the ratio would have been 3. Since more of the lower half of glucose was converted to lactic acid, the ratio would have been greater than 3 if carbon atom 4 did not randomize. To explain a ratio less than 3, it is probable that a partial randomization occurred between carbon atoms 4 and 6. Experiments are under way to prove this point by converting 1-C^{14}-gluconic acid and 3,4-C^{14}-gluconic acid to lactic acid.

On the basis of the data reported below, the reaction mechanism proposed by Evans should be modified to



Experimental

3,4-C^{14}-D-glucose.—This was prepared by isolating the glycogen from the liver of a fasted rat injected with 1 millicurie of $NaHC^{14}O_3$. After hydrolysis of the glycogen with sulfuric acid, the hydrolyzate was passed through ion exchange columns (Amberlite 100-H and Duolite A-4). Paper chromatography showed this to be free of radioactive impurities. Glucose prepared in this way has been shown[5] to be labeled only in the two central carbon atoms.

1-C^{14}-D-glucose.—This compound was kindly supplied by Dr. J. C. Sowden. It was prepared by the condensation of C^{14}-nitromethane with arabinose.[6] Paper chromatography indicated that this preparation was free of radioactive impurities.

The lactic acid was synthesized according to the method of Plunkett and Evans.[7] About 100 milligrams of glucose

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[1] Nef, *Ann.*, **357**, 301 (1907).

[2] Nef, *ibid.*, **376**, 1 (1910).

[3] Evans, *Chem. Rev.*, **31**, 537 (1942).

[4] Evans and Hass, THIS JOURNAL, **48**, 2703 (1926).

[5] Topper and Hastings, *J. Biol. Chem.*, **179**, 1255 (1949).

[6] Sowden, *Science*, **109**, 229 (1949).

[7] Plunkett and Evans, THIS JOURNAL, **60**, 2847 (1938).

was placed in a small vial and covered with 2 ml. of 3 *N* KOH. The vial was corked after being flushed with nitrogen gas. The vial and contents were placed in a water-bath at 50 ± 1° for forty-eight hours. After this period of time, the solution was made acid to phenol red and extracted with ether for thirty-six hours. About 5 ml. of water was added and the ether removed on a steam-bath. After the lactic acid was steam distilled to remove volatile impurities, it was degraded by the method of Wood, Lifson and Lorber.[8] Starting with 100 mg. of glucose, about 45 mg. of lactic acid was obtained. The lactic acid was determined by the method of Friedemann.[9] The C^{14} values are expressed in millimicrocuries (1×10^{-3} microcuries) per milligram of carbon.

TABLE I

DEGRADATION OF C^{14} LACTIC ACID FORMED DURING THE ACTION OF 3 *N* KOH ON GLUCOSE

Carbohydrate	Carbon Atoms of Lactic Acid COOH	CHOH	CH_3
3,4-C^{14}-D-glucose	8.73	0.69	3.64
1-C^{14}-D-glucose	3.60	0.22	3.60

Acknowledgment.—The author wishes to acknowledge the capable assistance of Mrs. Frances A. Bennett in these experiments.

Summary

1. The action of 3 *N* KOH on 1-C^{14}-D-glucose gave an equal distribution of label in the carboxyl carbon and beta carbon of lactic acid. This is in agreement with the theory of Evans.

2. During the action of 3 *N* KOH on glucose, about 42% of the lactic acid was derived from carbon atoms 1, 2 and 3 of glucose while the lower half of glucose yielded 58% of the lactic acid.

3. The action of 3 *N* KOH on 3,4-C^{14}-glucose yielded more activity (2.4:1) in the carboxyl carbon than in the beta carbon of lactic acid. This is not in agreement with the theory of Evans. The significance of this ratio was discussed.

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[8] Wood, Lifson and Lorber, *J. Biol. Chem.*, **159**, 475 (1945).

[9] Friedemann, *ibid.*, **76**, 75 (1928).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, QUEEN'S UNIVERSITY]

The Hydrolysis of 1-Nitro-4-methyl-2-nitramino-2-imidazoline

BY A. F. MCKAY[1] AND S. J. VIRON

Previously, it was reported[2] that although 1-nitro-2-nitramino-2-imidazoline had good power as an explosive, its sensitivity and ease of hydrolysis were detrimental to its use. It was found later that 1-nitro-2-nitramino-4-methyl-2-imidazoline[3] (I) also hydrolyzes readily. Since a knowledge of the course of hydrolysis of the latter compound might prove of importance as an aid in the syntheses of compounds of this series with greater stability, its behavior on hydrolysis was investigated.

When 1-nitro-2-nitramino-4-methyl-2-imidazoline (I) is refluxed with water three different compounds are obtained. One of the products, 1-nitro-4-methyl-2-imidazolidone (II) was identified by nitration to the known compound 1,3-dinitro-4-methyl-2-imidazolidone[4] (V). The second compound 1-(1-methyl-2-nitraminoethyl)-3-nitrourea (III) was converted to 1-(1-methyl-2-nitraminoethyl)-3-phenylurea by the method of T. L. Davis and K. Blanchard.[5] This same compound was obtained by treating 1-nitro-4-methyl-2-imidazolidone with aniline. If 1-nitro-2-nitramino-4-methyl-2-imidazoline (I) is completely hydrolyzed or 1-nitro-4-methyl-2-imidazolidone (II) and 1-(1-methyl-2-nitraminoethyl)-3-nitrourea are hydrolyzed, the end-product is 2-amino-3-nitraminopropane (IV).

Attempts to convert 1-(1-methyl-2-nitraminoethyl)-3-nitrourea (III) into 1-nitro-4-methyl-2-imidazolidone (II) in aqueous solution were unsuccessful. Thus the hydrolysis of 1-nitro-2-nitramino-4-methyl-2-imidazoline follows two routes to the product 2-amino-3-nitraminopropane (IV) one through compound (II) and the other through compound (III).

Acknowledgment.—The authors wish to thank the Defence Research Board of Canada for full support of this work and a grant-in-aid to one of them (S. J. V.).

Experimental[6]

1-Nitro-2-nitramino-4-methyl-2-imidazoline.—1-Nitro-2-nitramino-4-methyl-2-imidazoline (m. p. 121.5–123.6°) was prepared in 63.3% yield by a previously described method.[4]

Hydrolysis of 1-Nitro-2-nitramino-4-methyl-2-imidazoline.—The hydrolysis of 1-nitro-2-nitramino-4-methyl-2-imidazoline (25 g., 0.132 mole) was accomplished by refluxing with 125 cc. of water, until all the solid had dissolved. After refluxing for a further period of fifteen minutes, the solution was cooled to room temperature and concentrated *in vacuo* to *ca.* 5 cc. A white solid (12.7 g.) separated which melted at 94.6–119.2° with decomposition. Three crystallizations from nitromethane (1 cc./g.) raised the melting point to 124.5–125° with decomposition. This product was identified as 1-(1-methyl-2-nitraminoethyl)-3-nitrourea.

Anal. Calcd. for $C_4H_9N_5O_5$: C, 23.2; H, 4.34; N, 33.8. Found: C, 23.2; H, 4.52; N, 33.3.

The filtrate from the first nitromethane crystallization was evaporated almost to dryness after which a second crop of crystals (m. p. 124.6–128.9°) was obtained, yield 2.3 g. (12.0%). This material was crystallized from ethyl

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[1] Defence Research Chemical Laboratories, Ottawa, Ontario.

[2] A. F. McKay and G. F Wright, THIS JOURNAL, **70**, 3990 (1948).

[3] A. F. McKay, R. H. Hall and G. F Wright, *ibid.*, in press.

[4] A. F. McKay and D. F. Manchester, *ibid.*, **71**, 1970 (1949).

[5] T. L. Davis and K. Blanchard, *ibid.*, **51**, 1790 (1929).

[6] All melting points are uncorrected.