Studies on the Chemical Decomposition of Simple Sugars. XVI. Pyruvic Acid Formation from D-Glucose-1-^{14}C*

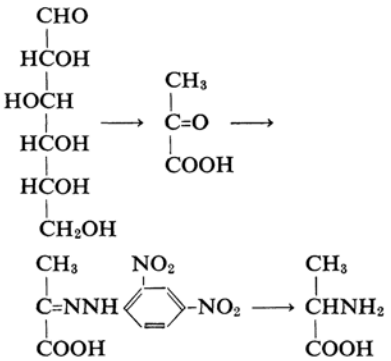
By Sinichi OTANI**

(Received February 20, 1965)

The fragmentation reaction of reducing sugars is well known in both acid and alkaline media,[1,2] but the mechanism of this reaction has not been satisfactorily elucidated. Especially, the formation of pyruvic acid has remained to be studied on account of its instability, its low yield, and the difficulty of isolating it. Nodzu et al.,[2,3] however, obtained pyruvic acid from a weakly alkaline and a weakly acid solution of glucose, and proposed a mechanism which included acetylformoin as a direct precursor of pyruvic acid. Recently, Hayami et al. effectively used the ^{14}C-labeled sugars[4,5] and the methylated sugars[6] for the investigation of the mechanism of the acetol formation, and presented another mechanism in which they inferred the formation of pyruvic acid, though they had no experimental evidence.

The present purpose was to obtain pyruvic acid by the decomposition of D-glucose-1-^{14}C in a weakly acid phosphate solution, and to determine the relative distribution of a label in pyruvic acid in order to elucidate the mechanism of the pyruvic acid formation.

The present author obtained a mixture of pyruvic acid 2, 4-dinitrophenylhydrazone and other kinds of acid hydrazone or osazone; he then reduced them to amino acids, while the pyruvic acid 2, 4-dinitrophenylhydrazone was reduced to alanine.[7] The alanine was separated[8] and subjected to the degradation, by which means the relative distribution of a label in pyruvic acid was determined.



Scheme 1.

The alanine was decomposed with ninhydrin in nitrogen gas after some modification of the method of Virtanen et al.[9] The methyl part and the center carbon of the alanine were converted into acetaldehyde, and the carboxylic acid part was converted into carbon dioxide,

* The present work was presented at the Symposium on the Mechanism of Organic Chemical Reaction of the Chemical Society of Japan, Nagoya, October, 1964.

** Present address: Department of Liberal Arts and Science, Kyoto University, Kyoto.

1) H. J. Dakin and H. W. Duddley, *J. Biol. Chem.*, **15**, 127 (1913); J. U. Nef, *Ann.*, **357**, 214 (1907); **376**, 1 (1910); **403**, 204 (1914); W. L. Evans, *J. Org. Chem.*, **1**, 1 (1937); R. Nodzu, This Bulletin, **10**, 122 (1935); R. Nodzu and K. Matsui, ibid., **10**, 467 (1935); R. Nodzu and R. Goto, ibid., **11**, 381 (1936); R. Goto, ibid., **15**, 103 (1940); R. Nodzu and R. Goto, ibid., **15**, 209 (1940); R. Goto, *J. Chem. Soc. Japan (Nippon Kwagaku Kwaisi)*, **64**, 999, 1054, 1183 (1943); L. Sattler and F. W. Zerban, *J. Am. Chem. Soc.*, **70**, 1975 (1948); M. Gibbs, ibid., **72**, 3964 (1950); E. Waldmann and V. Prey, *Monatsh.*, **82**, 856 (1951); **83**, 65 (1952); V. Prey, E. Waldmann, H. Berbalk and F. Sommer, ibid., **85**, 1186 (1954); V. Prey, E. Waldmann, H. Berbalk and F. Ludwig, ibid., **84**, 551 (1953); R. Weidenhagen, *Zucker*, **12**, 244 (1959).

2) R. Nodzu, K. Matsui, R. Goto and S. Kunichika, *Mem. Coll. Sci. Kyoto Imp. Univ.*, **20**, 197 (1937).

3) R. Nodzu, *J. Biochem. Soc. Japan (Nippon Seikagaku Kaishi)*, **18**, 237 (1944).

4) R. Goto, J. Hayami, K. Kudo and S. Otani, This Bulletin, **34**, 753 (1961).

5) J. Hayami, ibid., **34**, 924 (1961).

6) J. Hayami, ibid., **34**, 927 (1961).

7) G. H. N. Towers, J. F. Thompson and F. C. Steward, *J. Am. Chem. Soc.*, **76**, 2392 (1954).

8) C. H. W. Hirs, S. Moore and W. H. Stein, *J. Biol. Chem.*, **192**, 669 (1951).

9) A. I. Virtanen, T. Laine and T. Toivonen, *Z. physiol. Chem.*, **266**, 193 (1940).

which was then caught in a sodium hydroxide solution and counted in the form of barium carbonate. Acetaldehyde was caught as an adduct of sodium bisulfite and converted into iodoform and formic acid. From the combustion of the iodoform, the radioactivity of the methyl carbon of the alanine was determined.

By the method mentioned above, the radioactivity of both terminal carbons of the alanine was determined, but it was impossible to determine of the center carbon directly. Therefore, the radioactivity of the center carbon was indirectly deduced from the results of the two terminal carbons and from that of the three carbon atoms of the alanine.

Experimental

Radioactive Sugars and Assay Method. — D-Glucose-1-^{14}C was prepared from D-arabinose by a cyanohydrin synthesis.[10]

For an assay, all samples were converted into barium carbonate by the Van Slyke-Folch wet combustion method. Barium carbonate was collected on a filter paper and counted, mostly in an infinite thickness, with a 2π-gas flow counter. In the case of the combustion of iodoform, the barium carbonate obtained was counted in a medium thickness and corrected for an infinite thickness.

The Decomposition of D-Glucose-1-^{14}C.—A solution of 20 g. of the labeled D-glucose in 200 ml. of a concentrated potassium acid phosphate buffer solution (40%, pH 6.6) was heated and distilled. Water was added drop by drop in order to maintain a constant volume. About 100 ml. of the distillate was collected every 45 min., and the distillation was continued for 9 hr.

The Isolation of Crude Pyruvic Acid 2, 4-Dinitrophenylhydrazone. — The distillation residue was made up to one and a half in volume by the addition of 6 N hydrochloric acid; the precipitate formed was then removed by filtration after having been kept overnight. To the filtrate was added 2 l. of a saturated solution of 2, 4-dinitrophenylhydrazine in 2 N hydrochloric acid, and the precipitate formed was collected and dried in a vacuum. About ten grams of the precipitate were extracted with about 20 l. of isopropyl ether. The ether solution was then extracted with an equal volume of a 6% aqueous solution of sodium bicarbonate. After the extract had been slightly acidified with 6 N hydrochloric acid, it was again extracted with about an equal volume of isopropyl ether. The ether solution was evaporated under reduced pressure to give a brown residue (1.2 g.). It was shown by paper chromatography (solvent; *n*-butanol - 0.1 N aqueous ammonium hydroxide - methanol 4 : 1 : 1 v/v) that the residue contained pyruvic acid 2, 4-dinitrophenylhydrazone in a considerably high concentration.

The Reduction of the Crude Pyruvic Acid 2, 4-Dinitrophenylhydrazone to Alanine.—2, 4-Dinitrophenylhydrazone (600 mg.) was suspended in water (ca. 40 ml.) and hydrogenated with Adams' catalyst, platinum dioxide (147 mg.) under the atmospheric pressure of hydrogen gas.

The Isolation of the Crude Alanine.— After the removal of the catalyst by filtration, the filtrate and washings (25 ml.) were passed through a column (1.6×18 cm.) of Dowex 50W-X8 in the H form (200—400 mesh). Hydrochloric acid (1.5 N) was used as the eluting solvent. The effluent, which was positive for a ninhydrin test,[11] was then concentrated and passed through a column of Amberlite IR-400 (acetate form) in order to remove the chloride anions, using 0.5 N acetic acid as the eluting solvent. The effluent was then collected, concentrated under reduced pressure, and dried to a yellow powder.

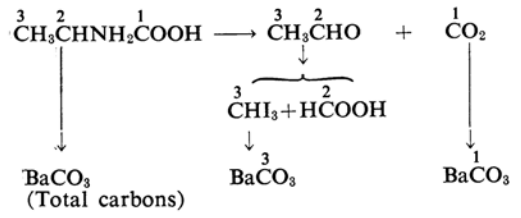
The Purification of Alanine.—This part was a modification of the method of Hirs, Moore and Stein.[8] An ammonium formate buffer solution (pH 3.7) was made by mixing 375 ml. of 2 N formic acid, 200 ml. of 1 N ammonium hydroxide, 400 ml. of ethanol and water to 1 l. Dowex 50W-X8 (200—400 mesh, 500 ml.) was placed in a column (3.5×55 cm.) with the aid of an ammonium formate buffer solution, which was used as an eluting solvent, and the crude alanine (ca. 50 mg.) was charged. The effluent was collected in about 10 ml. fractions with a flow-rate about 30 ml./hr. Aliquots (0.5 ml.) of the effluents were then analyzed by means of the ninhydrin test after the sublimation of ammonium formate. The fractions showing a positive reaction were collected and concentrated under reduced pressure. A yellow residue was obtained after the sublimation of ammonium formate. This residue was decolorized with active carbon and crystallized from a thick aqueous solution by adding anhydrous ethanol.

The Degradation of Alanine. — The procedure was carried out under a stream of nitrogen and under oxygen-free conditions. In a three-necked flask, 62.6 mg. of alanine, 168 g. of ammonium sulfate, and 11.5 g. of citric acid were dissolved in 150 ml. of water; the solution was then heated. After it had been boiled, 60 ml. of a 1% aqueous solution of ninhydrin was added drop by drop from a dropping funnel; the solution was then kept boiling for 90 min. The acetaldehyde formed was swept into 40 ml. of a 1% aqueous solution of sodium bisulfite passing through the Allihn condenser and caught in the form of an adduct. Carbon dioxide was introduced into 20 ml. of a 0.1 N sodium hydroxide solution, which was connected to the sodium bisulfite solution. The sodium bisulfite solution was treated with a 10% sodium hydroxide solution and a 10% iodine solution, and the iodoform formed was collected using a centrifugal separator, washed with water, and converted in a wet condition into barium carbonate by the Van Slyke-Folch wet combustion method. The carbon dioxide, caught in a sodium hydroxide solution, was treated with 360

10) H. S. Isbell, J. V. Karabinos, H. L. Frush, N. B. Holt, A. Schwebel and T. T. Galkowski, *J. Res. Natl. Bur. Std.*, **48**, 163 (1952).

11) E. W. Yemm and E. C. Cocking, *Analyst*, **80**, 209 (1955).

mg. of barium chloride; barium carbonate was thus obtained.



Scheme 2. Degradation process.

TABLE I. RADIOASSAY DATA

Sample	Carbon atom(s)	Radioactivity c. p. m./mol.
D-Glucose-1-^{14}C	Total carbons	562*2
Pyruvic acid*1	Total carbons	162*2
	CH_3	133*4
	C=O	*5
	COOH	14*6

*1 Degraded in the form of alanine.
*2 Probable error was less than 1%.
*3 Probable error was less than 4%.
*4 Probable error was less than 2%.
*5 Not determined.
*6 Probable error was less than 15%.

Results and Discussion

The results reported herein indicate that, in the case of D-glucose-1-^{14}C, the radioactivity is mostly found in the methyl carbon of the alanine. This means that the C-1 of the glucose was converted into the methyl carbon of the pyruvic acid.

In the case of the formation of acetol,[5-6] accompanied by pyruvic acid, the C-1 and C-6 of the original glucose were converted into the methyl carbon of the acetol, while the C-3 and C-4 of the original glucose were converted into the hydroxymethyl carbon of the acetol. In addition, the acetol-1-^{14}C, which was formed from D-glucose-3, 4^{14}-C_2, was found to preserve the molar radioactivity of the original glucose. This indicates that, in the formation of acetol, the C-1, 2, and 3 and the C-4, 5, and 6 of the original glucose behaved as units, and that no skeletal rearrangement in these C_3 units was involved. In the case of pentoses,[5] both terminal carbons were converted into the methyl carbon of the acetol. Hayami[6] has determined the mechanism of the formation of acetol on the basis of findings concerning labeled sugars and methylated sugars; he inferred that pyruvic acid should be formed by a similar mechanism. His inference consisted of two essential points, which he applied also to the case of the formation of acetol. One was β-hydroxy-carbonyl elimination, after the isomerization to 3-ketose or 4-ketose; the other was hydrolytic cleavage between the C-3 and C-4 of glucose. The present results support Hayami's inference.

From the common finding of these experiments with pyruvic acid and acetol, it may be deduced that the C-6 of glucose is converted into the methyl carbon of pyruvic acid, while the C-3 and C-4 are converted into the carbon of the carboxylic acid group of pyruvic acid.

From this point of view, comparing the molar radioactivity of the original D-glucose-1-^{14}C with that of the alanine found in the present study, the results show that the lower half of the glucose skeleton contributes more than the upper half to the formation of pyruvic acid.

Further experiments using other labeled sugars are, however, necessary to explain the mechanism of the formation of pyruvic acid.

The author wishes to express his thanks to Professor Ryozo Goto for his constant guidance and encouragement and to Dr. Jun'ichi Hayami for his kind suggestions throughout the course of this work. The author wishes also to thank Dr. Yo Miyagi for his assistance in synthesizing the labeled sugar.

Institute of Chemistry
Faculty of Science
Kyoto University
Sakyo-ku, Kyoto