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Studies on the Chemical Decomposition of Simple Sugars. XVII. Pyruvic Acid Formation from D-Glucose-6-^{14}C*

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The formation of pyruvic acid from D-glucose-6-^{14}C in a concentrated potassium acid phosphate buffer solution (40%, pH 6.6), was studied. It was found that the C-6 of the glucose was converted into the methyl carbon of pyruvic acid. In view of the formation of pyruvic acid from D-glucose-1-^{14}C, the C-1 and C-6 of the glucose were converted into the methyl carbon of pyruvic acid. This reaction mechanism was similar to that of the formation of acetol (J. Hayami, This Bulletin, **34,** 927 (1961)). The mechanism presented may be summarized as follows: 1) The formation of α-diketose by β-hydroxy-carbonyl elimination after isomerization to 3-ketose or 4-ketose. 2) The formation of acetylformoin by a second β-hydroxy-carbonyl elimination after isomerization to the β-diketose. 3) The hydrolytic cleavage of the acetylformoin.

The formation of pyruvic acid from carbohydrate has never been well studied because of its instability, the low yield, and the difficulty of isolation. Nodzu et al.[1] found pyruvic acid as the 2, 4-dinitrophenylhydrazone in the decomposition of D-glucose, and proposed[2] a mechanism of pyruvic acid formation in which acetylformoin was included as a direct precursor of pyruvic acid. Recently, Hayami et al.[3] effectively used ^{14}C-labeled sugars and methylated sugars for an investigation of the formation of acetol which was formed along with pyruvic acid, and presented a mechanism of the formation of pyruvic acid. In the preceding report,[4] the formation of pyruvic acid from D-glucose-1-^{14}C was examined. The degradation of the alanine which had been derived from pyruvic acid 2, 4-dinitrophenylhydrazine revealed that the C-1 of the D-glucose was converted into the methyl carbon of pyruvic acid.

In the present study, D-glucose-6-^{14}C was decomposed and the relative distribution of a label in the pyruvic acid was determined in order to obtain further evidence of the mechanism of pyruvic acid formation.

Experimental

The Assay Method of Radioactivity.—A sample was converted into barium carbonate by Van Slyke-Folch wet combustion. The barium carbonate was collected on filter paper and counted in an infinite thickness with a 2π-gas flow counter.

The Decomposition of D-Glucose-6-^{14}C.—D-Glucose-6-^{14}C (20 g.) in 200 ml. of a concentrated potassium acid phosphate buffer solution (40%, pH 6.6) was heated and distilled in a way described earlier.[4]

The Isolation of Crude Pyruvic Acid 2, 4-Dinitrophenylhydrazone, and the Reduction of Pyruvic Acid 2, 4-Dinitrophenylhydrazone to Alanine.—These procedures were also carried out in ways described earlier.[4]

The Purification of Alanine.—The crude alanine (16 mg.) was recrystallized from ethanol.

The Degradation of Alanine.—The experiment was carried out in a way described earlier,[4] using 80 mg. of active alanine.

Results and Discussion

In the case of D-glucose-6-^{14}C, radioactivity was mostly found in the methyl carbon of the alanine. This means that the C-6 of D-glucose was converted into the methyl carbon of pyruvic acid. Considering the findings regarding D-glucose-1-^{14}C in the preceding report,[4] it may be concluded

TABLE I. RADIOASSAY DATA OF PYRUVIC ACID*1

Carbon atom(s)	Radioactivity c.p.m./mol.
Total carbons	16*2
CH_3	14*3
C=O	—*4
COOH	1*5

*1 Degraded in the form of alanine.
*2 Probable error was less than 6%.
*3 Probable error was less than 13%.
*4 Not determined.
*5 Probable error was less than 50%.

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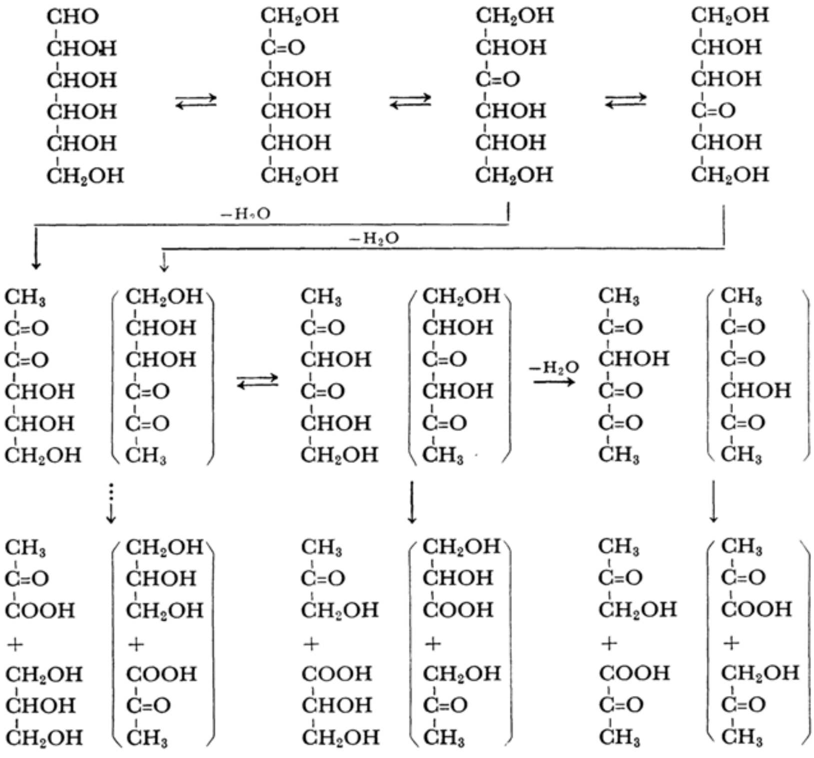
[1] R. Nodzu, K. Matsui, R. Goto and S. Kunichika, *Mem. Coll. Sci. Kyoto Imp. Univ.*, **20**, 197 (1937).

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

[3] R. Goto, J. Hayami, K. Kudo and S. Otani, This Bulletin, **34**, 753 (1961); J. Hayami, ibid., **34**, 924, 927 (1961).

[4] S. Otani, ibid., **38**, 1873 (1965).



Scheme 1

that the C-1 and C-6 of the glucose were converted into the methyl carbon of pyruvic acid.

In the case of acetol,3) along with the formation of pyruvic acid, the C-1 and C-6 of the original glucose were converted into the methyl carbon of the acetol, and 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. Therefore, in the formation of acetol, the C-1, 2, 3 and the C-4, 5, 6 portions of the original glucose behaved as units; no skeletal rearrangement in these C_3 units was involved. Considering the simultaneous formation of acetol and of pyruvic acid from glucose,1) it seems quite possible that pyruvic acid is formed by a mechanism similar to that of acetol. The mechanism of the pyruvic acid formation can be expressed as shown in Scheme 1.

3-Ketose or 4-ketose, formed by the enolization-ketonization route, is subjected to dehydration by β-hydroxycarbonyl elimination. This conclusion is based on the facts that the methyl carbon of pyruvic acid4) and of the acetol3) was found to be converted from the terminal carbon atoms, and that the acetol formation3) was depressed in the case of 3-*O*-methyl-D-glucose, whereas it was accelerated in the cases of 1-*O*-methyl-D-fructose and 6-*O*-methyl-D-glucose.

The first three steps are known as the Lobry de Bruyn - Alberda van Ekenstein transformation.5) Since Lobry de Bruyn et al.6) found the transformation between glucose, mannose and fructose in aqueous alkaline solutions in 1895, the transformation reactions of most reducing sugars have been studied, and the transformation has been recognized to proceed through 1, 2-enediol. Later, transformations by C-3- and C-4-epimerization were observed. For instance, Hough et al.7) observed the formation of D-psicose from D-glucose by the action of ammonia. Sowden et al.8) isolated (DL+D)-sorbose from the transformation products of D-glucose in the presence of a strong basic ion-exchange resin.* This means that enolization-ketonization proceeds

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5) J. C. Speck, Jr., "Advances in Carbohydrate Chem.," **13**, 63 (1958).

6) C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *Rec. trav. chim.*, **14**, 203 (1895), **15**, 92 (1896), **16**, 257, 262, 274, 282 (1897), **18**, 72 (1899).

7) L. Hough, J. K. N. Jones and E. L. Richards, *J. Chem. Soc.*, **1953**, 2005.

8) M. G. Blair and J. C. Sowden, *J. Am. Chem. Soc.*, **77**, 3323 (1955).

9) W. G. Berl and C. E. Feazel, ibid., **73**, 2054 (1951).

* It was thought that (DL+D)-sorbose could be formed by the aldol condensation of the glyceraldehyde and dihydroxyacetone formed by the fragmentation. In fact, glyceraldehyde gave DL-sorbose and DL-fructose in the presence of alkali.9) Sowden et al.10) isolated (DL+D)-sorbose-1-^{14}C from the reaction mixture of D-glucose-1-^{14}C, and from the relative distribution of a label of the enantiomorphs they obtained data showing that the racemic ketose was formed almost exclusively by the enolization-ketonization mechanism, while the fragment-recombination played only a minor role in the isomerization.

through all of the secondary carbon atoms of the chain. Therefore, 1, 2-, 2, 3-, 3, 4-, 4, 5-enediols are possible intermediates in the alkaline isomerization of hexose.

The transformation reaction is usually studied in the presence of bases and placed in the category of alkaline isomerizations, and a sugar is considered to proceed in the form of an anion. However, some instances were found in the presence of acid. This is understandable in view of the facts that the enolization is subjected to a general acid and base catalysis and that the transformation proceeds via enolization.[5] For instance, Ost[11] and Ohno et al.[12] reported the isomerization of D-glucose into D-fructose in dilute sulfuric acid at room temperature. Petuely,[13] observing the isomerization between D-glucose and D-fructose in an acid-catalyzed reaction, considered the protonated sugars to be reaction intermediates. In the formation of the furfural derivatives in the presence of acids, 1, 2-enediol is generally thought to be formed in the first step[14]; in this case, protonation has to be thought of as an important reaction process. Spoehr et al.[15] observed the transformation between D-glucose, D-mannose and D-fructose in a disodium hydrogen phosphate buffer solution (pH 6.69). Ashmarin et al.,[16] using acetate, succinate and formate buffers at pH 4.2, found D-glucose to be transformed to D-fructose. Englis et al.[17] heated D-glucose in a potassium dihydrogen phosphate solution (pH 6.4—6.6) in an autoclave and observed the formation of ketoses the main part of which was D-fructose. Wolfrom et al.[18] obtained (DL+D)-sorbose and (DL+D)-allose from D-fructose in an aconitic acid - potassium hydroxide system (initial pH 8.00; after 24 hr., pH 6.15). In addition to these results, the author observed that D-fructose was formed from D-glucose by heating it in the same medium for one or two hours under the same conditions as those in which pyruvic acid and acetol were formed.[19] From these results, enolization-ketonization can be considered to occur in the present experimental conditions. The mechanism of enolization-ketonization in these nearly neutral solutions has been little discussed. However, Ashmarin et al.[16] proposed that anions of the weak acids were acting as bases, according to Brønsted's conception.

Lobry de Bruyn et al.[6] obtained an unfermentable reducing substance, which they called glutose, as a 3-ketose in an alkali-isomerization mixture of glucose or fructose. Sattler et al.,[20] however, observed that the glutose from D-fructose was a mixture of fructose anhydride and difructose dianhydride. Recently, Sera[21] reported that the so-called "glutosazone" was an impure glucosazone. Thus, 3-ketose has never been proved directly to be formed in an isomerization reaction. When Schaffer synthesized[22] D-manno-3-heptulose and isomerized[23] it in a diluted calcium hydroxide solution at room temperature for a week, he could not detect it in the reaction solution. Stoodley[24] and Sera[25] synthesized a 3-ketopentulose, 3-xylulose. They observed that 3-xylulose was very unstable and that it was easily converted into *erythro*-2-pentulose and *threo*-2-pentulose, even without alkali.* These facts showed that 3-ketose was reactive under the conditions of the Lobry de Bruyn-Alberda van Ekenstein transformation and that the direct detection of 3-ketose in the transformation reaction was impossible.

The next reaction, dehydration by β-hydroxy-carbonyl elimination, is generally observed in carbohydrate chemistry. The formation of saccharinic acids[27] in the presence of bases is explained by a benzylic acid-type rearrangement after the dehydration. The formation of furfural derivatives in the presence of acid is also explained by the dehydration.[14,28] Under the present experimental conditions, methylglyoxal was formed from glyceraldehyde by the same type of dehydration.[1]

The α-diketose thus formed isomerizes to the β-diketose without being subjected to the benzylic acid-type rearrangement, and acetylformoin is formed by the second dehydration by β-hydroxy-carbonyl elimination. About the isomerization of α-diketone to β-diketone, Hesse et al.[29] recognized that pentane-2-ol-3, 4-dione isomerized

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10) J. C. Sowden and R. R. Thompson, ibid., **80**, 1435 (1958).

11) H. Ost, *Angew. Chem.*, **18**, 1170 (1905).

12) Y. Ohno and K. Ward, Jr., *J. Org. Chem.*, **26**, 3928 (1961).

13) F. Tetuely, *Monatsh.*, **84**, 298 (1953).

14) F. H. Newth, "Advances in Carbohydrate Chem.," **6**, 83 (1951).

15) H. A. Spoehr and H. H. Strain, *J. Biol. Chem.*, **85**, 365 (1929).

16) P. A. Ashmarin and Y. S. Belova, *Archi. sci. biol.* (*U. S. S. R.*), **42**, 61 (1936); P. A. Ashmarin and A. D. Braun, ibid., **42**, 53 (1936).

17) O. T. Englis and D. J. Hanahan, *J. Am. Chem. Soc.*, **67**, 51 (1945).

18) M. L. Wolfrom and J. N. Schumacher, ibid., **77**, 3318 (1955).

19) S. Otani, unpublished result.

20) L. Sattler and F. W. Zerban, *Ind. Eng. Chem.*, **37**, 1133 (1945).

21) A. Sera, This Bulletin, **35**, 2031 (1962).

22) R. Schaffer, *J. Am. Chem. Soc.*, **81**, 2838 (1959).

23) R. Schaffer, *J. Org. Chem.*, **29**, 1473 (1964).

24) R. J. Stoodley, *Can. J. Chem.*, **39**, 2593 (1961).

25) A. Sera, This Bulletin, **35**, 2033 (1962).

* Yuen et al.[26] synthesized a 3-ketohexose, DL-*xylo*-3-hexulose, but its transformation reaction has not yet been studied.

26) G. U. Yuen and J. M. Sugihara, *J. Org. Chem.*, **26**, 1598 (1961).

27) J. C. Sowden, "Advances in Carbohydrate Chem.," **12**, 36 (1957); H. S. Isbell, *J. Res. Natl. Bur. Stand.*, **32**, 45 (1944); J. C. Sowden and E. K. Pohlen, *J. Am. Chem. Soc.*, **80**, 242 (1958); J. C. Sowden, M. G. Blair and D. J. Kuenne, ibid., **79**, 6450 (1957); G. Machell and G. M. Richards, *J. Chem. Soc.*, **1960**, 1924, 1932, 1938.

28) J. Kenner and G. N. Richards, ibid., 2921 (1956); W. A. Bonner and M. R. Roth, *J. Am. Chem. Soc.*, **81**, 5454 (1959); T. G. Bonner, E. J. Bourne and M. Ruszkiewicz, *J. Chem. Soc.*, **1960**, 787; M. L. Mednick, *J. Org. Chem.*, **27**, 398 (1962); E. F. L. J. Anet, *J. Am. Chem. Soc.*, **82**, 1502 (1960); *Austral. J. Chem.*, **13**, 396 (1960); **14**, 295 (1961); *Chem. & Ind.*, **1961**, 345; **1962**, 262.

29) G. Hesse and H. S. Stahl, *Chem. Ber.*, **89**, 2414, 2424 (1956).

to pentane-3-ol-2, 4-dione in the presence of proton or hydroxide ion, and reported the isomerization between butane-1-ol-2, 3-dione, butane-2-ol-1, 3-dione, and butane-3-ol-1, 2-dione. Simon,[30] using ^{14}C-labeled sugars, proposed a mechanism including α-diketose → β-diketose → acetylformoin processes in the formation of 2, 4-dihydroxy-3-piperidino-5-methyl-2-cyclopentanone from 1-deoxy-1-piperidino-D-fructose.

The last step is the hydrolytic cleavage of acetylformoin to form pyruvic acid and acetol. Acetylformoin was synthesized by Nodzu et al.,[31] and found to give pyruvic acid and acetol in a good yield.[32] Compounds with a structure similar to acetylformoin were found to be cleft. For instance, Combes[33] observed the formation of acetol from pentane-3-ol-2, 4-dione by alkali. Blatt et al.[34] and Karrer et al.[35] reported that dibenzoylcarbinol gave benzoylcarbinol and benzoic acid by the use of sodium hydroxide or sodium carbonate. Juni et al.[36] also observed that 3-methylpentane-3-ol-2, 4-dione gave butane-2-ol-3-one and acetic acid by sodium hydroxide. House et al.[37] confirmed the formation of propionic acid and pentane-2-ol-3-one by the sodium hydroxide cleavage of 4-methylheptane-4-ol-3, 5-dione. The hydrolytic cleavage of the carbon-carbon bond between the C-3 and C-4 in the β-diketose stage was the acetol formation process proposed by Hayami et al.[3] The cleavage in the stage of the α-diketose may be conjectured to take place and to give pyruvic acid and glycerin, but there is no experimental data for it. In the case of D-glucose-1-^{14}C,[4] compared with the molar radioactivity of the original labeled glucose and that of the alanine, the lower half of the D-glucose contributed to the formation of the pyruvic acid more than the upper half. The result is compatible with the mechanism in which the acetylformoin is a precursor of pyruvic acid and the acetol, because 3-ketose is thought to be more favorable than 4-ketose in the isomerization reaction.

Thus, the formation of the pyruvic acid can be summarized as follows:

1) The formation of α-diketose by β-hydroxy-carbonyl elimination after isomerization to 3-ketose or 4-ketose.

2) The formation of acetylformoin by a second β-hydroxy-carbonyl elimination after isomerization to the β-diketose.

3)The hydrolytic cleavage of the acetylformoin.

Compared with other carbohydrate reactions in solution, the furfural formation requires neither the transformation nor the benzylic acid-type rearrangement; the saccharinic and the isosaccharinic acid formations need both the *trans*formation and the rearrangement, and the metasaccharinic acid formation needs the rearrangement, but no transformation. In the present case, it seems probable that the transformation is required without the rearrangement.

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[30] H. Simon, ibid., **95**, 1003 (1962).

[31] R. Nodzu and S. Kunichika, This Bulletin, **15**, 211 (1940).

[32] K. Kitamura, Graduation Thesis, Kyoto Imperial University (1938).

[33] A. Combes, *Compt rend.*, **111**, 421 (1890).

[34] A. H. Blatt and W. L. Hawkins, *J. Am. Chem. Soc.*, **58**, 81 (1936).

[35] P. Karrer, J. Kebrle and K. M. Thakkar, *Helv. Chim Acta*, **33**, 1711 (1950).

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[37] H. O. House and W. F. Gannon., *J. Org. Chem.*, **23**, 879 (1958).