*Studies on the Chemical Decomposition of Simple Sugars. X. Acetol Formation from ^{14}C-Labeled Hexoses**

By Ryozo GOTO, Jun'ichi HAYAMI, Kenichi KUDO and Sinichi OTANI

(Received November 17, 1960)

As is well known, carbohydrates, especially monosaccharides, have many reactive functional groups, and these compounds show various kinds of reactions. In an aqueous solution, monosaccharides show the following fourma in types of reactions, which depend upon the pH of the reaction medium.

These are a) the "Lobry de Bruyn-Alberda Van Eckenstein reaction[1]", b) the formation of furfural derivatives[2a] in acid medium, c) the reverse aldolization[3] which gives rise to the formation of smaller fragments, a possible recombination reaction of these fragments which may occur, and d) an acid formation in an alkaline medium to yield lactic and saccharinic acids[4a].

Each of these reactions has been well-examined for a long time, and detailed mechanisms for these reactions were proposed. Recently, some of them have been confirmed chemically and also radiochemically[1,2b,4b].

Besides these reactions, there is another decomposition reaction, for example, when an aqueous solution of hexoses (pH 3~11) is heated, sugars are decomposed and some steam volatile products are formed. From the decomposition mixture Dakin[5], Nef[6], and Evans[7,8] have obtained methylglyoxal phenylosazone, and they concluded that the reaction product might be methylglyoxal. However, it is easily understood that methylglyoxal phenylosazone can be derived from either acetol or lactic aldehyde as well as from methyl glyoxal. Therefore methylglyoxal cannot be (concluded to be) a sole product. But with the use of semicarbazide, it is possible to distinguish methylglyoxal from acetol.

* Presented at the 11th Annual Meeting of the Chemical Society of Japan, Tokyo, April, 1958 and the 12th Annual Meeting of the Chemical Society, Kyoto, April 1959.

1) J. C. Speck, Jr., "Advances in Carbohydrate Chem.", Vol. 13 (1958), p. 63.

2) a) F. H. Newth, ibid., Vol. 6, (1951), p. 83, and references cited therein; J. Kennerand G. N. Richards, *J. Chem. Soc.*, **1956**, 2921.

b) W. A. Bonner and M. R. Roth, *J. Am. Chem. Soc.*, **81**, 5454 (1959).

3) W. L. Evans, *Chem. Revs.*, **31**, 537 (1942).

4) a) J. C. Sowden, "Advances in Carbohydrate Chem.", Vol. 12, (1957), p. 36; R. L. Whistler and J. N. BeMiller, ibid., Vol. 13, (1958), p. 289; G. Machell and G. N. Richards, *J. Chem. Soc.*, **1960**, 1924, 1932, 1938.

b) J. C. Sowden et al., *J. Am. Chem. Soc.*, **79**, 6450 (1957); J. C. Sowden and E. K. Pohlen, ibid., **80**, 242 (1958).

5) H. J. Dakin and H. W. Duddley, *J. Biol. Chem.*, **15**, 127 (1913).

6) J. U. Nef, *Ann.*, **357**, 214 (1907); **376**, 1 (1910); **403**, 204 (1914).

7) W. L. Evans, *J. Org. Chem.*, **1**, 1 (1937) and references cited therein.

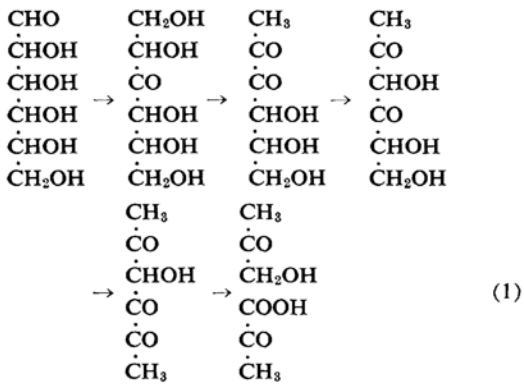
8) C. Enders et al., *Naturwiss.*, **29**, 46 (1941); *Biochem. Z.*, **312**, 349 (1942).

Nodzu[9] and Goto[10] have examined the decomposition products of glucose in a weakly acid and a weakly alkaline solution and isolated acetol semicarbazone instead of methylglyoxal bissemicarbazone. In a series of extensive experiments, they confirmed the fact that a) hexose[11a,b], methylpentose[10], and pentose[10] gave acetol accompanied by small amounts of diacetyl and of lactic aldehyde[11c] and these sugars gave substantially no methylglyoxal, b) tetrose[10] gave only diacetyl and did not produce acetol and c) triose[11d] gave methylglyoxal and diacetyl.

Recently, Sattler[12] observed the formation of acetol by the decomposition of simple sugars in a weakly alkaline aqueous solution. Prey[13], also, thoroughly examined the alkaline degradation of simple sugars and observed the formation of acetol.

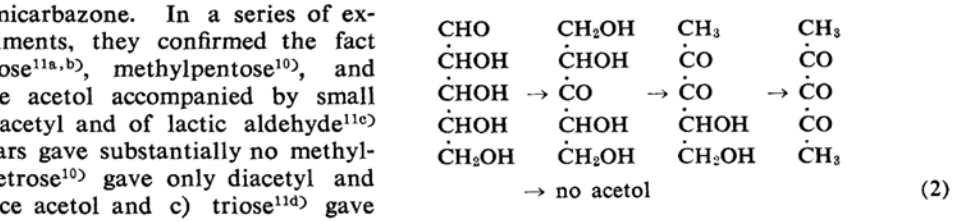
As it has been reasonably accepted that hexose and pentose can yield trioses through a reverse-aldolization[3], and that trioses in turn are easily dehydrated to methylglyoxal, the supposition of the formation of methylglyoxal which was claimed by early workers would be rather natural. Therefore, the formation of acetol, instead of methylglyoxal, seemed to be a novel reaction of a sugar. But little has been known regarding the mechanism of acetol formation from simple sugars.

Nodzu et al.[11d] noticed the formation of acetol accompanied by pyruvic acid and under the supposition of their simultaneous formation, the following mechanism (Scheme 1) which included acetylformoin[14] as a direct precursor of acetol was proposed. Acetylformoin is an acyloin formed from methylglyoxal.



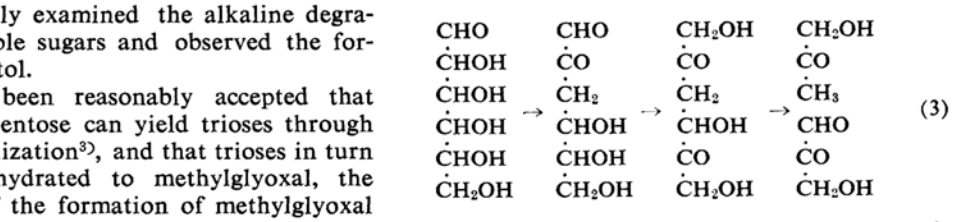
(1)

Later on, Goto inferred from Nodzu's mechanisms, that if it was applied to a pentose, it would lead to the formation of triketopentane as is shown in the following scheme:



(2)

Since no acetol is derived from this triketopentane, he propounded an alternative mechanism for the formation of acetol. His mechanism for this reaction is illustrated in Scheme 3:



(3)

These mechanisms, however, lack any positive evidence. For the better understanding of the mechanism of acetol formation, more detailed knowledge is required. To establish the nature and to confirm the precise mechanism for the acetol formation, the authors undertook the syntheses of ^{14}C-labeled monosaccharides and examined the decomposition of these ^{14}C-labeled monosaccharides.

In the present study, glucose-1-^{14}C, glucose-3,4-^{14}C, and glucose-6-^{14}C were decomposed in a concentrated phosphate buffer to yield acetol, and the relative distribution of a label in the acetol was determined.

Experimental

Radioactive Sugars and Assay Method. — D-Glucose-1-^{14}C was prepared from D-arabinose by a cyanohydrin synthesis[15]. D-Glucose-3,4-^{14}C was prepared from rat liver glycogen injected with $NaH^{14}CO_3$, according to Wood's method[16] with minor modifications. D-Glucose-6-^{14}C was prepared from 1,2-*O*-isopropylidene-D-*xylo*-dialdopentofuranose[17]. In the course of synthesis, deionization at an ice temperature was undertaken with Amberlite type I IRCG-120, instead of ordinary IR-120, to secure a satisfactory exchange.

For an assay, except for specified ones, all sample were converted into barium carbonate by Van Slyke-Folch wet conbustion. Barium carbonate was collected on a filterpaper and counted in an infinite

9) R. Nodzu, This Bulletin, **10**, 122 (1935). (weakly acid region.)
10) R. Goto, ibid., **15**, 209 (1940); *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kwagaku Kwaisi*), **64**, 999, 1054, 1183 (1943). (weakly alkaline region.)
11) a) R. Nodzu and K. Matsui, This Bulletin, **10**, 467 (1935); b) R. Nodzu and R. Goto, ibid., **11**, 381 (1936); c) R. Goto, ibid., **15**, 103 (1940); d) R. Nodzu, et al., *Mem. Coll. Sci. Kyoto Imp. Univ.*, **A20**, 197 (1937).
12) L. Sattler and F. W. Zerban, *J. Am. Chem. Soc.*, **70**, 1975 (1948).
13) V. Prey and E. Waldman, *Monatsh.*, **83**, 55 (1952).
14) R. Nodzu and S. Kunichika, This Bulletin, **15**, 211 (1940).
15) H. S. Isbell et al., *J. Research N. B. S.*, **48**, 163 (1952).
16) H. G. Wood et al., *J. Biol. Chem.*, **159**, 475 (1945).
17) R. Schaffer and H. S. Isbell, *J. Research N. B. S.*, **56**, 191 (1956).

thickness under an end-window type G. M. counter. Probable errors of the counting were within 3% except for specified ones.

Decomposition of Labeled Sugars.—A solution of 5 g. of D-glucose in 200 ml. of concentrated potassium acid phosphate buffer (40%, pH 6.7) was heated and distilled, fresh water was added to maintain a constant volume. Two hundred and fifty mililiters of distillate was collected every 90 min., and distillation was continued until the distillate showed only a faint iodoform reaction, (total volume of distillate 5 l., 284 mg. as acetol). The amount of acetol contained in the distillate was determined by iodometric titration according to Nodzu[9] (1 ml. of 0.1 N I_2 solution corresponds to 0.73 mg. of acetol).

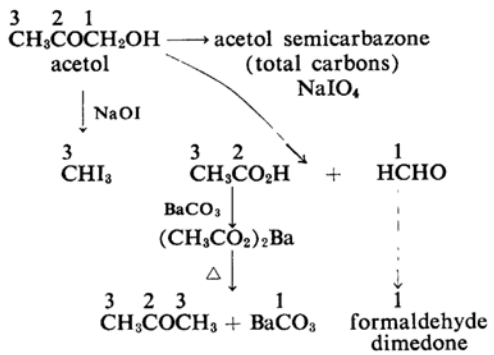
By the above titration, the methyl carbon of acetol was converted into iodoform. This compound was collected and assayed for activity.

Acetol Semicarbazone.—The combined distillate cited above was treated with semicarbazide hydrochloride and with sodium acetate (5 fold equivalents of acetol). The mixture was heated over a boiling water bath for 30 min., cooled and concentrated to a small volume under reduced pressure. Semicarbazone was separated and fractionally recrystallized from hot water. Successive recrystallizations gave 70 mg. of acetol semicarbazone as colorless needles, m. p. 199~200°C. The melting point showed no depression when mixed with an authentic sample.

Degradation of Acetol.—*Formaldehyde Dimedone from Acetol.*—An aqueous solution of acetol was treated with 50% excess of sodium metaperiodate in sodium bicarbonate buffer, and stood overnight at room temperature. The aqueous solution was concentrated under reduced pressure, and from the distillate, formaldehyde was precipitated as formaldehyde dimedone. It was recrystallized from an aqueous ethanol to give colorless needles, m. p. and mixed m. p. 190~191°C.

Separation and Degradation of Barium Acetate.—The residue from the periodate oxidation was treated with a carrier acetate, acidified, and was steam distilled. The iodic and periodic acid in the distillate were removed by silver carbonate[18], and formic acid was removed by oxidation with mercuric oxide*, according to Osburn's method[19]. The acetic acid was refluxed for a further 15 min. After cooling and filtration, acetic acid was distilled out with steam.

Scheme 4



Note: Numbers correspond to the one in the present acetol.

Table I. Radioassay data for ^{14}C-labeled glucose (radioacetol from glucose-1-^{14}C)

	Sample	Carbon atom(s)	Radioactivity μc./mol.
Glucose-1-^{14}C			435
Acetol	Acetol semicarbazone	all	203
	Formaldehyde dimedone	CH_2OH	0
	$BaCO_3$ from Ba acetate	CO	—*1
	Iodoform	CH_3	+*2

Table II. Radioassay data for ^{14}C-labeled glucose (radioacetol from glucose-3,4-^{14}C)

	Sample	Carbon atom(s)	Radioactivity μc./mol.
Glucose-3,4-^{14}C			31.7
Acetol	Acetol semicarbazone	all	32.6
	Formaldehyde dimedone	CH_2OH	33*3
	$BaCO_3$ from Ba acetate	CO	—*1
	Iodoform	CH_3	—*4

*1 Free from radioactivity, a carrier was used.
*2 Radioactivity was present.
*3 Probable error was less than 10%.
*4 Free from radioactivity.

Table III. Radioassay data for ^{14}C-labeled glucose (radioacetol from glucose-6-^{14}C)

	Sample	Carbon atom(s)	Radioactivity μc./mol.
Glucose-6-^{14}C			616
Acetol	Acetol semicarbazone	all	141
	Formaldehyde dimedone	CH_2OH	35
	—*1	CO	—*1
	Iodoform	CH_3	106*2

Table IV. Radioassay data for ^{14}C-labeled glucose (radioacetol from glucose-1-^{14}C, pH 11)

	Sample	Carbon atom(s)	Radioactivity μc./mol.
Glucose-1-^{14}C			435
Acetol	Acetol semicarbazone	all	173
	Formaldehyde dimedone	CH_2OH	73
	$BaCO_3$ from Ba acetate	CO	—*3
	Iodoform	CH_3	108

*1 Not determined.
*2 Indirectly calculated.
*3 Almost free from radioactivity, a carrier was used.

* About 250 mg. of crude sodium acetate (with carrier), was dissolved in 50 ml. of water, 3 drops of sulfuric acid and 5 g. of mercuric oxide were added, and the mixture was refluxed. After 20 min., 3.5 ml. of phosphoric acid (85%) and 2 ml. of water were added, and the mixture

18) P. W. Clutterbuck and F. Reuter, *J. Chem. Soc.*, 1935, 1467.
19) O. L. Osburn et al., *Anal. Chem.*, 5, 247 (1933).

thus purified, was converted into barium salt, and recrystallized from water for an assay and a degradation. Barium acetate was degraded to acetone and barium carbonate by pyrolysis[20] in the usual way. Acetone was converted into 2, 4-dinitrophenylhydrazone and assayed directly.

The course of the degradation of acetol is summarized in Scheme 4, and radioactivity assay data are given in Tables I—IV respectively.

Results and Discussion

The contents of assay data which are shown in Tables I—IV can be summarized as follows:

1) In the case of D-glucose-1-^{14}C, radioactive acetol of about a half molar activity of the original glucose was formed. Periodate oxidation of the acetol provided formaldehyde (corresponding to carbinol carbon of the acetol), which was almost free from activity. The other carbons—methyl and carbonyl carbon—were converted, at the same time, into acetic acid. The acetic acid was degraded by the usual method of pyrolysis and afforded radioactive acetone and nonactive barium carbonate. These facts show that acetol-3-^{14}C was formed from glucose-1-^{14}C.

2) In the case of D-glucose-3, 4-^{14}C, radioactive acetol was obtained which preserved the original molar activity of the reactant glucose. The acetol was degraded to radioactive formaldehyde and nonactive acetic acid. This means that radioactivity was present at the primary carbinol carbon of the acetol. From these facts, it is quite possible that acetol-1-^{14}C is produced from D-glucose-3, 4-^{14}C.

3) D-Glucose-6-^{14}C, as was expected from the results of Tables I and II, afforded acetol, labeled mainly at the methyl carbon. But it should be noted that between the methyl and the carbinol carbon an appreciable randomization of a label is observed. This randomization was confirmed by another repeated run. But, as the label was found mainly at the methyl carbon, it might be possible to say that D-glucose-6-^{14}C produces acetol-3-^{14}C.

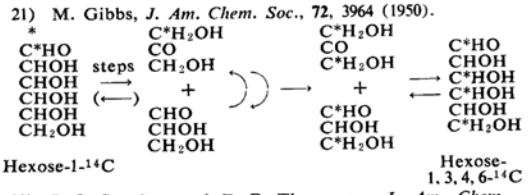
The patterns of the label cited above would reveal that, in the formation of acetol from glucose (hexose), C-1 and C-6 of the original glucose are converted into the methyl carbon of an acetol, while C-3 and C-4 of the original glucose are converted into the carbinol carbon of an acetol.

It is also remarkable that the acetol-1-^{14}C, which was produced from D-glucose-3, 4-^{14}C, preserves the molar radioactivity of the original glucose. This, coupled with the pattern of the label cited above, implies that, in the formation of acetol, C-1, 2, 3 and C-4, 5, 6 of the original glucose behave as a unit, and that no skeletal rearrangement in these C_3 units is involved. These facts further exclude the possibility of a random scission of the carbon-chain of a glucose in this reaction. This is because according to the mechanism of a random scission, universally labeled acetol might be produced, but it is not the case.

The randomization of a label might be attributed to the reverse-aldolization[3,21] and the recombination reaction of a hexose. This is because, as Sowden[22] pointed out, glucose-1-^{14}C and/or glucose-6-^{14}C could be converted into a hexose-1, 3, 4, 6-^{14}C through a reverse-aldolization and a recombination reaction of a hexose*. These reverse-aldolization and recombination reactions are shown to prevail in a high pH region, and in the present instance, radiactive acetol formed from glucose-1-^{14}C at pH 11 shows an appreciable randomization of a label (Table IV), where the carbinol carbon shows about 2/3 activity of the methyl carbon.

On the other hand, in a neutral or a weakly acid medium, the reverse aldolization and the recombination of a hexose have been believed not to occur[24,25]. And also, the lower half of a hexose was suspected to behave somewhat differently from the upper half of a hexose[21]. Therefore, at this stage, it is better not to attribute the randomization of a label in the case of glucose-6-^{14}C (pH 6.7, Table III) to the reverse aldolization (and recombination) of a hexose.

The pattern and the relative distribution of the label found in the present investion, would give the contribution ratio of the upper and the lower half of a hexose molecule[26]. For example Table III shows that 77% of the acetol would come from the nonactive upper half and 23% would come from the active lower half of the glucose-6-^{14}C. But, as the substantial nature of the randomization of the label remains unresolved, the validity of these figures of contribution ratio contains much ambiguity, and will require further examination.



Hexose-1-^{14}C — Hexose-1, 3, 4, 6-^{14}C

20) S. Aronoff et al., *Science*, **110**, 476 (1949).

21) M. Gibbs, *J. Am. Chem. Soc.*, **72**, 3964 (1950).

22) J. C. Sowden and R. R. Thompson, *J. Am. Chem. Soc.*, **80**, 1435 (1958).

23) V. Prey et al., *Monatsh.*, **85**, 1186 (1954).

24) A. A. Bothner-By and M. Gibbs, *J. Am. Chem. Soc.*, **72**, 4805 (1950).

25) H. G. Hers et al., ibid., **76**, 5160 (1954).

26) H. F. Bauer and C. Teed, *Can. J. Chem.*, 33, 1824 (1955); F. Weygand and G. Billek, *Z. Naturforsch.*, **12b**, 601 (1957).

The authors wish to express their sincere thanks to Professor Teruaki Simamoto of the Pharmaceutical Institute of Kyoto University who offered much convenience and suggestions for the the use of animals for experiments.

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