

[CONTRIBUTION FROM THE RADIOCHEMISTRY LABORATORY, DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

The Isomerization of D-Glucose by Alkali in D₂O at 25°¹BY JOHN C. SOWDEN AND ROBERT SCHAFFER²

The Lobry de Bruyn and Alberda van Ekenstein isomerization reaction of D-glucose to D-fructose and D-mannose is shown to occur in heavy water with the introduction of carbon-bound deuterium into the products in accord with the classical enediol mechanism. By the use of radioisotopic dilution analyses for D-fructose and D-glucose and of a corrected phenylhydrazone precipitation method for D-mannose, the extents of formation of these products in the reaction mixture were determined also, and the combination of data was correlated with the reactivities of the individual sugars.

The products obtained by Lobry de Bruyn and Alberda van Ekenstein,³ Nef,⁴ Lewis and co-workers,⁵ Wolfrom and co-workers,⁶ and ourselves⁷ on interaction of reducing sugars with aqueous alkali, usually are explained by assuming that the reaction proceeds through an enediol intermediate. However, it has been reported^{8,9} that when the isomerization reaction takes place in heavy water, introduction of carbon-bound deuterium into the isomerized sugar, in accordance with the predictions of the enediol mechanism, does not occur. Thus, with D-glucose in alkaline D₂O at 25°, Fredenhagen and Bonhoeffer⁸ could detect no significant incorporation of deuterium, while at temperatures above 25° non-reproducible data were obtained. Similarly, Goto⁹ reported an 85% conversion of D-glucose to D-fructose in alkaline D₂O without introduction of carbon-bound deuterium into the product.

In an effort to find evidence for a mechanism that would explain this reported carbohydrate isomerization in heavy water without exchange of carbon-bound hydrogen by deuterium, Bothner-By and Gibbs¹⁰ employed 1-C¹⁴-D-glucose to explore the possibility of rearrangement of the carbon skeleton during isomerization by alkali and found that such rearrangement does not occur.

Recently, Topper and Stetten¹¹ have reported that, in contradiction to the observations of Fredenhagen and Bonhoeffer and of Goto, carbon-bound deuterium is, indeed, incorporated during the alkaline isomerization of D-glucose in heavy water. An expansion of the classical enediol mechanism to include *cis*- and *trans*-enediols with individual functions also was proposed by Topper and Stetten to explain their experimental observations. Thus, they pictured the transformation of D-glucose to D-mannose as occurring *via* the following steps: D-glucose \rightleftharpoons *trans*-enediol \rightleftharpoons D-fructose \rightleftharpoons *cis*-enediol \rightleftharpoons D-mannose.

(1) Abstracted in part from the thesis of Robert Schaffer presented in partial fulfillment for the degree Doctor of Philosophy, Washington University, October, 1950.

(2) National Bureau of Standards, Washington, D. C.

(3) 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**, 147 (1899); **19**, 5 (1900).

(4) J. U. Nef, *Ann.*, **357**, 294 (1907); **376**, 1 (1910); **403**, 204 (1913).

(5) M. L. Wolfrom and W. L. Lewis, *THIS JOURNAL*, **50**, 837 (1928); R. D. Greene and W. L. Lewis, *ibid.*, **50**, 2813 (1928).

(6) M. L. Wolfrom and co-workers, *ibid.*, **68**, 122, 578, 1443, 2342 (1946).

(7) J. C. Sowden and R. Schaffer, *ibid.*, **74**, 499 (1952).

(8) H. Fredenhagen and K. T. Bonhoeffer, *Z. physik. Chem.*, **A181**, 392 (1938).

(9) K. Goto, *J. Chem. Soc. Japan*, **63**, 217 (1942).

(10) A. A. Bothner-By and M. Gibbs, *THIS JOURNAL*, **72**, 4805 (1950).

(11) Y. J. Topper and D. Stetten, Jr., *J. Biol. Chem.*, **189**, 191 (1951).

We also had obtained independent evidence that carbon-bound deuterium is incorporated into the sugar products during the isomerization of D-glucose in alkaline heavy water. However, our results, reported herein, are in agreement with the *classical* enediol mechanism for the alkaline isomerization of reducing sugars. By comparison of the extents of deuterium incorporation into the D-fructose and D-mannose and by examination of the distribution of deuterium on the D-fructose carbon chain, we conclude that D-fructose is not necessarily an intermediate in the conversion of D-glucose to D-mannose by aqueous alkali.

Experimental

The Isomerization of D-Glucose in Alkaline Heavy Water.—A suspension of 0.087 g. of calcium hydroxide in 50.0 g. of 99.8% deuterium oxide was shaken for 18 hours at 0° and then for 16 hours at 25°. An amount of 9.00 g. of D-glucose was dissolved in 49.42 g. of the filtered, alkaline heavy water and the solution, whose volume was 50.3 ml., was transferred to polarimeter tubes and kept at 25°. The mutarotation data observed for the solution are shown in Table I.

TABLE I

MUTAROTATION OF MOLAR D-GLUCOSE AT 25° IN D₂O SATURATED WITH Ca(OD)₂

Time, hr.	[α] ²⁵ _D	Time, hr.	[α] ²⁵ _D
0.8	53.6	117	34.0
20	49.1	166	29.0
46	45.0	240	23.3
72	40.4	335	16.5
94	37.0	504	10.9

Isolation and Preparation of Products for Deuterium Analysis.—After a reaction time of 21 days at 25°, samples of 1.255 g., 4.314 g. and 40.0 ml. of the reaction solution were taken for D-glucose, D-fructose and D-mannose isolations, respectively. The previously described methods⁷ were employed for the isolation and crystallization of the three sugars. Just prior to the initial crystallizations, each of the three sirupy sugars was evaporated four times to dryness with intervening additions of several volumes of water in order to eliminate all deuterium not stably bound to carbon. The crystalline deuterated products were diluted with the corresponding non-deuterated sugars and then recrystallized again prior to the mass analyses.¹²

Due to the high concentration of exchangeable hydrogen in the D-glucose and to the contribution of hydrogen by the calcium hydroxide, the concentration of the deuterium oxide in the solvent was reduced from 99.8% to 95%. This correction was applied in calculating the number of deuterium atoms per molecule in the deuterated products from their observed deuterium contents. The analytical results and calculations are shown in Table II.

Quantitative Analysis for D-Glucose, D-Fructose and D-Mannose.—The previously described methods⁷ were employed. Radioisotopic dilution analysis served for determination of D-glucose and D-fructose concentrations, while D-mannose was determined by the corrected phenylhydrazone precipitation procedure.

(12) We are indebted to Dr. H. S. Anker, Department of Biochemistry, The University of Chicago, for the deuterium analyses.

TABLE II

DEUTERIUM CONTENT AND CONCENTRATION OF PRODUCTS FROM THE ISOMERIZATION OF D-GLUCOSE IN ALKALINE D₂O AT 25° AFTER TWENTY-ONE DAYS

Product	Specific radio-activity, cts./min./mg.	Amount in aliquot, ⁷ mg.	Concentration, % of products	Dilution by non-deuterated sugar	D content, ¹¹ atom % excess	D content, atom % excess, undiluted	D atoms/molecule
D-Glucose	357.1	128.5	66.5	13.2:1	0.080 ± 0.01	1.06	0.13
D-Fructose	169.0	189.7	28.6	64.8:1	.212 ± .01	13.74	1.74 ± 0.1
D-Mannose	...	54	0.8	36.4:1	.303 ± .01	11.03	1.39 ± .1
Diacetonefructose	0	7.73 ± .30	7.73	1.63 ± .1
K Diacetone-2-ketogluconate	0	0.77 ± .02	0.77	0.14 ± .01

After the addition of 400 mg. of known D-mannose to the 40 ml. sample of reaction solution, there was obtained 661 mg. of D-mannose phenylhydrazone, corresponding to 54 mg. of D-mannose initially present in the sample.

The observed concentrations of the three sugars in the reaction solution are shown in Table II.

The Distribution of Deuterium in the D-Fructose Carbon Chain.—A sample of the undiluted, deuterated D-fructose was converted to 2,3,4,5-diisopropylidene-D-fructose according to the directions of Pacsu, Wilson and Graf.¹³ The diacetonefructose, m.p. 95–96°, then was oxidized with alkaline potassium permanganate solution, as described by Ohle and co-workers,¹⁴ to 2,3,4,5-diisopropylidene-2-ketogluconic acid. The latter was isolated as the anhydrous, crystalline potassium salt, $[\alpha]_D^{20} -36.5^\circ$ in water, by crystallization from absolute ethanol with the addition of absolute ether. The salt did not lose weight when heated in a high vacuum over phosphorus pentoxide at 140°.

The comparative deuterium contents of the diacetonefructose and the potassium diacetone-2-ketogluconate, shown in Table II, indicate that approximately 92% of the deuterium in the deuterated fructose is located at carbon-1.

Discussion of Results

Correlation with the Classical Enediol Mechanism.—The data reported herein are consistent with the classical enediol mechanism of alkaline isomerization of reducing sugars. Compared with our results⁷ obtained from isomerizations conducted at 35° with aqueous sodium hydroxide, the deuterium content of each sugar is consistent with the previously observed order of reactivity of the three sugars.

The relatively high deuterium content of the D-fructose, 1.74 ± 0.1 atoms of deuterium per molecule, results from the high reactivity of this sugar, which was shown in the previous study to be the preferred product as well as the most reactive of the three hexoses. The initially formed D-fructose, upon reverting to the enediol, presumably would give rise to molecules of the intermediate containing some carbon-bound deuterium. From the latter, multiply-labeled D-fructose could form.

The importance of the conversion of D-fructose to the labeled intermediate is shown by the observed isotopic content of the D-mannose, 1.39 ± 0.1 atoms of deuterium per molecule, since multiply-labeled D-mannose could only have formed from enediol containing carbon-bound deuterium.

The greater preference for the formation of D-glucose than D-mannose from the intermediate, observed in our previous work, is again apparent in this experiment. Assuming that the *re-formed* D-glucose has its deuterium distributed in similar

fashion to the D-mannose, the minimum amount of D-glucose formed from the intermediate can be estimated as $\frac{1.06}{11.03} \times 66.5 =$ approximately 6% of the

sugar content of the isomerization mixture. This is nearly eight times the observed yield of D-mannose.

The possibility of enolization of D-fructose to a 2,3-enediol introduces the opportunity for formation of some D-fructose labeled with deuterium at carbon-3. The presence of a minor degree of labeling at other than carbon-1 of D-fructose was confirmed by observation of retention of some 8% of deuterium in the transformation: deuterated D-fructose → deuterated potassium diacetone-2-ketogluconate. Since the labeling at other than carbon-1 of the D-fructose is of such minor nature, introduction of deuterium at other than carbon-1 and carbon-2 of the D-glucose and D-mannose may be presumed to be negligible. This is confirmed by the observation of Topper and Stetten¹¹ that the transformation: deuterated D-glucose → potassium D-arabonate results in complete loss of deuterium.

Deviations from the *cis, trans*-Enediol Mechanism of Topper and Stetten.—The reasoning that led Topper and Stetten to propose the *cis, trans*-enediol mechanism also led them to the conclusion that a maximum of one deuterium atom could be incorporated into carbon-1 of D-fructose in the course of its formation from D-glucose in alkaline heavy water. Our examination of the distribution of label in the deuterated D-fructose, however, shows that in a D-fructose containing 1.74 deuterium atoms per molecule 1.5 deuterium atoms are incorporated at carbon-1.

A further outstanding deviation of our experimental results from the mechanism proposed by Topper and Stetten is apparent from the observed relative degree of labeling of the D-fructose and D-mannose. According to the enediol mechanism, D-fructose must incorporate one carbon-bound D atom per molecule during its initial formation in heavy water from the enediol. On reverting to an enediol, it then can lose a *maximum* of one D atom per molecule. Indeed, it appears reasonable to assume that at least a significant percentage of the label will be retained in this step and that the resulting enediol will contain carbon-bound deuterium. The formation of D-mannose from an enediol in heavy water must also involve incorporation of one D atom per molecule. Thus, if D-fructose were a *necessary* intermediate in the transformation D-glucose → D-mannose in heavy water, then the D-mannose would contain *at least* as high a concentration of carbon-bound deuterium as that observed

(13) E. Pacsu, E. J. Wilson, Jr., and L. Graf, *THIS JOURNAL*, **61**, 2875 (1939).

(14) H. Ohle, I. Koller and G. Behrend, *Ber.*, **58**, 2577 (1925); H. Ohle and G. Behrend, *ibid.*, **60**, 1159 (1927); H. Ohle and R. Wolter, *ibid.*, **63**, 843 (1930).

for D-fructose. While the estimated deuterium content per molecule for the D-mannose is the least accurate of our experimental results, due to uncertainty in the dilution factor occasioned by the low concentration of D-mannose produced in the isomerization reaction, nevertheless, our results indicate that the D-fructose contains a *higher* concentration of carbon-bound deuterium than does the D-mannose. Thus some D-mannose must arise directly from the enediol initially formed from D-glucose.

Possible Applications of Deuterated D-Fructose.—In addition to providing substantiative evidence for the classical enediol mechanism, the isomerization of D-glucose in alkaline heavy water comprises

an easy and inexpensive route to isotopically labeled D-fructose. Assuming that labeling at other than carbon-1 and carbon-3 is negligible, the distribution of deuterium in the labeled D-fructose also is known. The isotopic content is such that considerable dilution is possible for studies, conducted in the absence of alkalinity, where it may be used for isotopic dilution analysis or as an auxiliary label for carbon-1 and carbon-3.

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[CONTRIBUTION FROM THE CHEMISTRY LABORATORY OF THE OHIO STATE UNIVERSITY]

Bicyclo[1,2,3]octanol-2

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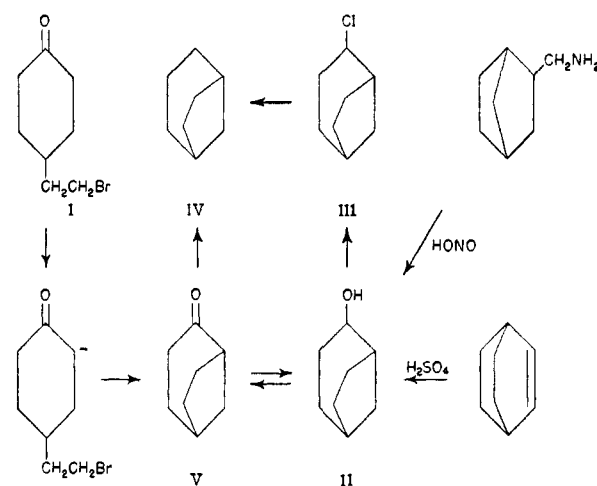
The internal condensation of 4-(β -bromoethyl)-cyclohexanone brought about by lithium leads to bicyclo[1,2,3]octanol-2 instead of the expected bicyclo[2,2,2]octanol-1. The proof of structure and synthesis of intermediates is described. Bicyclo[1,2,3]octanol-2 is also formed by the sulfuric acid hydration of bicyclo[2,2,2]octene.

In an effort to prepare bicyclo[2,2,2]octanol-1 desired for studies on the behavior of bridgehead alcohols,³ we attempted the intramolecular condensation of 4-(β -bromoethyl)-cyclohexanone (I). No reaction took place with magnesium but with lithium an alcohol, II, $C_8H_{14}O$, was isolated in very small yield along with a small amount of a ketone, V, $C_8H_{12}O$, and a large resinous fraction.

The alcohol was saturated and was exceedingly volatile. These properties suggested a bicyclic structure. On treatment with thionyl chloride and pyridine, II yielded a chloride, III, which gave an immediate precipitate when treated with aqueous alcoholic silver nitrate. Reduction of III with sodium in alcohol yielded a hydrocarbon, IV, C_8H_{14} , m.p. 136.8–137.2°. This hydrocarbon was undoubtedly bicyclo[1,2,3]octane.⁴ The same hydrocarbon was obtained by oxidizing II to the ketone, V, followed by reducing to IV by the thioacetal hydrogenolysis method.⁵ The lower melting points recorded are undoubtedly due to only small amounts of impurity since this type of hydrocarbon has a large cryoscopic constant.

The alcohol, II, was shown to be bicyclo[1,2,3]octanol-2 by comparison of its phenylurethan⁶ with that prepared from an authentic sample.⁷ In addition,

we found that II resulted in good yield from the hydration of bicyclo[2,2,2]octene⁸ with 80% sulfuric acid. It is interesting to note that rearrangement from the bicyclo[2,2,2]octyl to the bicyclo[1,2,3]octyl structure occurred in this hydration whereas hydrogen bromide adds to bicyclo[2,2,2]octene without rearrangement.^{4d,9} However, 2-bromobicyclo[2,2,2]octane has been shown to rearrange to 2-bromobicyclo[1,2,3]octane on treatment with silver bromide.^{4d}



The formation of the alcohol, II, from I may be explained as follows: either an organolithium derivative or lithium itself converts I into an enolate which then undergoes an intramolecular cyclization to yield the ketone, V; the ketone is then reduced to II, again either by an organolithium intermediate fact that compounds in this bicyclic series have large cryoscopic constants.

(8) We are indebted to Dr. H. Walborsky for a sample of bicyclo[2,2,2]octene.

(9) H. Meerwein and K. von Emster, *Ber.*, **55** 2500 (1922).

(1) Taken from the Ph.D. thesis of Mrs. Yu, O.S.U., 1950.

(2) This research was supported by the Office of Naval Research.

(3) P. D. Bartlett and L. H. Knox, *THIS JOURNAL*, **61**, 3184 (1939).

(4) (a) G. Komppa, *et al.*, *Ann.*, **521**, 242 (1936), reported the m.p. as 133° as did (b) J. W. Barrett and R. P. Linstead, *J. Chem. Soc.*, 611 (1936); (c) K. Alder and E. Windemuth, *Ber.*, **71**, 2404 (1938), reported 141°; (d) W. von E. Doering and M. Farber, *THIS JOURNAL*, **71**, 1515 (1949), reported 139.5–141°.

(5) M. L. Wolfrom and J. V. Karabinos, *ibid.*, **66**, 909 (1944).

(6) We are indebted to Dr. Doering for a sample of his authentic phenylurethan, m.p. 128–129.5°. We also prepared an authentic sample of phenylurethan from alcohol synthesized as in ref. 7.

(7) K. Alder and E. Windemuth, *Ber.*, **71**, 1939 (1938), and Doering and Farber, ref. 4d, report a melting point of 183–184° for this alcohol. The sample we obtained melted at 173.6–174.8° in a sealed tube. Again the impurity present (a small ketonic impurity as indicated by the infrared absorption spectrum) was surely not large as judged by the