Studies on the Chemical Decomposition of Simple Sugars. XIII. Separation of the So-called Glutose (a 3-Ketohexose)

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In 1895 Lobry de Bruyn and Alberda van Ekenstein(1) found that glucose, when treated with a dilute alkaline solution, gave a equilibrium mixture containing original glucose, mannose, and fructose. This isomerization of reducing sugars is well known as the Lobry de Bruyn-Alberda van Ekenstein transformation(2,3). Through this transformation, all of the known reducing sugars and some non-carbohydrate substances such as α-hydroxyketones and -aldehydes have been converted into their epimers or diastereomers through the action of inorganic or organic bases, or, in some cases, enzymes.

According to present thinking(2,3), this classical transformation is base-catalysed; it has usually been supposed to proceed through an enolization which gives rise to an 1,2-enediol or enediolate anion intermediate. The 1,2-enediol or enediolate anion so produced may either revert to the starting aldose (i. e., glucose), be converted into the epimeric aldose (i. e., mannose), or be further transformed to the corresponding ketose (i. e., fructose).

The transformation, however, may further proceed and, if so, the 2,3-enediol or enediolate anion can be produced; this is probably converted into the corresponding 2-ketoses (i. e., fructose and allulose) or 3-ketohexoses. Lobry de Bruyn and Alberda van Ekenstein(1) could separate an unfermentable, reducing substance from the equilibrium mixture which was produced by the action of alkalis on glucose or fructose. They mistakenly considered allulose to be a fermentable sugar, so they described the unfermentable, reducing substance as a 3-ketose and named it "glutose", though they did not provide any supporting experimental evidence.

Their unfermentable, reducing substance was imagined to be the same as the unfermentable portion of cane molasses. Goto(4) prepared an osazone from the unfermentable, reducing substance obtained from invert sugar by the action of dibasic sodium phosphate. After repeated fractional recrystallization, he obtained; osazone A, m. p. 172~176°C* (163

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1) 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).

2) J. C. Speck, Jr., "Advances in Carbohydrate Chemistry", 13, 63 (1958).

3) R. L. Whistler and J. N. BeMiller, ibid., 13, 289 (1958).

4) R. Goto, Graduation Thesis, Kyoto Univ. (1931) unpublished.

* Further recrystallization gave osazones: A_1, m. p. 178~180°C and A_2, m. p., 185~186°C.

parts); osazone B, m. p. 190∼194°C (325 parts; after recrystallization, m. p. 193∼194°C); and others (about 170 parts). He also considered that the so-called "glutose" was not 3-ketohexose but a more complex mixture which was produced from sugar by means of rearrangements, dehydrations, decompositions and polymerizations. Sattler and Zarban[5] observed that the "glutose" from fructose was a mixture of fructose anhydride and difructose dianhydride.

In accordance with the research program in our laboratory on the decomposition of simple sugars, the separation of "glutosazone" has been investigated. When D-glucose was treated with oxygen-free saturated lime-water, an unfermentable, reducing substance was obtained (about 11%); the osazone of this, prepared in the usual way, melted at 194°C. The "glutosazone" from cane molasses* also melted at 192∼193°C. With liquid chromatography by silica-gel-Celite (5 : 1, wt./wt.), using petroleum ether (40∼70°C) as a developer, the osazone of m. p. 194°C was separated into 3 parts. From the top zone D-glucosazone (m. p., 207∼208°C) and from the lower zone methylglyoxal bisphenylhydrazone (m. p. and mixed m. p., 152°C) were isolated. The composition of these two was 37 : 1 in a typical run. The middle zone contained a trace of an unidentified substance. From these results, it is claimed that the so-called "glutosazone" is substantially glucosazone contaminated with among other things, methylglyoxal bisphenylhydrazone; this is consistent with the results of Sattler and Zarban[5].

Experimental

Preparation of the Unfermentable, Reducing Substance.—Thirty grams of D-glucose was dissolved in oxygen-free, saturated lime-water (100 ml.), and the solution (initial pH 10.0) was allowed to stand or to boil gently. After 22 hr. at room temperature, the solution turned yellow in color and the pH value of the solution fell to 9.2. The solution was neutralized with 0.1 N oxalic acid, and the precipitated calcium oxalate was filtered off with the aid of Celite**. Then the filtrate was diluted with distilled water to about 600 ml. To this aqueous solution (pH 7.15), 90 g. of baker's yeast*** was added, and the mixture was kept in an incubator at 30°C. After 72 hr. the yeast was removed by filtration, and the filtrate was neutralized with dilute sodium hydroxide and freed from protein by the addition of a slight exess of lead acetate solution. After filtration, the filtrate was bubbled with hydrogen sulfide gas to remove any dissolved lead salt. The precipitate was filtered and the filtrate was concentrated under reduced pressure at 40∼50°C to about 100 ml. of syrup. To this syrup, an equal volume of alcohol was added, and the mixture was heated on a water bath for 15 min., whereupon the flocculent precipitate appeared. After filtration, the filtrate was concentrated as above. After this purification process with alcohol had been repeated three times, the resulting filtrate was decolorized with active carbon and concentrated under reduced pressure at 40∼50°C to a syrup.

This syrup was freed from water by repeated azeotropic distillation by adding a benzene-alcohol mixture. The syrup obtained was slight yellow in color and was not fermented by baker's yeast; yield, 3.5 g. With repeated reprecipitations of this syrup from methanol-acetone, a light yellow, hygroscopic solid was obtained. The reducing power of this unfermentable substance was 10.3% of that of D-fructose (Fehling's solution).

Treatment of the unfermentable, reducing substance with phenylhydrazine hydrochloride and sodium acetate, under the usual conditions, produced a yellow osazone which melted at 148∼152°C when crude. After three recrystallizations from aqueous alcohol, yellow crystals were obtained; m. p., 194°C.

Found: N, 15.8%. Calcd. for $C_{18}H_{22}O_4N_4$: N, 15.6%.

Many authors have reported on the melting points of the so-called "glutosazone": 194∼195[5], 165[1,5], 207[6] and 163∼166°C[7].

Chromatographic Separation of the So-called "Glutosazone". — A portion of "glutosazone" (720 mg.) was dissolved in a minimum volume of ethanol, and the solution was placed at the top of a chromatographic column (2.6×30 cm.) in which acid-washed silica gel-Celite (5 : 1, wt./wt.) had been packed. The development was carried on with petroleum ether (40∼70°C) until the separation of the yellow zones was complete. The content was extruded, and each zone was cut and extracted with boiling ethanol. The solvent was distilled off, and the residual crystalline mass was weighed, and recrystallized from aqueous ethanol. The results obtained are shown in Table I.

TABLE I. CHROMATOGRAPHIC SEPARATION OF "GLUTOSAZONE"

No.	Zone	Wt., mg.	Remark
1	Top	630	D-Glucosazone, m. p. 208∼209°C*
2	Middle	trace	Orange yellow powder
3	Lower	17	Methylglyoxal bisphenylhydrazone, yellow crystal; m. p. and mixed m. p., 152°C*,**

* Recrystallized from aqueous ethanol.

** Found: N, 22.0%. Calcd. for $C_{15}H_{16}N_4$: N, 22.2%.

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[5] L. Sattler and F. W. Zarban, *Ind. Eng. Chem.*, 37, 1133 (1945).

** Johns-Manville Co., New York, N. Y.

*** Kaneka No. 2612409, Kanegahuti Chem. Ind. Co., Ltd.

[6] H. A. Spoehr et al., *J. Biol. Chem.*, **85**, 305 (1929).

[7] H. A. Spoehr and P. C. Wilbur, ibid., **69**, 421 (1926).

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