# XLVI. THE HAGEDORN-JENSEN METHOD APPLIED TO VARIOUS SUGARS.

## RELATION OF REDUCING POWER TO CONFIGURATION.

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(*Received December 30th, 1929.*)

The increasing knowledge of sugar structure and its physiological significance has prompted a close scrutiny of the methods for the quantitative analysis of reducing sugars. Most of the methods are based on the oxidation of the sugars. The amount of oxygen consumed permits a comparison of the oxidising reagents. The products of oxidation are in most instances not uniform, thus the oxygen factors are only pseudo-stoichiometrical [Sobotka, 1926], but a comparison of these factors with the degree of oxidation in the possible reaction products conveys a general idea of their nature.

Lobry de Bruyn and Nef and, particularly, Evans [1929] have dealt with the numerous products obtained by alkaline rearrangement and oxidation of carbohydrates. These researches have established beyond doubt what may be termed progressive enolisation as the key to sugar destruction. By extending the range of substrates over the field of methylated glucoses it has been demonstrated [Sobotka, 1926] that the methoxy-group is an obstacle in the progress of enolisation in the Bertrand solution and more so in the less alkaline Fehling reagent.

Purely steric differences, as between glucose and galactose, give rise to merely insignificant deviations with the usual alkaline copper solutions. Micro-methods like Bang's according to Rohny [1928] and the Folin-Wu method according to Greenwald *et al.* [1924] yield rather divergent figures for hexoses other than glucose. It has been found that alkaline mercury solutions oxidise quantities of aldoses and ketoses above 10 mg. at a remarkably constant rate of 6·0 oxygen equivalents. This linear function was entirely distorted for smaller amounts *ceteris paribus* (unpublished experiments). Such irregularities in the lowest sections of the sugar-oxygen curves are at least in part due to a passive state of the complex metal ion in absence of certain organic groups; their bearing upon steric differences of the sugars is important but not obvious.

A constant difference between glucose and galactose was realised over a wide range of concentrations when employing potassium ferricyanide as the oxidising agent. The Hagedorn and Jensen method has been adapted to

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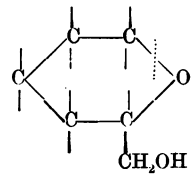
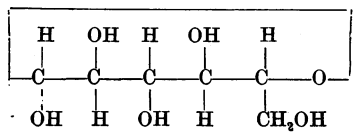
amounts of glucose of 1–10 mg. by Issekutz and Both [1927] and up to 4 mg. by Hanes [1929]. We adopted Hanes's accurate and convenient volumetric procedure. Pucher and Finch [1928] seem to have been the first to describe the low reduction power of galactose with the Hagedorn-Jensen reagent. Their results were recently confirmed by Hawkins [1929] for the gasometric ferricyanide reagent.

The complex iron salt of the mildly alkaline Hagedorn-Jensen reagent probably approaches the conditions of physiological sugar oxidation more than any copper, mercury, bismuth or permanganate solution. One may attribute special significance to its steric predilections.

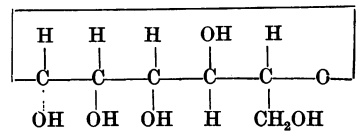
By unrolling the cylinder of the accepted pyranoid formulas of the hexoses [Haworth, 1929], we obtain the following symbols for the *d*-hexoses:



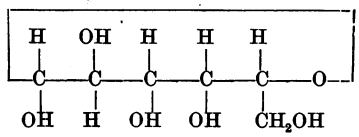
I.



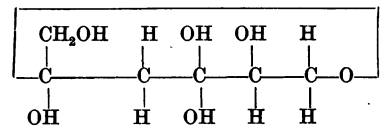
II. β-Glucose



III. β-Mannose



IV. β-Galactose



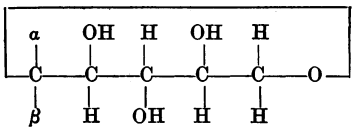
V. β-Fructose

Disregarding the labile steric configuration on the first C atom ($C_1$) glucose shows an alternation of the hydroxyls throughout the molecule: $C_2$ *trans* $C_3$ *trans* $C_4$. Accordingly in mannose and rhamnose: $C_2$ *cis* $C_3$ *trans* $C_4$, and in galactose: $C_2$ *trans* $C_3$ *cis* $C_4$.

The average reducing power of mannose is only slightly less than that of glucose. The isomerism along the $C_2$—$C_3$ link is therefore not important for the stability of the hexose chain towards ferricyanide. In all other instances the increased reactivity of the *trans*-configuration is conspicuous. The great difference of approximately 24 % for glucose minus galactose must be attributed to the *trans*-configuration on $C_3$—$C_4$.

Haworth [1929] pointed out that the asymmetry of the fifth carbon atom may be interpreted in the above ring formula (I) as a *trans* or *cis* location of the side chain —$CH_2OH$ with respect to the hydroxyl on the fourth carbon atom. It is not possible to ascertain the influence of this isomerism as the number of hexoses available for comparison is limited; but it seems probable that this end of the carbon chain is of no importance for the reducing power.

In order to extend this investigation we tested three of the four possible aldopentoses.



VI.

*d*-Xylose (the Greek letters indicate the location of OH on $C_1$ in the isomeric forms)

Omitting the *cis-trans*-isomerism between $C_1$ and $C_2$ ($\alpha$–$\beta$ forms), they show the following differences:

| | |
|---|---|
| Xylose (VI) | $C_2$ *trans* $C_3$ *trans* $C_4$ |
| Arabinose | $C_2$ *trans* $C_3$ *cis* $C_4$ |
| Ribose | $C_2$ *cis* $C_3$ *cis* $C_4$. |

Arabinose with an oxygen equivalent for ferricyanide of 4·18 resembles galactose with 4·04; xylose with 4·56 equivalents ranges below the analogous hexose, glucose, with 5·31. The decrease of reducing power by 8·3 % and 7·3 % from xylose to arabinose to ribose permits the conclusion that in the oxidation of aldopentoses, the influence of *trans* or *cis* position between $C_2$ and $C_3$ is equal to that between $C_3$ and $C_4$. This finding, together with the lower absolute reducing power of pentoses, shows that the progress of enolisation and oxidation does not reach beyond $C_3$ in the five-carbon sugars under the experimental conditions. The oxygen equivalent of ribose of less than 4 (= two atoms oxygen) barely allows for the formation of a keto-pentonic acid and for the cleavage into formic acid and erythronic acid. This is in sharp contrast to the pivotal $C_3$—$C_4$ link of the hexoses.

The Lobry de Bruyn rearrangement leads to the formation of talose and tagatose from galactose. Determinations of optical rotation, copper and ferricyanide reduction from Lobry de Bruyn equilibria of glucose and of galactose reveal parallel changes. Hence, in the ketohexoses the reducing power of tagatose ($C_3$ *cis* $C_4$ *trans* $C_5$) is essentially lower than that of fructose (V) ($C_3$ *trans* $C_4$ *cis* $C_5$). This corroborates the assumption that the considerable differences in the reducing power of hexoses are due mainly to the *cis-trans*-distribution of the hydroxyls on the third and fourth carbon atom.

The reducing power of rhamnose is identical with that of glucose or mannose in amounts of 2 mg. or less; in higher concentrations, it exhibits considerable deviations.

Despite the identity of their reducing moiety we observed a slight increase of maltose over lactose. This may be due to the difference of the other moiety set free by partial hydrolysis which is in turn indicated by the high absolute equivalent of more than 8 equivalents of oxygen.

The difference between the reducing power of glucose and galactose with respect to $K_3Fe(CN)_6$ may be applied with great advantage to the analysis of mixtures, as in selective glycolysis, in the hydrolysis of saponins, etc. In combination with the polariscope, the accuracy of these analyses proved to be almost doubled.

Table I. *Titration data and oxygen equivalents.*

| Sugar mg. | Glucose cc. | Fructose cc. | Mannose cc. | Galactose cc. | Glucose + Galactose cc. | Glucose + Fructose cc. | Xylose cc. | Arabinose cc. | Ribose cc. | Rhamnose cc. | Maltose cc. | Lactose cc. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 0·01 *N* thiosulphate | | | | | | | |
| 0·5 | 1·45 | 1·55 | 1·45 | 1·02 | 1·21 | — | 1·55 | 1·40 | 1·25 | 1·63 | 1·33 | 1·22 |
| 1·0 | 2·91 | 3·10 | 2·92 | 2·19 | 2·46 | 2·88 | 3·11 | 2·72 | 2·55 | 3·13 | 2·44 | 2·44 |
| 1·5 | 4·40 | 4·70 | 4·30 | 3·40 | 3·75 | 4·34 | 4·65 | 4·15 | 3·79 | 4·86 | 3·63 | 3·49 |
| 2·0 | 5·91 | 6·28 | 5·87 | 4·50 | 5·00 | 5·87 | 6·07 | 5·55 | 5·11 | 6·49 | 4·96 | 4·54 |
| 2·5 | 7·38 | 7·83 | 7·46 | 5·64 | 6·33 | 7·32 | 7·49 | 7·05 | 6·39 | 7·67 | 6·08 | 5·78 |
| 3·0 | 8·93 | 9·39 | 8·63 | 6·86 | 7·65 | 8·87 | 9·06 | 8·41 | 7·76 | 9·27 | 7·23 | 6·89 |
| 3·5 | 10·55 | 10·90 | 10·38 | 8·12 | 9·24 | 10·36 | 10·59 | 9·92 | 9·14 | 10·51 | 8·47 | 7·92 |
| 4·0 | — | 12·48 | 11·42 | 9·12 | 10·49 | 11·91 | 11·83 | 11·28 | 10·49 | 11·56 | — | 8·88 |
| 4·5 | — | — | — | 10·41 | — | — | — | 12·29 | 11·76 | 12·10 | — | 9·92 |
| | | | | | Oxygen equivalents | | | | | | | |
| 0·5 | 5·22 | 5·58 | 5·22 | 3·67 | 4·35 | — | 4·65 | 4·20 | 3·75 | 5·35 | 9·58 | 8·80 |
| 1·0 | 5·26 | 5·58 | 5·26 | 3·94 | 4·43 | 5·19 | 4·66 | 4·08 | 3·82 | 5·14 | 8·79 | 8·79 |
| 1·5 | 5·28 | 5·64 | 5·16 | 4·08 | 4·50 | 5·21 | 4·65 | 4·15 | 3·79 | 5·31 | 8·72 | 8·38 |
| 2·0 | 5·32 | 5·65 | 5·28 | 4·05 | 4·50 | 5·28 | 4·55 | 4·16 | 3·83 | 5·32 | 8·92 | 8·18 |
| 2·5 | 5·31 | 5·62 | 5·36 | 4·06 | 4·56 | 5·28 | 4·49 | 4·23 | 3·83 | 5·04 | 8·76 | 8·33 |
| 3·0 | 5·36 | 5·63 | 5·18 | 4·12 | 4·59 | 5·32 | 4·53 | 4·21 | 3·88 | 5·07 | 8·68 | 8·27 |
| 3·5 | 5·42 | 5·61 | 5·32 | 4·15 | 4·73 | 5·33 | 4·54 | 4·25 | 3·89 | 4·91 | 8·71 | 8·14 |
| 4·0 | — | 5·62 | 5·14 | 4·11 | 4·72 | 5·37 | 4·44 | 4·23 | 3·93 | 4·67 | — | 7·99 |
| 4·5 | — | — | — | 4·16 | — | — | — | 4·10 | 3·92 | 4·40 | — | 7·94 |
| Average | 5·31 | 5·62 | 5·24 | 4·04 | 4·55 | 5·28 | 4·56 | 4·18 | 3·85 | — | — | — |
| % deviation from glucose | 0 | +5·8 | −1·3 | −23·9 | −14·3 | −0·5 | −14·1 | −21·3 (−8·3)* | −27·5 (−15·6)* | — | — | — |

* % deviation from xylose.

## Experimental.

The procedure of Hanes was followed, but 0·01 *N* sodium thiosulphate was used for all titrations. In Table I are given the titration data of duplicate determinations and the oxygen equivalents for the sugars studied. All sugars, except the two disaccharides, were anhydrous; maltose and lactose were the monohydrates. The stock solutions were freshly prepared and checked by the Bertrand method and by optical rotation. These concentrations were 10·00 ± 0·03 %. Decimal dilutions were made for the determinations and any deviation from 10·00 % in the stock solution was taken into account. The variation in reducing power with concentration of sugar is shown by the curves in Fig. 1.

10 % solutions of glucose and galactose were set up in 0·1 *N* NaOH; one portion of each was neutralised immediately, another after 24 hours at room temperature. In another experiment they were exposed for 12 hours to 0·5 *N* NaOH at 30°. The $[\alpha]_D$ of the glucose changed from + 52° to + 11° in the former and to − 4° in the latter experiment, the $[\alpha]_D$ of the galactose from + 81° to + 56° and + 43° respectively. The apparent diminution of ferricyanide-reducing power was 5·4 % for glucose and 7·8 % for galactose in the second experiment, whereas the 0·1 *N* alkali did not decrease the reducing titre of either solution for ferricyanide. As fructose shows a smaller reduction than glucose by Bertrand's method [Rohny, 1928] but a greater one with ferricyanide, the

quotient $\frac{\text{Hanes}}{\text{Bertrand}}$ changes from 1·00 to 1·035 and 1·09 in the glucose experiments, and from 1·00 to 1·055 and 1·07 in those with galactose. From these findings it may be concluded that the Lobry de Bruyn alkali rearrangement of galactose into the epimeric talose and the ketose tagatose causes merely slight changes of ferricyanide-reducing power analogous to those observed in the glucose-mannose-fructose group. The average ferricyanide-reducing power of the alkaline equilibrium mixture derived from galactose remains more than 20 % below that from glucose.

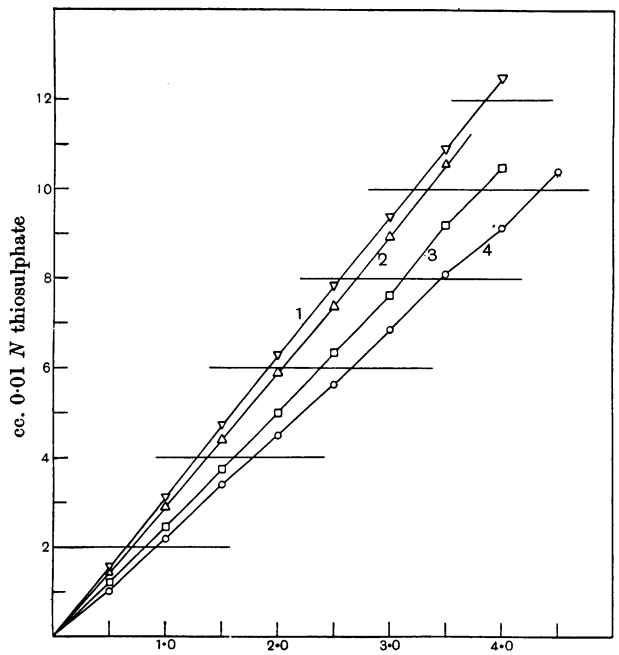



Fig. 1. Titration data for (1) Fructose (▽), (2) Glucose (△), (3) Galactose + glucose (1 : 1) (□) and (4) Galactose (○).

The reducing power of invert sugar is practically that of glucose instead of the expected average of glucose plus fructose. This parallels Rohny's [1928] findings for the Bertrand method. For the glucose-galactose mixture the curve approaches the mean value, but is slightly closer to the galactose curve. It is easy to correct the interpolations incident to the analysis of varying mixtures.

## Summary.

1. Significant differences in the reducing power of several sugars with $K_3Fe(CN)_6$ are demonstrated.

2. These differences are correlated with *trans* and *cis* configuration of the sugars in the following manner. In aldo- and keto-hexoses the configuration

between the third and the fourth carbon atom is the determining factor: in aldopentoses the configurations of $C_2$—$C_3$ and $C_3$—$C_4$ share the influence on the reducing power.

This is illustrated by the following examples:

glucose/mannose, isomerism on $C_2$—$C_3$, no difference in reducing power;
glucose or mannose/galactose $C_3$—$C_4$, *trans* > *cis*;
arabinose/ribose $C_2$—$C_3$, *trans* > *cis*;
xylose/arabinose $C_3$—$C_4$, *trans* > *cis*.

We wish to thank Dr P. A. Levene of the Rockefeller Institute for Medical Research, for a specimen of pure ribose.

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