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Preliminary communication

Chemoselective reduction of D-fructose in the presence of D-glucose

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The reaction of reducing sugars with phenol–sulfuric acid has been known for many years to be a useful colorimetric method for the quantitative, spectrophotometric determination of carbohydrates in biological mixtures[1]. A recent report by Boratynski[2] described a modification of the original procedure which allows for the selective determination of ketoses and D-glucose in the presence of other aldoses. The method was based on the presence of acetone as a third component in the phenol–sulfuric acid mixture. Moreover, it has also been possible to eliminate the interference of D-glucose in the test by simply adding boric acid to the assay reagents[2]; with this modification, the new colorimetric method permitted the selective detection of ketoses. We therefore became interested in the possibility of reversing the chemoselectivity of the test in order to determine only the aldoses in complex mixtures containing both aldoses and ketoses. Furthermore, with the modifications proposed by Boratynski[2], such chemoselectivity would render the assay specific for glucose. This could allow the monitoring of the sucrose inversion process and the quantitation of D-glucose in a high-fructose syrup by a colorimetric method.

To achieve this goal, we relied on the chemoselective reduction of ketoses in the presence of aldoses. The alditols thus produced were known not to react in the original[1] or the modified[2] colorimetric assays. Sodium borohydride and such lanthanide cations as cerium(III) have been shown to reduce ketones selectively in the presence of aldehydes, and we now report on the results of this choice of reducing conditions in the carbohydrate field.

The assays were performed as follows. To reaction mixtures (4.5 mL) containing either D-glucose or D-fructose (750 μg, 4.16 μmol) kept in an ice–water bath were added solutions (1.2 mL) of cerium(III) chloride heptahydrate (0, 3, or

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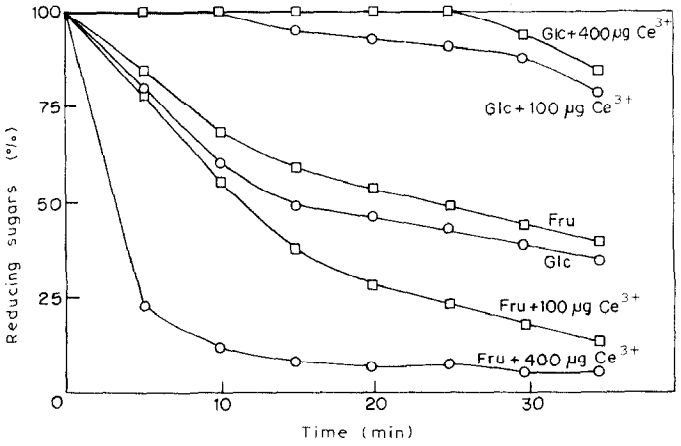
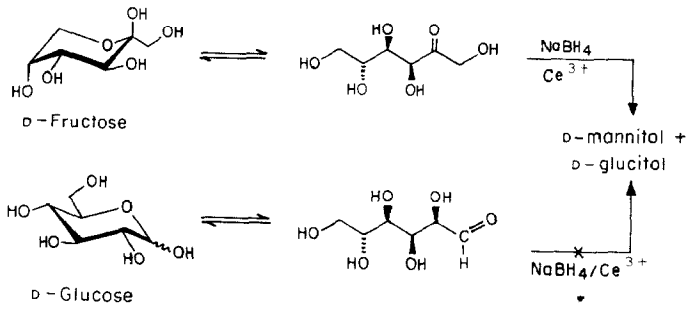


Fig. 1. Time course of reduction of D-glucose and D-fructose with sodium borohydride with and without cerium(III) chloride.

12 mg; 0, 8.05, or 32.2 μmol) and a freshly prepared solution (0.3 mL) of sodium borohydride (3 mg, 79.3 μmol) in 0.05M NaOH. Aliquots (200 μL) containing up to 25 μg (138 nmol) of carbohydrates were withdrawn at intervals (see Fig. 1) and quenched by the addition of 1.5M HCl (25 μL). The samples were then processed to determine the amount of reducing sugars by the original procedure of Dubois *et al.*[1] (λ_{max} 490 nm) or by the modification of Boratynski[2] (λ_{max} 568 nm). Briefly, the aliquots were rapidly mixed with 0.4 mL of the phenol–acetone reagent [5% acetone in 2% (w/v) aq. phenol]. Concentrated sulfuric acid (1.5 mL) was then added all at once, and the samples were immediately vortexed. The samples were allowed to stand for 60 min at 37°, and the absorbances were measured at 568 nm against blanks prepared in the absence of sugars. The percentages of reducing sugars remaining were calculated by comparison with samples lacking the borohydride.

The relative rates of reduction are illustrated in Fig. 1. The selective reduc-



Scheme 1.

tion of D-fructose to D-glucitol plus D-mannitol, and the "protection" of D-glucose against reduction (see Scheme 1) appeared optimal at a 3–4-fold excess of the cerium salt over the sodium borohydride present. In the absence of cerium(III) cations, the reduction of the aldehyde group in D-glucose was slightly faster than the reduction of the ketone group in D-fructose. The reduction of D-glucose started after an initiation period of 10 min, when there was an equivalent amount of cerium salts over the borohydride. This initial period could be extended to 20 min by using a 3–4-fold excess of cerium. At this time, the D-fructose was totally reduced. Interestingly, the cerium(III) had also catalyzed the reduction of D-fructose, thus accelerating its disappearance when compared to the reaction in the absence of the lanthanide cations. A reaction time of 20–25 min at 0°, with a large excess of the cerium salts appeared most suited for the minimal decomposition of D-glucose and the maximal reduction of D-fructose. Hence, a molar ratio of 1:8:5 for the carbonyl:cerium(III):hydride ions is highly recommended in order to achieve the high chemoselectivity desired. Selective reduction of the D-fructose in a mixture of D-fructose with D-glucose was accomplished.

It is also of interest that the requirement for an excess of the lanthanide ions might be explained by the catalytic effect of these ions on the reaction of sodium borohydride with alcohols[3].

The reasons for the selectivity observed are not yet fully understood. However, a reasonable explanation, proposed by Luche and Gemal[3], is that the aldehydes, which are more prone to hydrate formation than the ketones, would be stabilized as *gem*-diols by the cations. On the other hand, as ~0.7% of the D-fructose in aqueous solutions exists as the acyclic ("open") form, and because this value is much greater than for D-glucose, which exists as only ~0.002% of the open form and 0.006–0.01% of the hydrate form[4–6], the rationale could simply be that more carbonyl would be exposed from the ketose than from the aldose. The various equilibria could also be more shifted to the open forms, as we observed an acceleration of the rate of reduction when cerium ions were present (see Fig. 1).

In conclusion, chemoselectivity in the reduction of D-fructose and D-glucose could be achieved when cerium(III) chloride was present together with sodium borohydride. When used in combination with the modified phenol–sulfuric acid method[2], the present assay[7] appears complementary to existing ones which are selective for the determination of ketoses[8–12]. The assay is specific for glucose, and does not require enzymic methodologies[13,14].

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