Identification of Sugars from Rates of Oxime Formation

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The concept of using precise kinetic measurements to aid in the characterization of organic compounds has been applied to sugars. Hydroxylamine reacts with sugars to form oximes; the rate of the reaction can be conveniently followed spectrophotometrically. At pH 6.5 and with an excess of hydroxylamine the rate of oxime formation follows apparent first-order kinetics and is sufficiently discriminating to allow identification of many sugars. Kinetic evidence obtained in this study indicates that the reaction of ketoses and aldoses with hydroxylamine proceeds through a carbinolamine addition compound intermediate, analogous to that proposed for other aldehydes and ketones.

IDENTIFICATION OF an unknown organic compound is usually carried out by a combination of qualitative elemental analysis, determination of the functional groups present in the molecule by chemical or spectroscopic means, determination of constants such as melting point, density, refractive index, *etc.*, and ultimately preparation of a derivative. In many cases a clear distinction between possible choices cannot be made on the basis of the classical approach (and the full range of possible spectroscopic methods is not always available), and therefore a further criterion for identification is desirable. An earlier publication (1) has introduced the concept of using precise kinetic measurements as an additional aid in the characterization of organic compounds. It is the purpose of the present report to extend the concept to another class of organic compounds, namely, carbohydrates.

Sugars represent a class of organic compounds that are difficult to characterize, due partly to their great water solubility, and also to the large number of functional groups in the molecules. Numerous methods have been proposed (2–6) for the identification of sugars, the most common being chromatographic techniques. These procedures usually involve the preparation and purification of a derivative; this is at best difficult, and in addition it may not be very discriminating and may be limited to a small number of compounds.

Phenylhydrazine, semicarbazide, and similar nitrogenous bases react with sugars to form compounds that, because of their low water solubility, can be used as derivatives for identification purposes. The rate of this reaction has been used for many years as a qualitative test for identifying sugars, *e.g.*, the rate of osazone formation using phenylhydrazine as the base. Since qualitative kinetic differences exist with this reaction, it was expected that reactions with other more water-soluble bases, such as hydroxylamine, would also show kinetic differences. Appreciable water solubility of the reactants and products is essential for a study such as this in order to have homogeneous reaction conditions and also to encompass as many members of the carbohydrate class as possible.

Gladding and Purves (7) utilized the reaction of hydroxylamine and sugars to determine quantitatively the purity of various starch and cellulose derivatives. The study indicated that the rate of oxime formation of sugars did follow apparent first-order kinetics, but was nondiscriminating in that sugars could be grouped into only two categories, fast and slow reactants. This last observation is to be expected in view of the reaction conditions used and the currently accepted mechanism for oxime formation (8).

Reactions between hydroxylamine and various aldehydes and ketones have been studied extensively (8), although to the authors' knowledge the reaction between hydroxylamine and sugars has not yet been reported. It is the aim of this study to investigate the feasibility of using the reaction between hydroxylamine and sugars as a method to distinguish between various sugars, and, in addition, to compare the mechanism of this reaction with the proposed mechanism for other aldehydes and ketones.

EXPERIMENTAL

Reagents and Apparatus—Hydroxylamine hydrochloride (Baker reagent grade) was used without further purification. Sugars were obtained from customary commercial sources, and their decomposition points were the sole criteria of purity (9). Representative sugars were repeatedly recrystallized to establish that impurities normally present in sugars had no effect on the rate of oxime formation. Water was glass-distilled from alkaline permanganate. All other chemicals employed were either analytical or reagent grade purity.

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pH measurements and adjustments were made on a Corning model 12 pH meter with an expanded scale using a Beckman-type E-3 wide range glass electrode. The pH meter-electrode system was standardized against a phosphate buffer as described by Bates (10).

Water-bath temperatures were maintained to ±0.1° with Sargent Thermonitor electronic relays.

Kinetic Procedure for Spectrophotometric Determinations—The standard buffer solution employed in all runs was prepared by weighing 0.1 mole (6.950 g.) of hydroxylamine hydrochloride and dissolving to a volume of approximately 95 ml. with water. This solution was titrated to a pH of 6.50 with a saturated sodium hydroxide solution, and brought to a precise volume of 100 ml. with water. The solution was equilibrated to 25 ± 0.1° and brought to a final pH of 6.50 with the addition of one or two drops of concentrated NaOH or HCl solution.

Six milliliters of buffer solution were introduced into a 2-cm. absorption cell (or 3 ml. of buffer into a 1-cm. cell) together with 50 μl. of a 1 *M* aqueous solution of sugar. Mixing of the solutions was accomplished by shaking the absorbance cell prior to placement into the cell compartment. Absorbance changes were measured on a Cary model 15 recording spectrophotometer with thermostated cell compartments. The appearance of all the sugar oximes was followed at 245 mμ with pure buffer as a blank.

By following the experimental conditions as specified a large excess of the free base, hydroxylamine, is present and pseudo first-order kinetics ensue. From semilogarithmic plots of $(A_\infty - A_t)$ (absorbance at time infinity minus absorbance at time t) against time or by the Guggenheim method (11), pseudo first-order rate constants were obtained.

The convenience of the relatively rapid rates of aldose-oxime formation enabled these reactions to be followed by a direct recording method on the instrument. Because of the slower rates of oxime formation for the ketoses (L-sorbose and D-fructose), a nonrecording technique was employed by measuring absorbance at 1,800-sec. intervals with the absorbance cells kept at 25 ± 0.1° in a constant-temperature bath between measurements.

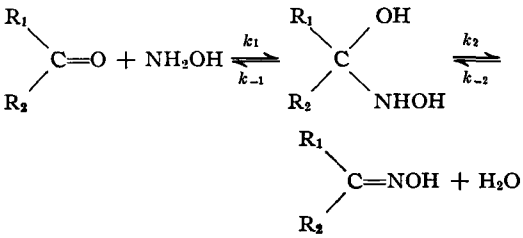
The procedure was altered slightly for some of the polysaccharides. The buffer solution was used as described above but 100 μl. of the sugar solution (cellobiose 0.5 *M*, lactose 0.5 *M*, and raffinose 0.25 *M*) was employed. This change in procedure was required by the decreased molar solubility of these di- and trisaccharides.

Formation and Characterization of Sugar Oximes—Mannose oxime was prepared by the method of Wolfrom and Thompson (12). The melting point of the oxime was found to be 174–176° [lit. m.p. 175–177° (12)]. Elemental analysis yielded N = 7.15%, calculated N = 7.175%. The molar absorptivity of mannose oxime was ϵ_{245} = 38.8.

Attempts to prepare fructose oxime by the method of Wohl (13) were considered unsuccessful. Repeated recrystallization of the product yielded a crystalline material, m.p. 120–122° [lit. m.p. 118° (13)]. However, elemental analysis for nitrogen content was more than two and a half times the calculated amount, and, in addition, the molar absorptivity was more than two and a half times the expected value. The authors assume that degradation of the fructose occurred during the reaction due to the rather harsh reaction conditions, and a species other than fructose oxime is formed.

KINETIC RESULTS AND DISCUSSION

Jencks (8), as well as others (14–16), has suggested that hydroxylamine, semicarbazide, and other similar nitrogeneous bases react with carbonyl functional groups to form additional compound intermediates which subsequently break down to product. The general reaction scheme for oxime formation can be written as:



It was also suggested that the characteristic bell-shaped pH profile obtained for reactions such as this was due to a change in rate-limiting step, which is attributed to two opposing effects. At pH values below the rate maximum there is a decrease in the concentration of the attacking free nitrogen base, and the formation of the carbinolamine addition compound is rate-determining. At pH values above the rate maximum general acid-catalyzed dehydration of the carbinolamine intermediate leading to the oxime is rate-determining (8). The pH-rate maximum is the result of a transition between these two opposing effects.

The partial pH profiles for formation of the oxime from an aldose and ketose exhibit the characteristic pH-rate maximum as shown in Fig. 1. No attempt was made to determine rates beyond the buffer capacity of hydroxylamine (below pH 4 and above pH 8), and the pH dependence is assumed to follow the profile reported for other carbonyl compounds.

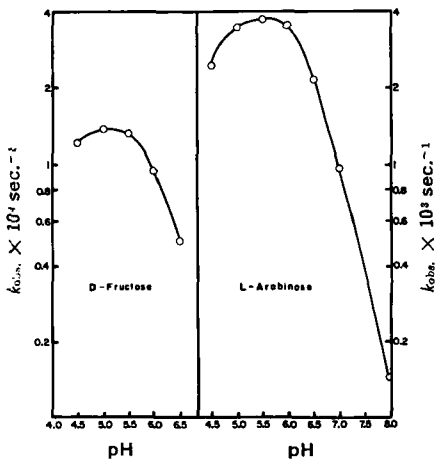


Fig. 1—Partial pH profiles for the formation of a keto- and an aldo-sugar oxime from the reaction of hydroxylamine and the respective sugar at 25° in aqueous solution.

Jencks (8) has found that the first-order rate constants for the reaction of hydroxylamine with ketones or aldehydes increase with the concentrations of free base and hydrogen ions so that the overall reaction is third order. However, at higher concentration of base the rate at a given pH becomes independent of the concentration of free base and approaches first-order kinetics; this was determined to be the case for the reaction between sugars and hydroxylamine (the terminal portions above pH 6, of the pH profiles shown in Fig. 1, have slopes that approach one). At acid pH (low concentration of nitrogen base) the first-order rate constants were found to increase with increasing free hydroxylamine, indicating that the reaction is second order with respect to hydroxylamine in this region, which is in agreement with published work.

Elemental analysis of mannose oxime indicates that only 1 mole of hydroxylamine is consumed per mole of oxime formed, unlike the reactions between sugars and other nitrogenous bases such as phenylhydrazine, which results in the addition of 2 moles of the base to 1 mole of sugar. The oxime reaction is second order with respect to hydroxylamine, but this is because the reaction is general acid-catalyzed and the second mole of hydroxylamine is serving as a general acid. From the above data the authors conclude that keto and aldo sugars react with hydroxylamine to form oximes *via* the same mechanism that is proposed for other aldehydes and ketones.

There is the possibility with aldo and keto sugars that tautomeric conversion from the cyclic ring structure to the open chain analog prior to reaction with hydroxylamine could be rate-limiting in this sequence (17). However, the rate of mutarotation for many of the sugars does not parallel the rate of oxime formation; *e.g.*, fructose shows the slowest rate of oxime formation, but is reported (7) to have a relatively fast rate of mutarotation. Although sugars in the solid state as well as in solution exist mainly in the cyclic ring structure, it is assumed, based on the above, that conversion to the open chain is not rate-determining in the reaction sequence.

Quantitative conversion of the aldo sugar to oxime occurred in the reaction, as calculated *via* the molar absorptivity of mannose oxime. The keto sugar oximes (fructose and sorbose) have molar absorptivities that are three times greater than the aldo sugars, as observed from the spectrophotometric data. It is assumed that the keto sugars are also quantitatively converted to oximes.

ANALYTICAL RESULTS

Figure 2 shows typical ultraviolet spectra of the formation of a sugar oxime at various time intervals. This time course absorbance profile demonstrates that the reaction can conveniently be followed at 245 mμ. Because of the absence of any appreciable absorbance by sugars in the concentrations employed and at a wavelength of measurement, the observed absorbance change was due solely to formation of the sugar oxime.

Choice of reaction conditions is always important in a study of this type. In this study reaction conditions are even more critical than usual in that the variables pH and hydroxylamine concentration affect not only the rate but also the order of the reaction. At pH values below the maximum, pseudo first-order conditions are approached but not rigorously adhered to because of the decreased concentration of the attacking free nitrogen base. At the pH maximum, spectral interference due to overlap of the reaction sequence is observed. At pH values above the maximum, pseudo first-order conditions rigorously hold. The choice of pH 6.50 for the kinetic determinations was based on two factors. First, pseudo first-order conditions exist. Second, the rates of oxime formation are sufficiently rapid at this pH so that the procedure is not self-prohibitive with respect to time.

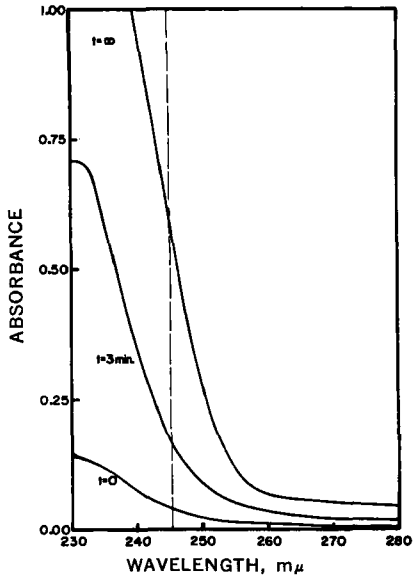


Fig. 2—Absorbance spectra of D-*arabinose-oxime ormation in 1* M *hydroxylamine buffer at pH = 6.50, 25°. Dashed line (245 mμ) indicates the wavelength at which the rates of oxime formation were followed.*

Table I lists the observed first-order rate con-

TABLE I—FIRST-ORDER RATE CONSTANTS FOR THE FORMATION OF OXIMES FROM THE REACTIONS OF VARIOUS SUGARS AND HYDROXYLAMINE IN AQUEOUS SOLUTION

Sugar	Decomposition Point	$k_{obs.} \times 10^3$ (sec.$^{-1}$)[a]	SD[b]	No. of Determinations
Raffinose	80 (hyd.)	0.673	0.023	4
D-Glucose	90 (hyd.)	0.208	0.009	4
D-Ribose	95	2.450	0.080	4
Maltose	100	0.180	0.010	4
D-Fructose	104	0.050	0.003	2
L-Rhamnose	105	0.841	0.037	7
D-Mannose	132	1.330	0.063	4
D-Xylose	145	1.360	0.030	6
D-Arabinose	158	1.890	0.105	4
L-Arabinose	160	1.920	0.089	9
L-Sorbose	160	0.023	0.002	2
D-Galactose	170	1.200	0.065	5
Gentiobiose	186	0.222	0.002	3
Lactose	203	0.322	0.004	4
Cellobiose	225	0.260	0.014	6

[a] At pH = 6.50 in 1.0 *M* hydroxylamine solution at 25°. [b] $SD = [\Sigma(x_i - \bar{x})^2/n - 1]^{1/2}$.

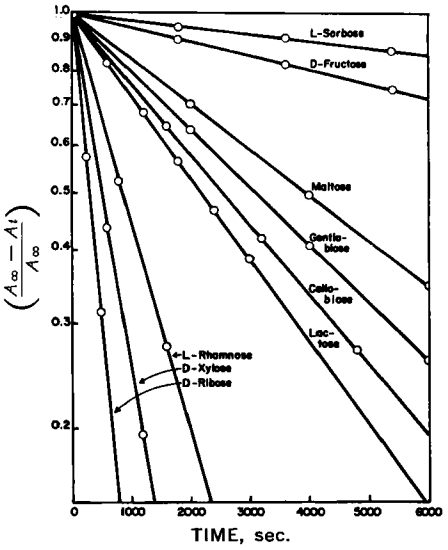


Fig. 3—Normalized representation of some sugar-oxime formations. The reactions were carried out in 1 M hydroxylamine, pH = 6.5 at 25°.

stants for sugar-oxime formations as well as sugar decomposition points. It should be noted that no rate constant for sucrose appears; sucrose does not form an oxime. Glyceraldehyde and ascorbic acid were also investigated, but the reactions did not appear to follow apparent first-order kinetics.

Figure 3 illustrates a normalized graphical representation of some sugar-oxime formations. This displays the approximate 125-fold difference in rate observed between the fastest and slowest oxime formations.

Variation of the ionic strength from 1.0 to 3.0 had a negligible effect on the rate. For this type of reaction only a secondary salt effect would be expected.

DISCUSSION

Utilizing kinetic measurements as an aid in the identification of the sugars as described above gives one the important advantage of a rapid and precise method without the necessity of a common derivative of the sugar. It is apparent from Table I that, with a combination of decomposition point and rate constant of oxime formation, it is possible to differentiate any sugar listed in the table.

The more than 10-fold difference in rate between the keto and aldo sugars is unexpected on the basis of steric and inductive effects. The structural difference between the ketoses and aldoses reported here is the presence of a hydroxymethyl group alpha to the carbonyl group in the ketoses and a hydrogen in the aldoses. Although the authors would predict a small steric effect for dehydration of the tetrahedral intermediate, this effect together with inductive effect cannot explain the difference in rate.

The procedure suggested here for identifying sugars is a relatively sensitive method. For example, a typical experiment with any of the aldoses included in this study would require approximately 5×10^{-5} mole of sugar, while the ketoses would require approximately 1.7×10^{-5} mole of sugar. Naturally this sensitivity could be increased 10-fold by using the expanded scale on the spectrophotometer. Many times only a limited amount of unknown sugar is available for identification, and therefore a sensitive characterization procedure is desirable.

Spectroscopic techniques (NMR, IR, *etc.*) are limited in differentiating among members of this class of compounds. The procedure suggested here for identifying sugars is rapid, sensitive, and quite discriminating. As with most solution kinetic measurements the limiting factor in reproducibility lies in the pH measurements.

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Keyphrases

Sugars—identification
Oxime formation rate—sugar identification
Hydroxylamine HCl—reactant
UV spectrophotometry—oxime formation monitoring