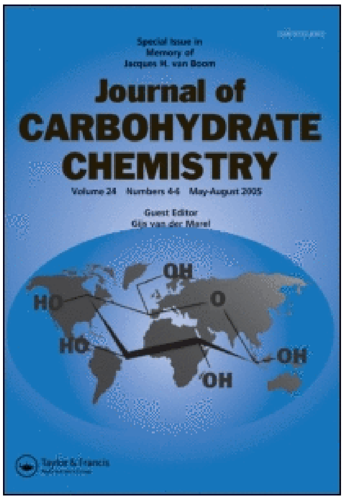

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CHROMIC OXIDATION OF 2-DEOXY-D-GLUCOSE.

COMPARATIVE STUDY WITH ALDOSES. PART I.

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ABSTRACT

A rate law for the oxidation of 2-deoxy-D-glucose (2DG) by Cr(VI) in perchloric acid has been derived. This rate law corresponds to the reaction leading to the formation of 2-deoxy-D-gluconic acid (2DGA). No cleavage to carbon dioxide takes place when a twenty-fold or higher excess of aldose over Cr(VI) is employed. Kinetic constants are interpreted in terms of the absence of an hydroxyl group at C-2 on the stability of the chromic ester formed in the first reaction step. Free radicals formed during the reaction convert Cr(VI) to Cr(V). The latter species was detected by EPR measurements.

INTRODUCTION

Chromate, the dominant form of chromium(VI) in neutral aqueous solutions, readily enters the cell using the cell's general anion transport system. Once chromate traverses the initial barrier of the cell membrane, it may be reduced by amino acids, ascorbic acid, carboxylic acids, molecules containing sulfhydryl groups and assorted other small molecules.[1-2] The metabolism of chromate involves the generation of reactive intermediates which ultimately bind to cellular constituents and disrupt their function in the cell.[3] Cr(VI) has a strong tendency to form chromic esters with substrates containing oxygen atoms, such as sugars and thiol groups, e.g. glutathione.[4-5]

Oxidation of different aldoses with Cr(VI) shows a difference in the respective oxidation rate, which could be attributed to the different arrangement of the hydroxyl groups in the molecule.[6] In these reactions, the complex formed between Cr(VI) and the sugar is the first step before the electron transfer, and the stability of the complex depends on the steric disposition of the hydroxyl groups. For example, comparing L-rhamnose and D-mannose oxidation, we observed that the kinetic equilibrium constant for chromic ester formation, K_1, is 2.63 and 5.40, respectively. On the other hand, the oxidation rate constants of these esters show relative inverse values.[6]

This difference could be attributable to the stability of the chromic esters if two or three hydroxyl groups are involved (OH from C-1 and C-2 (L-rhamnose), OH from C-1, C-2 and C-6 (D-mannose)).[6]

Assuming that only some hydroxyl groups of the sugar contribute to the formation and stabilization of the chromic ester previous to the oxidation process [in which Cr(VI) bind OH of C-1], we can to expect that the stabilization of the chromic ester would be much lower with 2-deoxy-D-glucose (2DG) than with L-rhamnose or D-mannose due to the absence of OH at C-2.[6]

In the present work we wish to demonstrate this hypothesis and to suggest a tentative reaction mechanism.

RESULTS AND DISCUSSION

Reaction Products. The concentration of Cr(VI) was kept constant at 8.0×10^{-4} M, while the deoxyaldose concentrations were varied between 0.02 and 0.32 M, at a constant ionic strength of 1.0 M, T= 50 °C. In the presence of excess of 2-deoxy-D-glucose (2DG) (kinetic conditions), CO_2 was never detected when a slow stream of pure nitrogen was flushed through the reaction mixture to sweep the carbon dioxide that might be produced into a barium hydroxide solution. 2-Deoxy-D-gluconic acid (2DGA) was the mayor oxidation product.

Kinetic Results. The pseudo-first order rate constants (the observed rate constants, k_{obs}) were determined from the linear part of the curve of ln Abs vs. time. The table summarizes values of k_{obs} for various concentrations of aldose at fixed concentrations of perchloric acid. Plots of k_{obs} vs. [2DG] at different fixed concentrations of hydrogen ion showed straight lines which pass through the origin. The second-order rate constants, k_1, were calculated from the slope of these straight lines. Plot of k_1 vs. $[H^+]^2$ shows that the acidity dependence of k_1 can be written as follows:

$$k_1 = k_o + k_H[H^+]^2$$

where $k_o = 2.38 \times 10^{-3}\ s^{-1}M^{-1}$ and $k_H = 5.03 \times 10^{-2}\ s^{-1}M^{-3}$.

Table. Pseudo-first order rate constants for the oxidation of 2-deoxy-D-glucose by Cr(VI) in acidic medium.

	$k_{obs}{}^{a} \times 10^{4}$, s^{-1}, for $[HClO_4]$, M, of					
[2DG], M	0.10	0.20	0.25	0.50	0.75	1.00
0.02				6.33	10.46	15.26
0.04		2.43		9.25	16.92	26.09
0.08	1.45	3.88	6.03	15.28	27.03	46.52
0.12	1.92	5.18	8.37	22.44	38.30	
0.16	2.65	6.36	11.68	27.41	50.93	87.49
0.20	3.45	8.33	15.03	34.40	62.27	113.27
0.24	4.35	8.89	18.23	38.10	76.74	130.20
0.32	5.63					

[a] Rate constants obtained from multiple determinations.

T = 50.0 °C, $[Cr(VI)]_o = 8.0 \times 10^{-4}$ M, I = 1.0 M, λ = 350 nm

According to these results, the rate law might be expressed as:

$$-d[Cr(VI)]/dt = k_{obs}[Cr(VI)] = (k_o + k_H[H^+]^2)[2DG][Cr(VI)] \qquad (1)$$

No spectrophotometric detection of Cr(V) at 750 nm was observed, this wavelength being typical of this intermediate[7] in spite of the fact that the concentration of the reaction mixture varies. Similar results were found when L-rhamnose and D-mannose were submitted to the same reaction conditions.[6] Failure to detect this intermediate may be attributed to a low molar extinction coefficient for the Cr(V) complex or to a low proportion of such complex at t_{max}, caused by high rates of disappearance of Cr(V) with respect to Cr(VI).[6]

At present, the proposed mechanism for the oxidation of 2DG by Cr(VI) in acidic medium is postulated as follows:

(2) $2DG + Cr(VI) \overset{Q_1}{\rightleftharpoons} \underset{I^-}{2DG\text{-}Cr(VI)}$

(3) $I^- \xrightarrow{k_2} Cr(IV) + 2DGA$

(4) $I^- \xrightarrow[k_3]{H^+} IH \xrightarrow[k_4]{H^+} IH^+ \xrightarrow[k_5]{} Cr(IV) + 2DGA$

According to this mechanism, the first step represents the formation of an anionic intermediate between Cr(VI) and 2DG. This intermediate I^- decomposes to the products through two parallel intramolecular two-electron transfer steps to yield Cr(IV) and 2DGA (one of these paths being catalized by acid).

Thus, if I^- behaves as a steady state, under our kinetic conditions the rate law could be written as:

$$-d[Cr(VI)]/dt = (k_2Q_1+k_aQ_1[H^+]^2)[Cr(VI)][2DG]/(1+k_2/k_{-1}+k_3/k_{-1}[H^+]) \qquad (5)$$

where $k_a=k_3k_4k_5$

In this way, if 1 is the greater term in the denominator, eq (5) will be simplified to the experimentally observed form, where $k_o= k_2Q_1$ and $k_H = k_aQ_1$.

Another mechanism which fit well with the experimental kinetic law is the direct reaction between Cr(VI) and 2DG yielding the redox products through two parallel redox steps:

(6) $Cr(VI) + 2DG \longrightarrow Cr(IV) + 2DGA$

(7) $Cr(VI) + 2DG \xrightarrow{H^+} \xrightarrow{H^+} Cr(IV) + 2DGA$

Anyway, both mechanims denote that 2DG binds Cr(VI) weakly, in accordance with no spectrophotometric nor kinetic indication of an ester formation.

After the slow redox steps equations 8, 9 and 10 may take place:

(8) $Cr(IV) + 2DG \longrightarrow Cr(III) + 2DG\cdot$

(9) $2DG\cdot + Cr(VI) \longrightarrow Cr(V) + 2DGA$

(10) $Cr(V) + 2DG \longrightarrow Cr(III) + 2DGA$

Cr(IV) reacts rapidly with another 2DG molecule affording a free deoxyaldose radical (2DG •), whose existence was demonstrated through the polymerization of acrylonitrile added to the reaction mixture.

Radicals react with Cr(VI) to yield the oxidation product and Cr(V) which is finally reduced to Cr (III) by reaction with another 2DG molecule.

No Cr(V) EPR signals were detected in the dilute solutions used for kinetics measurements. The fact that no signals were observed does not necessarily mean that they were not present. It may mean that the sensitivity of the instrumentation used was not sufficient to detect the Cr(V), at the concentration present. Given the high concentrations of chromium that were required to detect the signals in other experiments, we believe that it is highly likely that Cr(V) was present in all the experiments, but that its signal was not detected due to low sensitivity of the measurements. When 2DG and Cr(VI) saturated solutions were used at pH =1.0 there appeared a signal at g_{iso}= 1.978, typical of Cr(V) intermediary[8-9] with a very low intensity.

This signal grew in intensity at pH= 7.0, which is in agreement with the low oxidation rate observed as pH increases. This means that the life time of the Cr(V)-complex increases as the proton concentration is lowered.

When aldoses are oxidized by Cr(VI) under the same experimental conditions, plots of k_{obs} vs. [aldose] at different acidities show curves saturated at high aldose concentrations,[6] revealing that a kinetically rather stable complex is formed between aldoses and Cr(VI), with $K_f \approx 2$-6, at 33 °C. Even though several linkage isomers might be formed by coordination of sugars with Cr(VI) via any pair of properly disposed vicinal groups, it seems reasonable to think that C1-OH is involved in the redox steps precursor complex. Thus, in aldoses, C2-OH favors stabilization of a five member ring complex with Cr(VI). On the other hand, for 2DG, the absence of a hydroxyl group at C2 compels the sugar to bind Cr(VI) through the anomeric OH only and, as a consequence, its ability to stabilize Cr(VI) [and eventually Cr(V)] is reduced; a fact which explains the difference in the kinetic behavior.

EXPERIMENTAL

Materials and Methods. 2-Deoxy-D-glucose (2DG) (Sigma) and potassium dichromate (BDH), were used without further purification. Stock solutions of perchloric acid were prepared from 70% perchloric acid (Merck, AR) and were analyzed by titration employing standard analytical methods.[10] Mixtures of sodium perchlorate and perchloric acid were used to maintain a constant ionic strength of 1.0 M at different hydrogen ion concentrations. Aqueous solutions were prepared in doubly distilled water.

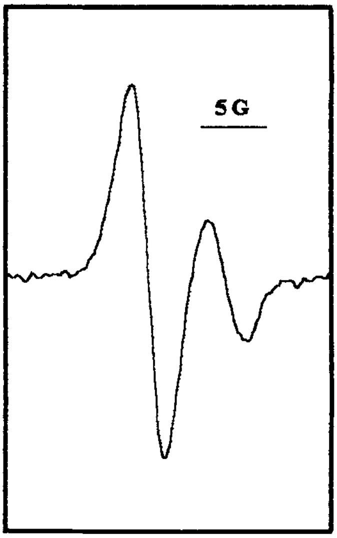

Fig. The EPR signal of a mixture of 2DG and Cr(VI) 4 min after mixing. 2:1 mole ratio, pH= 7.0, T= 25 °C.

Kinetic Measurements. Rates of disappearance of Cr(VI) were measured spectrophotometrically at 350 nm with a Gilford Response II spectrophotometer with fully thermostated cell compartments, using a 1 cm path-length cell. The substrate is transparent at this wavelength, and neither Cr(III) nor the oxidized products has any appreciable absorption at this wavelength. The concentration of Cr(VI) was kept constant at 8.0 x 10^{-4} M, while the aldose concentration was varied between 0.02 and 0.32 M (ratios of [aldose]/ [Cr(VI)] from 25 to 400). Each reaction was initiated by addition of potassium dichromate solution to 2DG solution containing the required amount of substrate for the desired 2DG/Cr(VI) mole ratio.

Product Analysis. Oxidation products were identified by paper chromatography which was performed in the descending mode with Whatman n° 1 paper and 1-butanol-acetic acid-water in three different relationship (4:1:1, 4:1:5 and 4:3:2) as eluting solvent, being the reference an authentic sample of 2-deoxy-D-gluconic acid. Spots were detected by employing alkaline silver nitrate,[11] 60% perchloric acid in acetone[12] and *p*-anisidine reagents.[13]

Free Radicals Detection. A solution of potassium dichromate (1.0 ml, 1.0 M) in 0.5 M $HClO_4$ was added to a mixture of 2-deoxy-D-glucose (1.0 ml, 0.6 M) in M $HClO_4$

and acrylonitrile (1.0 ml) at 50 °C. After 30 min a white precipitate of a polymer appeared slowly. The control experiment (no deoxyaldose or oxidant agent present) did not show the formation of precipitate.

EPR Spectroscopy were recorded on a Bruker spectrometer model ER-200. The g values were measured by reference to diphenylpicrylhydracyl (DPPH, g= 2.0036). Spectra were acquired at a frequency of ca. 9.8 GHz, with a center field of 3550 G, modulation of 100 kHz (amplitude: 5.0 G), sweep width of 200 G. The cavity and sample were kept at 25 °C. Aqueous neutral solutions saturated with $Cr_2O_7^{2-}$ and 2DG were used for Cr(V) detection.

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