

KINETICS AND MECHANISM OF THE OXIDATION OF D-GALACTOSE BY COPPER(II) IN ACIDIC MEDIUM

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(Received 9 August 1994; accepted 12 September 1994)

Abstract—The oxidation of D-galactose by Cu^{II} has been studied in acetate buffers within the pH range 4–4.75 at 110°C. The observed pseudo-first-order rate constant is given by $k_{\text{obs}} = \{k_a + k_b/[\text{H}^+] + k_c/[\text{H}^+]^2\}[\text{gal}]/\{1 + (k_d + k_e/[\text{H}^+])[\text{gal}]\}$ where $k_a = 2.77 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$, $k_b = (1.22 \pm 0.11) \times 10^{-7} \text{ s}^{-1}$, $k_c = (1.12 \pm 0.16) \times 10^{-12} \text{ M s}^{-1}$, $k_d = 2.08 \text{ M}^{-1}$, $k_e = (2.75 \pm 0.33) \times 10^{-5}$. This rate law corresponds to the formation of galactonic acid and Cu^I when a 10 fold or higher excess of D-galactose over Cu^{II} is employed. The results are discussed in terms of a possible mechanism of oxidation of the enediol of galactose by CuOAc⁺, present in solution.

After Ca^{II} binding, Cu^{II} binding to carboxylates has been the most studied. This is in part due to several observations of biologically significant interactions between Cu^{II} and sugars. Cu^{II} carbohydrate complexes have been extensively studied because of their potential efficacy in the treatment of rheumatoid arthritis.^{1,2} However, the possibility of redox reactions between sugars and Cu^{II} may lead to the production of free radicals involved in carcinogenic and ageing cellular processes.³ We have therefore studied the oxidation of sugars by Cu^{II}.⁴ In this work we have investigated the kinetics of oxidation of D-galactose (Scheme 1) at low pH in the absence of oxygen.

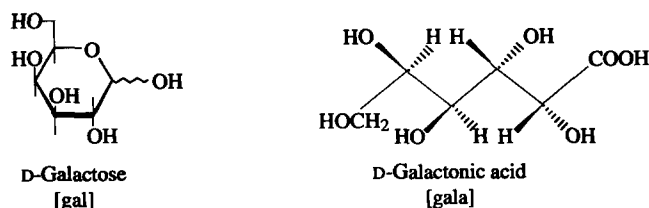
EXPERIMENTAL

Kinetics

The reaction mixture was prepared by mixing Cu(ClO₄)₂ in appropriate solutions of NaOAc–AcOH and variable amounts of D-galactose (gal)

(Sigma grade) (Scheme 1) in order to obtain the desired ratio of oxidant/reductant. Ionic strength was maintained at $I = 0.59 \text{ M}$ throughout all experiment employing sodium perchlorate (Fluka grade). Oxygen was purged from the solutions by bubbling nitrogen through the solutions protected by serum caps. Residual dioxygen in the nitrogen was removed by passing the nitrogen through scrubbers containing pyrogallol. All solutions were prepared with deionized water which had been distilled from alkaline permanganate in glass apparatus. The sealed solutions were brought to the desired temperature within 15 s using an electrically regulated thermostat. A set of 20 ampoules for each gal/Cu^{II} ratio was prepared. The reaction was quenched by quickly cooling the solutions. The Cu₂O produced in the reaction was filtered off and 50% NH₃ was added to the filtrate. Unreacted Cu^{II} was determined spectrophotometrically as the [Cu(NH₃)₄]²⁺ complex ($\lambda_{\text{max}} = 620 \text{ nm}$, $\epsilon = 42 \text{ M}^{-1} \text{ cm}^{-1}$) on a Shimadzu UV-140 double beam spectrophotometer. The nature of the precipitate was confirmed by treating it with hydrochloric acid in the presence of glycerine to stabilize Cu^I, thereby avoiding its dismutation to Cu^{II} and Cu⁰. The complete dissolution of the precipitate confirms the formation of Cu₂O and the absence of Cu⁰. A large excess of gal (10–100 fold) was used for all the

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Scheme 1.

kinetic measurements. Pseudo-first-order rate constants were determined from the slope of $\ln[\text{Cu}^{\text{II}}]$ vs time plots. All the rate constants are the average of three determinations at each set of conditions.

The pH was checked with a chemcadet pH meter calibrated against standard NBS buffer solution provided by the Cole-Palmer Instruments Co., at temperatures between 70 and 95°C, pH values at 110°C were obtained by extrapolation.

Product analysis

Qualitative identification of carbohydrates oxidation products was carried out by paper chromatography using a mixture of 4 : 1 : 5 *n*-butanol–AcOH–H₂O as eluent. Paper chromatograms were developed by two reagents, silver nitrate/NaOH/Na₂S₂O₃⁵ and β -naphthol–sulphanilamide,⁶ specific for the detection of aldonic acids. In this way, D-galactonic acid (gala) (Scheme 1) was identified, by comparison with an authentic sample, as the only oxidation product, either when an excess of oxidant or organic substrate was employed.

The stoichiometry of the oxidation of gal by Cu^{II} was determined by mixing gal and Cu^{II} solutions to final concentrations of 0.1 and 0.01 M respectively, at pH = 4.75, $T = 110^\circ\text{C}$, for 40 min. The amount of reacted Cu^{II} per mole of sugar was determined by indirect iodometry and it was found to be 2 ± 0.2 mol Cu^{II} per mol of gal.

Detection of free radicals

A solution of Cu(ClO₄)₂ (5 ml, 0.01 M) was added to a mixture of gal (0.05 mmol) and acrylonitrile (0.03 mol) to a final volume of 7 cm³. The reaction was allowed to go to completion at 110°C (judged by the change in colour of the solution). A white gel appeared slowly. Blanks experiments in the absence of gal or Cu^{II} did not show gel formation.

RESULTS AND DISCUSSION

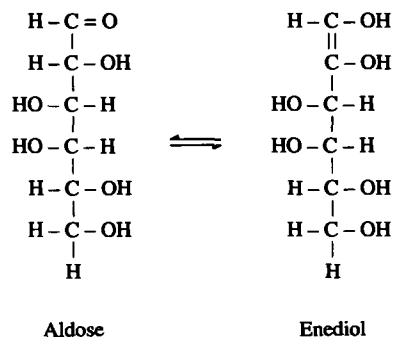
The possibility of gal isomerization after heating at 110°C in the pH range used in this work was

evaluated. Paper chromatography of heated gal solutions did not show any isomer formation as compared with authentic samples. The UV–vis spectra of gal over the whole pH range used in this work shows an absorbance band at 284 nm, either at 25°C or after heating at 110°C. A minor band appears at 325 nm after heating in the pH range 4–6 and in basic medium (pH = 10, $T = 110^\circ\text{C}$, [gal] = 0.0555 M). This means that aldehyde⁷ as well as enediol⁸ (Scheme 2) are present in the reaction mixture and the last may be responsible of the particular kinetic behaviour of galactose, as discussed below.

Within the range of concentrations of this study, no precipitate of cupric hydroxide was produced by mixing the reactants at pH 4–5, and the reaction mixture was perfectly homogeneous before the reactions started. However, it did not remain homogeneous throughout because of the formation of cupric oxide as a heterogeneous reaction product during the kinetic runs. No autocatalysis by cuprous oxide was observed. This was confirmed by adding Cu₂O to the mixture without any change in the experimental data.

When the oxygen was not removed from the solution, oxidation started after a slight induction period, which was essentially eliminated when N₂ was bubbled through before the reaction was initiated.

The pseudo-first-order oxidation rate (k_{obs}) was studied as a function of the concentration of gal (0.1–0.8 M) and pH (4–4.75) at 110°C (Table 1).



Scheme 2.

Table 1. Values of k and K at different pH

pH	4.00	4.25	4.50	4.60	4.75
$k \times 10^3$ (s ⁻¹)	1.42	2.50	2.53	3.02	3.59
K (M ⁻¹)	2.56	2.22	3.26	3.01	3.67

Curves of k_{obs} vs gal concentration show saturation at high [gal], typical of an intermediate complex formation (Fig. 1). Plots of k_{obs}^{-1} vs [gal]⁻¹ are given in Fig. 2.

The effect of the [gal] on k_{obs} can be written as:

$$k_{\text{obs}} = kK[\text{gal}]/(1 + K[\text{gal}]). \quad (1)$$

Values of k and K for different pH are given in Table 1 and it is easily observed that either k or K decay with acidity. Plots of k and K vs [H⁺]⁻¹ give straight lines with positive intercepts (Figs 3 and 4).

$$k = a + b/[\text{H}^+] \quad (2)$$

$$K = c + d/[\text{H}^+] \quad (3)$$

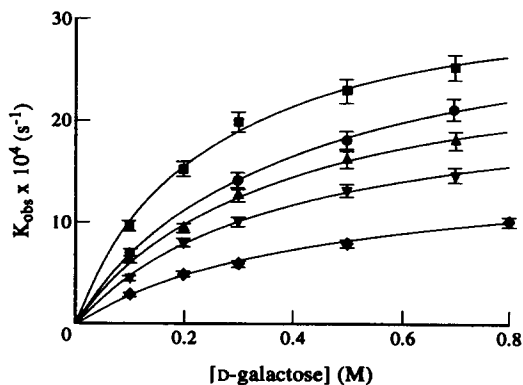


Fig. 1. Effect of [D-galactose] on k_{obs} . $T = 110^\circ\text{C}$; $I = 0.59$ M; [NaOAc] = 0.056 M; ■ pH 4.75; ● pH 4.60; ▲ pH 4.50; ▼ pH 4.25; ◆ pH 4.00.

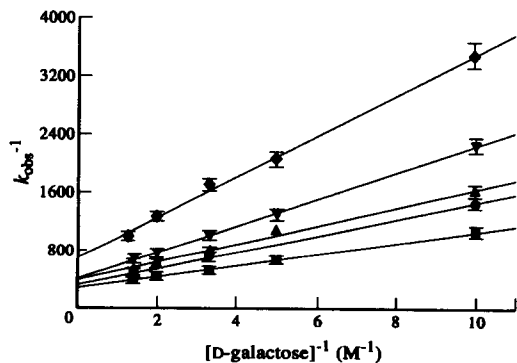


Fig. 2. Plots of k_{obs}^{-1} vs [gal]⁻¹. $T = 110^\circ\text{C}$; $I = 0.59$ M; [NaOAc] = 0.056 M; ■ pH 4.75; ● pH 4.60; ▲ pH 4.50; ▼ pH 4.25; ◆ pH 4.00.

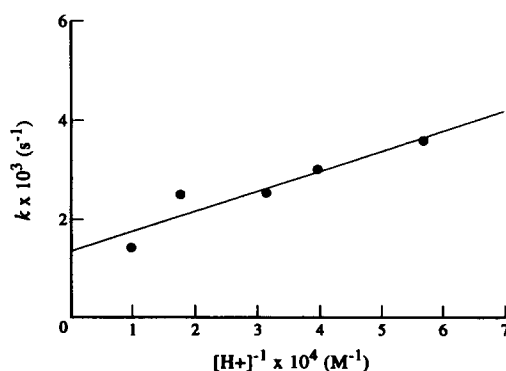


Fig. 3. Effect of acidity on k . $T = 110^\circ\text{C}$; $I = 0.59$ M; [NaOAc] = 0.056 M.

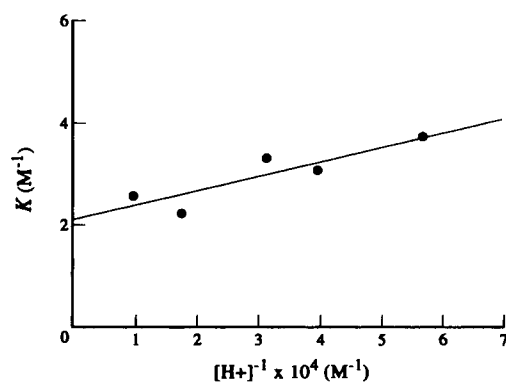


Fig. 4. Effect of acidity on K . $T = 110^\circ\text{C}$; $I = 0.59$ M; [NaOAc] = 0.056 M.

where $a = 1.3 \times 10^{-3}$ s⁻¹, $b = (4.09 \pm 0.35) \times 10^{-8}$ M s⁻¹, $c = 2.08$ M⁻¹ and $d = (2.75 \pm 0.33) \times 10^{-5}$.

The complete rate law for the reaction thus is given by:

$$\begin{aligned} -d[\text{Cu}^{\text{II}}]/dt &= k_{\text{obs}}[\text{Cu}^{\text{II}}]_{\text{T}} \\ &= (a + b/[\text{H}^+])(c + d/[\text{H}^+])[\text{gal}] \\ &\quad \times [\text{Cu}^{\text{II}}]_{\text{T}} \{1 + (c + d/[\text{H}^+])[\text{gal}]\}^{-1} \quad (4) \end{aligned}$$

or

$$\begin{aligned} k_{\text{obs}}[\text{Cu}^{\text{II}}]_{\text{T}} &= \{k_a + k_b/[\text{H}^+] + k_c/[\text{H}^+]^2\} \\ &\quad \times [\text{gal}][\text{Cu}^{\text{II}}]_{\text{T}} \{1 + (k_d + k_e/[\text{H}^+])[\text{gal}]\}^{-1} \end{aligned}$$

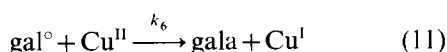
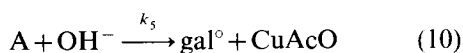
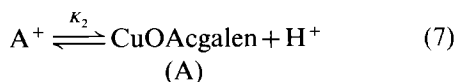
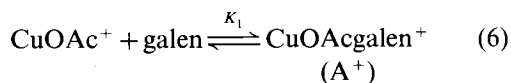
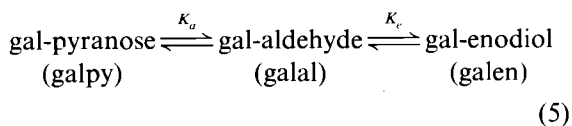
where $[\text{Cu}^{\text{II}}]_{\text{T}}$ represents the total Cu^{II} concentration and $k_a = 2.76 \times 10^{-3}$ M⁻¹ s⁻¹, $k_b = (1.22 \pm 0.11) \times 10^{-7}$ s⁻¹, $k_c = (1.12 \pm 0.16) \times 10^{-12}$ M s⁻¹, $k_d = 2.08$ M⁻¹ and $k_e = (2.75 \pm 0.33) \times 10^{-5}$. These values of k_a , k_b , k_c , k_d and k_e were used to find k_{calc} in Table 2, which are in good agreement with experimental data over the concentration range studied.

Table 2. Observed and calculated pseudo-first-order rate constants for different acidities

[gal] (M)	$k_{\text{obs}}(k_{\text{calc}}) \times 10^4 \text{ (s}^{-1}\text{)}$					
	0.1	0.2	0.3	0.5	0.7	0.8
pH						
4.00	2.94(3.31)	4.90(5.57)	5.89(7.20)	7.92(9.40)	—	10.0(11.3)
4.25	4.55(4.21)	7.99(6.98)	10.0(8.95)	13.1(11.6)	14.6(13.2)	—
4.50	6.29(5.98)	9.41(9.73)	12.6(12.3)	16.1(15.6)	18.0(17.8)	—
4.60	7.01(7.13)	—	14.1(14.4)	18.0(18.1)	21.0(20.4)	—
4.75	9.60(9.66)	15.2(15.3)	19.8(18.9)	22.8(23.4)	25.8(26.0)	—

$T = 110^\circ\text{C}$; $[\text{NaOAc}] = 0.056 \text{ M}$; $I = 0.59$.

According to all experimental data, we propose the mechanism in Scheme 3:



Scheme 3.

Other studies made in basic solution have showed that enediols have higher ability to coordinate Cu^{II} ⁹⁻¹² previous to redox steps. Thus, in this mechanism we propose the enediol as the reactive form of gal, which forms a kinetically rather stable complex A⁺. A little inspection of structural characteristics of gal favours this supposition. Even when the pyranose form is the major one in solution, the conformation and disposition of hydroxyl groups in the cyclic form of gal are not the best arrangement to bind Cu^{II} to give a detectable intermediate complex where C₁OH should be implied.^{13,14} However, if the open chain form is considered, coordination ability towards Cu^{II} should be enhanced through a 1,2-enediol *cis* configuration which is able to give a five-membered ring intermediate complex.¹⁵ On the other hand, we consider

CuOAc^+ as the reactive species. It has been reported that, under our experimental conditions, dissolved copper exists mainly as CuOAc^+ ^{16,17} and binuclear complexes, which are formed at higher pH than those used here,^{18,19} may be disregarded. Further, in acetate buffer, CuOAc^+ has already been reported to be an active species in oxidation reactions.²⁰

Complex A⁺ is in equilibrium with A which may be formulated as an enediol-Cu^{II} complex from which a proton has been removed, presumably from Cu^{II} bound water or from a hydroxyl group on galactose enediol. Complexes A⁺ and A lead to the dissociation of a galactose radical from Cu^I through an intramolecular electron-transfer process (eqs (8) and (9)) or a base catalysed path (eq. (10)). If it assumed that eqs (5)–(7) involve rapidly established equilibria, then the pseudo-first order rate constant in Scheme 3 is given by:

$$v = \{k_4 K[\text{gal}] + k_3 K_2 K[\text{gal}]/[\text{H}^+] + k_5 K_2 K K_w[\text{gal}]/[\text{H}^+]^2\} [\text{Cu}^{\text{II}}] \quad (12)$$

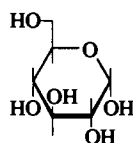
where $K = K_1 K_a K_e$

$$[\text{Cu}^{\text{II}}]_{\text{T}} = [\text{Cu}^{\text{II}}](1 + K[\text{gal}] + K_2 K[\text{gal}]/[\text{H}^+]). \quad (13)$$

Then replacing Cu^{II} in eq. (12)

$$v = \{k_4 K[\text{gal}] + k_3 K_2 K[\text{gal}]/[\text{H}^+] + k_5 K_2 K K_w[\text{gal}]/[\text{H}^+]^2\} [\text{gal}][\text{Cu}^{\text{II}}]_{\text{T}} \times \{1 + K[\text{gal}] + K_2 K[\text{gal}]/[\text{H}^+]\}^{-1}. \quad (14)$$

In a previous work we have studied oxidation of D-glucose (glc) (Scheme 4) by CuOAc^+ under the same experimental conditions, and the effect of glc concentration on k_{obs} did not show saturation at high (glc).⁴ This different kinetic behaviour between the oxidation of glc and gal by Cu^{II} requires some



D-Glucose
[glc]

Scheme 4.

comments. In aqueous solution, the aldehyde free form proportion is 0.02% for gal and 0.002% for glc.¹⁵ In the pH range under study glc does not show the enediol band, even after heating. However, this band ($\lambda_{\text{max}} = 312 \text{ nm}^{21}$) appears when heating at $\text{pH} \geq 7$ and in kinetic studies on oxidation of glc by Cu^{II} in basic media an enediol-Cu^{II} intermediate species has been proposed.⁹⁻¹² The higher proportion of aldehyde for gal and the presence of the enediol in the pH range used here support our hypothesis of enediol as responsible for the formation of intermediate A and, consequently, for the saturation of k_{obs} vs [gal] curves, not observed for glc.

Polymerization after addition of acrylonitrile supports the formation of gal^o. These radicals are formed in the slow steps and may rapidly react with Cu^{II} to yield Cu^I and gala as final products (eq. (11)), which is in accordance with stoichiometry of 2 mol of Cu^{II} per mol of gal.

The proposed mechanism enables us to think that, at higher pH, the equilibrium (7) ($K_2 = 1.3 \times 10^{-5}$) will be displaced towards the right and A will be the major species; thus, most of the intramolecular one-electron transfer will occur rapidly through the base catalysed step (10). On the contrary, at higher proton concentrations than used here, the oxidation will take place from A⁺ and through step (8). Examining experimental values of parameters it may be noted that $k_4 = k_3 \ll k_5$. So, the extremely slow reaction at $\text{pH} < 4$ may be explained by a pathway consisting of eqs (5), (6), (8) and (11) of the general mechanism in Scheme 3.

Acknowledgements—We thank the National Research Council of Argentina (CONICET), The Third World Academy of Science (TWAS), The International Science Foundation (IFS) and The National University of Rosario (UNR) for financial support.

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