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Note

Regio- and stereo-selectivity in homogeneous catalytic hydrogenation of 2,5-diketo-D-*threo*-hexonic acid

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Abstract

2,5-Diketo-D-*threo*-hexonic acid (2,5-diketo-D-gluconic acid, **1**), a crucial intermediate in the microbial production of L-*threo*-hex-2-enono-1,4-lactone (L-ascorbic acid, vitamin C), was isolated from the fermentation broth of bacterium *Erwinia citreus* ATCC 31623, and its regio- and stereo-selective hydrogenation, catalyzed by the water-soluble Ru(II) complex of tris(*m*-sulfophenyl)phosphine (TPPTS), was performed. The effect of hydrogen pressure, temperature, pH, and catalyst-to-substrate ratio on regio- and stereo-selectivity of the process was studied. At low pH, over 90% regioselectivity in favor of the reduction of the 5-keto group in **1** was achieved, affording L-*xylo*-2-hexulosonic acid (2-keto-L-gulonic acid, **2**) as the main product. Maximal diastereoselectivity, i.e. ratio between **2** and 2-keto-D-gulonic acid (**3**) expressed as diastereomeric excess (d.e.%), amounted to ca. 50% and was not influenced by any of the above reaction parameters. © 1997 Published by Elsevier Science Ltd.

Keywords: 2,5-Diketo-D-*threo*-hexonic acid (''2,5-diketo-D-gluconic acid''); L-*threo*-Hex-2-enono-1,4-lactone (L-ascorbic acid, vitamin C); Ru(II) Tris(*m*-sulfophenyl)phosphine [Ru(II)–TPPTS]

Production methods for L-*threo*-hex-2-enono-1,4-lactone (L-ascorbic acid, vitamin C) vary nowadays from chemical technologies based on the classic Reichstein–Grussner method [1] and its variations [2], to microbial methods [3], mostly based on genetically modified strains [4,5]. The latter comprise two crucial routes involving the cloned host, i.e. three oxidation steps of D-glucose to 2,5-diketo-D-*threo*-hexonic acid (''2,5-diketo-D-gluconic acid'', 2,5-DKG, **2**) by genus *Erwinia* or *Gluconobacter*, and reduction of 2,5-diketo-D-gluconic acid by 2,5-DKG reductase of different microbial origins (enzyme as yet not EC registered) [6]. The activity of the former enzyme is not crucial for the overall process, whereas the reducing

Abbreviations: (note that none of these abbreviations or names are officially recognized IUPAC–IUBMB names) 2,5-DKG: 2,5-diketo-D-gluconic acid; 2-KLG: 2-keto-L-gulonic acid; 2-KDG: 2-keto-D-gluconic acid; 5-KDG: 5-keto-D-gluconic acid; 5-KDM: 5-keto-D-mannonic acid; acids: aldonic acids

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activity of the recombinant cells is limited by the lower activity of this specific reductive enzyme. It was then of interest to study hydrogenation of 2,5-DKG by chemical homogeneous catalysis.

Chemical hydrogenation of carbohydrates, comprising aldoses, ketoses, and aldonic acids, is traditionally performed heterogeneously, using metal catalysts on various solid supports [7–9]. Heterogeneous catalysts, however, usually require high hydrogen pressures and elevated temperatures of aqueous solutions of carbohydrates in order to achieve acceptable rates, i.e. to limit conversion time. Besides, heterogeneous metallic catalysts are generally scarcely regioselective, and, in particular, they are not stereoselective. We therefore envisaged the use of the water-soluble homogeneous catalytic system consisting of the Ru(II) complex with tris(*m*-sulfophenyl)phosphine (TPPTS), a water-soluble triphenylphosphine ligand.

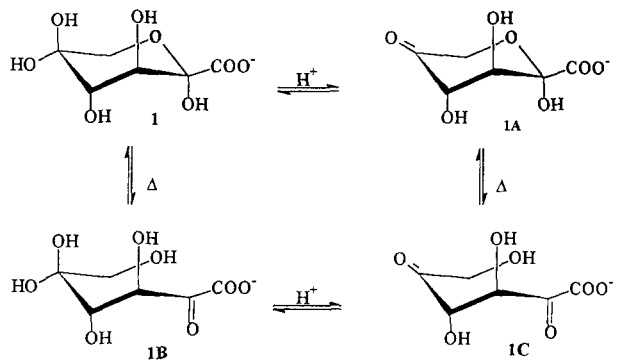
Water-soluble complexes with phosphines have been developed only recently. They can be prepared from the water-soluble phosphine ligands, as are hydroxyphosphines, carboxyphosphines, aminophosphines, or Rh complexes of the sulfophosphines. The sodium salt of tris(*m*-sulfophenyl)phosphine (TPPTS) has been introduced by Kuntz in hydroformylation [10]. Basset et al. [11] prepared various complexes of Ru(I) and Ru(II) with this ligand, and applied these complexes as hydrogenation catalysts in water. We recently reported the selective hydrogenation of D-mannose over the more stable C-2 epimer D-glucose [12] using the Ru–TPPTS complex.

These results are now extended to the regio- and stereo-selective hydrogenation of 2,5-diketo-D-gluconic acid, in order to explore the possibility of inserting this step into the sequence of microbial reactions that transform D-glucose into L-ascorbic acid.

2,5-Diketo-D-gluconic acid was obtained by cultivation of *Erwinia citreus* ATCC 31623 on D-glucose. During cultivation, this bacterium very efficiently converted D-glucose to 2,5-DKG with up to 98% yield. Isolated 2,5-DKG from fermentation broth showed specific rotation of $[\alpha]_D^{24}$ −54.1° (C 1, H_2O) a value very close to that found by Wakisaka [13].

2,5-DKG possesses two keto groups which can be hydrogenated either partially, leading to four possible isomeric keto-aldonic acids, or completely, leading to four aldonic acids (Scheme 1).

However, ^{13}C NMR studies [14] strongly suggest that in aqueous solution 2,5-DKG exists in a hydrated pyranose form (**1**), with little or no keto-form present



Scheme 1.

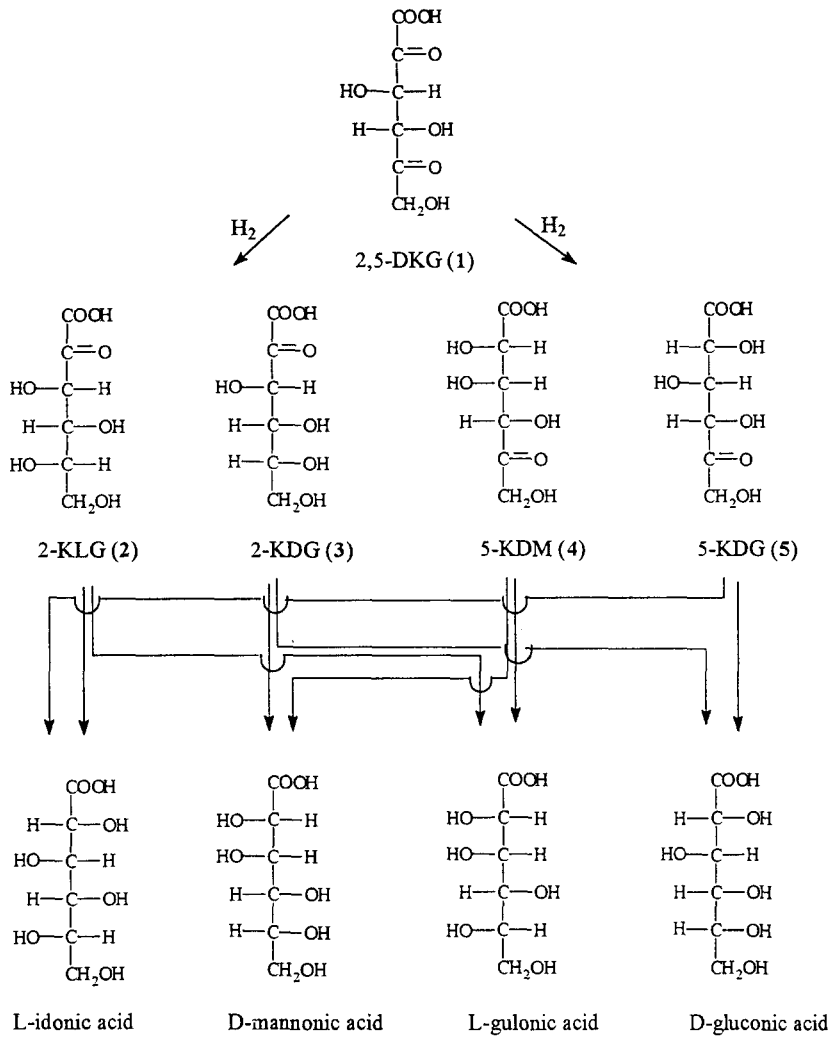
(Scheme 2). It is obvious that hydrogenation, according to the proposed mechanism [11], can proceed only by the attack of the catalytic complex on the 5-keto group of dehydrated form **1A** or on the 2-keto group of the open structure **1B**.

The first series of hydrogenation experiments, summarized in Table 1, demonstrates the influence of pH and temperature on the reaction rate and regioselectivity. At pH above 3, the reaction was very slow at room temperature (entries 4–6), while at elevated temperature, products of the reduction of the 2-keto group were formed. This series of experiments thus revealed that the regioselectivity can be controlled by temperature, and, in particular, by pH. Low pH favors dehydration, shifting the equilibrium towards the hydrated 5-keto form of 2,5-DKG, which is then reduced affording 2-keto aldonates, while high temperature favors opening of the pyranose ring promoting hydrogenation of 2-keto group.

By contrast, the stereoselectivity was not affected either by pH or temperature. The ratio of 2-keto-L- and -D-gulonic acid (**2**/**3**) remained unaltered, as shown by the experiments in Table 1.

Table 2 shows the results of the experiments performed at pH 1, which was found to be optimal. Higher temperature increased the reaction rate, but did not influence the ratio of diastereomeric products (entries 1 and 3). Experiments with varying quantities of the catalyst revealed notable sensitivity of the reaction on the catalyst/substrate ratio; at or above 1% molar concentration of the catalyst, the conversion of **1** was nearly complete in 1–3 h.

Typical progress curves for the reduction at low pH and 27 °C are shown in Fig. 1. Products of the hydrogenation of the 2-keto group, 5-KDG (**4**) and 5-KDM (**5**), were not detected during the course of the reduction at pH 1. We assume that, if formed, they are further hydrogenated to aldonic acids (Scheme 1).



Scheme 2.

Progress curves revealed that 2,5-DKG was completely consumed after less than 2 h at 60 °C (Fig. 2). At 60 °C and 10 bar (1 MPa), hydrogen diffusion to the reaction solution seemed to become rate determining, therefore we enhanced the pressure to 50 bar (5 MPa) and complete conversion was achieved already after 30 min (Fig. 3). By shortening the reaction time, formation of aldonic acids was limited as

Table 1
Influence of pH on the conversion and selectivity in Ru–TPPTS catalyzed hydrogenation of 2,5-DKG

Entry	pH	Temp. (°C)	Time (h)	Conv. (%)	Selectivity % (yield %)				
				2,5-DKG	2-KLG	2-KDG	5-KDG	5-KDM	Acids
1	1.1	27	5	95	63 (60)	21 (20)	0	0	15
2	1.5	27	7	90	55 (50)	19 (17)	0	0	23
3	2.0	27	8	76	38 (29)	13 (10)	6	5	26
4	3.1	27	6	33	traces	0	64 (21)	36 (12)	traces
5	5.2	27	6	7	0	0	57 (4)	43 (3)	0
6	7.0	27	4	0	0	0	0	0	0
7	7.0	70	3	96	0	0	42 (41)	28 (27)	29 (28)
8	1.0	5	6	38	68 (26)	29 (11)	0	0	3 (1)

Table 2
Influence of catalyst concentration, temperature, and pressure of hydrogen at pH 1.0 on the conversion and selectivity in Ru–TPPTS catalyzed hydrogenation of 2,5-DKG

Entry	Catalyst (mol. %)	Temp. (°C)	H_2 (bar)	Time (h)	Conv. (%)	Selectivity % (yield %)		
					2,5-DKG	2-KLG	2-KDG	Acids
1	3.5	27	10	7	100	68 (68)	22 (22)	10 (10)
2	1.2	40	10	5	99	70 (70)	21 (21)	8 (8)
3	3.5	40	10	2	100	68 (68)	21 (21)	11 (11)
4	1.2	50	10	1	97	66 (64)	20 (18)	16 (15)
5	0.5	50	10	6	74	71 (53)	22 (16)	7 (5)
6	1.2	60	10	1.5	98	67 (66)	19 (18)	15 (14)
7	1.2	60	50	0.5	98	74 (73)	21 (20)	5 (5)
8	0.25	60	50	4	66	68 (45)	22 (15)	9 (6)

compared to the reaction performed at the same temperature but at lower hydrogen pressure.

In conclusion, we have shown that the Ru–TPPTS complex can hydrogenate 2,5-diketo-D-gluconic acid at low pH with acceptable (over 90%) regioselectivity, but the experimental parameters used did not allow stereoselectivity higher than 50% d.e. in favor of 2-keto-L-gulonic acid. Such diastereoselectivity precluded any isolation of 2-KLG by crystallization at the preparative scale, whereas chromatographic separation of the Aminex A-27 and A-29 columns, used previously [15], or of the Aminex HPX-87, used in this work, is also inconvenient for any large-scale separation of the product.

1. Experimental

Bacterial cultivation.—For production of "2,5-diketo-D-gluconic acid", bacterium *Erwinia citreus* ATCC 31623 was used. Composition of cultivation medium (per liter) was: yeast extract (Difco) 5.0 g, pepton (Difco) 3.0 g, KH_2PO_4 (Kemika, Zagreb) 1.0 g, $MgSO_4 \cdot 7H_2O$ (Kemika, Zagreb) 200 mg, and D-glucose (Kemika, Zagreb) 100 g.

Cultivation was performed in 10 L working volume bioreactor (Biostat ED, B. Braun Int.). During cultivation, the temperature, pH, stirrer speed, and aeration rate were controlled at 28.0 ± 0.2 °C, 4.50 ± 0.02, 800 rpm, and 10 L/min, respectively. For pH control, 10 M NaOH was used.

2,5-DKG isolation.—After fermentation culture, the broth was centrifuged for biomass removal. The dark brown supernatant liquid was decolorized by activated carbon. A transparent liquid was obtained by filtration of suspended carbon. This liquid was stored at −20 °C and used for further experiments. HPLC analysis of this liquid showed a single peak corresponding to 2,5-DKG standard. The standard of 2,5-DKG was obtained according to the modified procedure of Shunichiro and Kiyoshi [16].

Analytical methods.—NMR spectra were recorded on a Varian XL-GEM 300 spectrophotometer. HPLC analyses were performed on a Hewlett–Packard instrument with 1050 pump, 1047A refractometric detector, and HP 3396A integrator, on an Aminex HPX

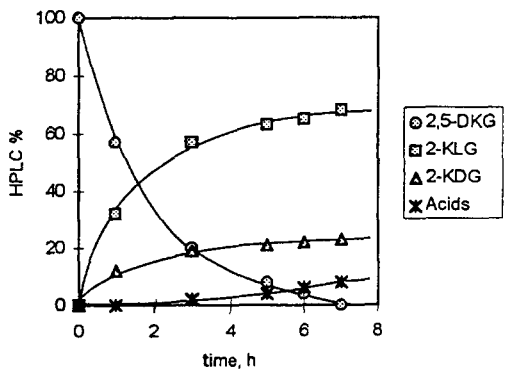


Fig. 1. Progress curve for hydrogenation of 2,5-DKG at pH 1.0, 10 bar (1 MPa) of hydrogen, and 27 °C.

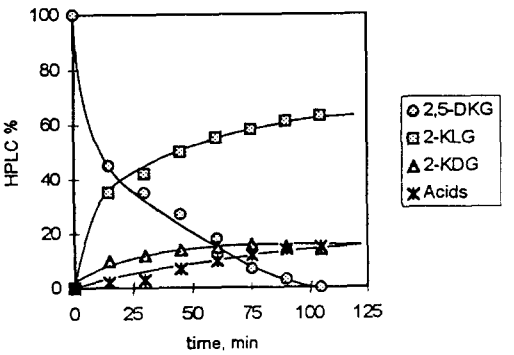


Fig. 2. Progress curve for hydrogenation of 2,5-DKG at 10 (1 MPa) bar of hydrogen and 60 °C.

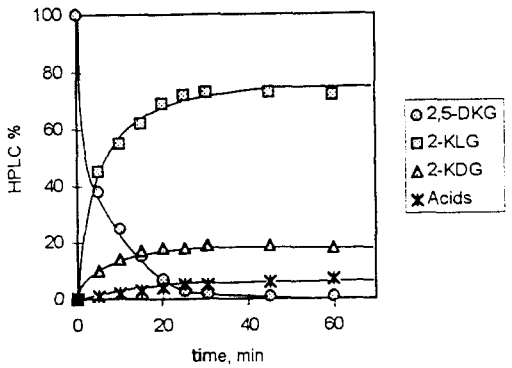


Fig. 3. Progress curve for hydrogenation of 2,5-DKG at 50 bar (5 MPa) of hydrogen and 60 °C.

87H column (Bio-Rad), thermostated at 40 °C, using 0.4 mL/min of 5 mM H_2SO_4 as the mobile phase. Under these conditions, aldonic acids were not separated and appeared as a single broad peak at 18.9 min R_t. Other R_t were: 2,5-DKG 10.85, 2-KLG 11.30, 2-KDG 11.86, 5-KDM 11.95, and 5-KDG 12.33 min.

TPPTS was obtained from Hoechst A.G. (Frankfurt) as ca. 33% aq soln. $RuCl_3 \cdot H_2O$ (Kemika) and NaI (Medica) were used as received. $RuCl_2(PPh_3)_3$ was prepared by a published method [11].

Preparation of the Ru–TPPTS complex.—TPPTS (2,4 mL of 33% aq soln) was dild with 10 mL of water and added dropwise, under vigorous stirring, to a soln of $RuCl_2(PPh_3)_3$ (0.54 g, 0,56 mmol) in THF (60 mL), preheated at 60 °C. The reaction mixture was stirred and heated under reflux for 30 min, then the organic phase was decanted, and the aq layer evaporated to dryness, resulting in a product (1.05 g) which was used as such as catalyst without further purification. ^{13}C NMR ($CDCl_3$): δ 127.55, 130.64 (J_{P-C} 6 Hz), 131.26 (J_{P-C} 24 Hz), 137.29 (J_{P-C} 16 Hz), 137.49 (J_{P-C} 14 Hz), 144.09 (J_{P-C} 7 Hz).

Hydrogenation of 2,5-diketo-D-gluconic acid.—Hydrogenations were performed in a 100-mL Parr M 4561 autoclave, connected to a temperature controller Parr series 4841.

In the standard experiment, 2,5-DKG (0.4 g, 1.7 mmol), Ru–TPPTS (0.05 g, 0.02 mmol), and sodium iodide (0.25 g, 1.6 mmol) were dissolved in water (40 mL). The pH was corrected either with 10% H_2SO_4 or with 5% NaOH. The soln was transferred to the autoclave, and when the temperature was stabilized, the reaction was started by adjusting the hydrogen pressure. Samples were taken through a sampling tube at regular time intervals, and analyzed by HPLC.

Acknowledgements

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