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Note

Enzymic oxidation of D-*arabino*-hexos-2-ulose (D-glucosone) to D-*arabino*-2-hexulosonic acid ("2-keto-D-gluconic acid")

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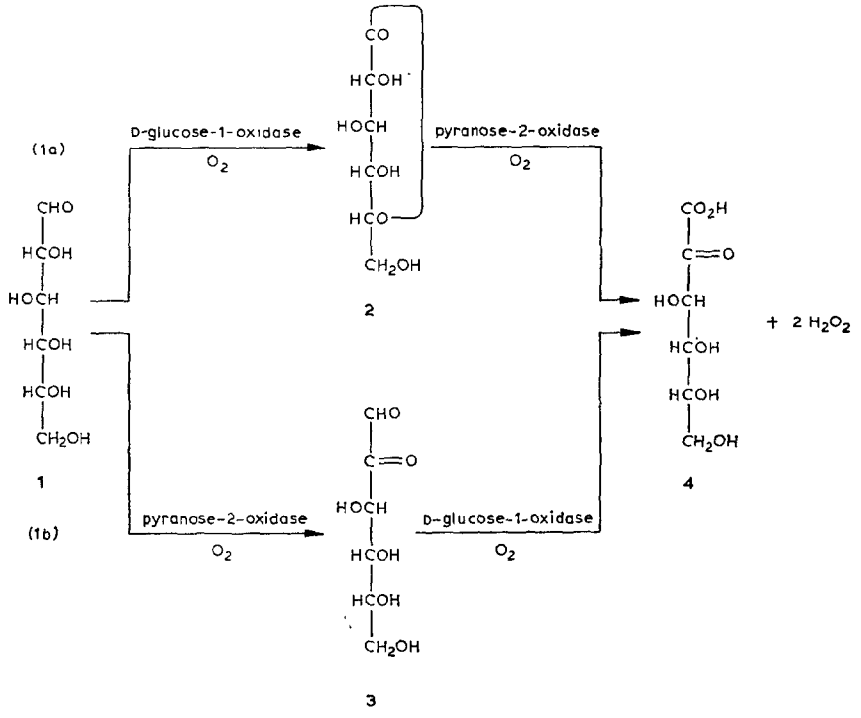
Scheme 1a presents a known enzymic pathway for forming D-*arabino*-2-hexulosonic acid ("2-keto-D-gluconic acid", **4**) from D-glucose (**1**). This pathway involves the intermediate D-glucono-1,5-lactone (**2**) and the enzymes D-glucose-1-oxidase (EC 1.1.3.4) and pyranose-2-oxidase (EC 1.1.3.10)[1,2]. We now propose an



Scheme 1. Two enzymic pathways from D-glucose (**1**) to "2-keto-D-gluconic acid" (**4**). [Both pathways require the same two enzymes. (a) Known pathway, involving D-glucono-1,5-lactone (**2**). (b) Proposed, alternative pathway, involving D-glucosone (**3**).]

alternative, enzymic pathway (see Scheme 1b) that requires the same two enzymes, but which proceeds through the intermediate D-glucosone (**3**).

In 1963, Bentley[3] reported that the enzyme D-glucose-1-oxidase oxidizes D-glucosone, but, because neither the identity of the oxidized product was determined nor D-glucosone free from residual D-glucose was used, later reports on D-glucose-1-oxidase no longer mention this activity[1]. The availability of pure D-glucosone[4] has now permitted us to discover the formation of "2-keto-D-gluconic acid" from D-glucosone by D-glucose-1-oxidase.

EXPERIMENTAL

Enzymic oxidation. — D-Glucosone (20 mg) and catalase (Sigma Chemical Co., Catalog No. C-10, 900 units, from bovine liver) were placed in a 100-mL, Erlenmeyer flask containing 0.1M potassium phosphate buffer, pH 6.0 (20 mL). D-Glucose-1-oxidase (Sigma Chemical Co., Catalog No. G-6500; 100 units, from *Aspergillus niger*) was then added, and the reaction was conducted for 6 h at 25°, with gentle stirring.

Analytical methods. — The oxidation of D-glucosone was monitored by high performance liquid chromatography (h.p.l.c.) as previously described[4]. "2-Keto-D-gluconic acid", D-glucosone, and other sugars and sugar acids are readily detected by this analytical method[5]. Confirmation of the identity of the product was made by comparing the chromatographic behavior and optical activity of the D-glucosone-oxidized product with those of an authentic sample of "2-keto-D-gluconic acid" (purchased from Sigma Chemical Company; hemi-calcium salt).

In addition to measurement by h.p.l.c., the oxidized product of D-glucosone co-chromatographed with authentic "2-keto-D-gluconic acid" in the following thin-layer chromatography (t.l.c.) system: Avicel-coated, glass plates; developed with 25:3:4 (v/v) 95% ethanol–water–25% ammonium hydroxide. Plates were sprayed with aniline phthalate reagent (1.15% of aniline, 2% of *o*-phthalic acid, and 1% of epichlorohydrin in 1:2 isopropyl alcohol–methanol).

A Perkin–Elmer Model 271 polarimeter was used to measure the specific rotation of the substrate and the product. Aqueous solutions containing 1mM potassium phosphate buffer, pH 6.0, were used.

RESULTS AND DISCUSSION

D-Glucose-1-oxidase converts D-glucosone into an oxidation product and hydrogen peroxide. The hydrogen peroxide can oxidize certain critical sites on the enzyme molecule (*e.g.*, sulfhydryl groups), damaging its function. Under the experimental conditions described herein, the hydrogen peroxide is decomposed to water and oxygen by the catalase. An alternative method of removing the H_2O_2 produced is to consume it to yield a useful co-product[6].

By comparing the chromatographic behavior of the D-glucosone-derived product

with that of an authentic sample of "2-keto-D-gluconic acid", its identity was readily achieved. Identical retention-times were obtained in the h.p.l.c. method. Identical distances of migration (R_F 0.42) and formation of an identical color (purple) were obtained in the t.l.c. method. (D-Glucosone showed a brown-colored spot at R_F 0.51.) The oxidation product of D-glucosone showed a specific rotation essentially equivalent to that of authentic "2-keto-D-gluconic acid"; $[\alpha]_D^{20}$ (degrees): D-Glucosone (substrate), −9.8; oxidation product of D-glucosone, −86.2; authentic "2-keto-D-gluconic acid", −85.4.

This enzymic reaction suggests that two pathways may exist to convert D-glucose into "2-keto-D-gluconic acid", rather than only that shown in Scheme 1a. It is even possible that the pathway shown in Scheme 1b, *i.e.*, *via* D-glucosone, may, in fact, be the major pathway in some micro-organisms. If the pathway shown in Scheme 1a proceeds at a higher rate than that shown in Scheme 1b, the pathway in Scheme 1b would go undetected.

ACKNOWLEDGMENTS

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