GALACTO-DIALDOSE PRODUCTION WITH AN ENZYME FROM THE MOLD POLYPORUS CIRCINATUS

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The wood mold Polyporus circinatus produces a galactose oxidase (1) which has now been purified 30-fold from the culture media. The enzyme resembles notatin (glucose oxidase) in several respects and can be employed for the determination of galactose with the peroxidase-chromogen system as in the procedure for glucose (2). However, in contrast to notatin, which leads to oxidation of the C-1 position of glucose, the galactose oxidase catalyzes oxidation of the C-6 position of galactose, yielding the dialdehyde of galactose. Compounds containing galactose in glycosidic linkage are excellent substrates for the enzyme.

The product formed in the oxidation of galactose yields a peak at 365 mμ in the diphenylamine reaction (3) and at 550 mμ in the carbazole reaction (4). It did not form a lactone and titration with iodine revealed the appearance

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Table I

Bromine Oxidation of the Enzymic Product Formed from Galactose

	I μmoles	II μmoles
Initial substrate	16.0	100.0
Consumed (enzyme assay)[1]	14.4	90.0
Aldehyde formed (I_2)[2]	14.2	87.1
Bromine oxidation		
Uronic acid (carbazole)	6.6	30.5
Mucic acid (weight)[3]	6.2	54.0

[1] Measured by assay with galactose oxidase.

[2] Iodine titration method (5). The value given represents increase over the initial level.

[3] The crystals formed on exposure to bromine were collected, washed and weighed. They were identified as mucic acid by melting point (218°, dec.) which was unchanged when mixed with authentic mucic acid, and by comparison of X-ray diffraction patterns. We are indebted to Dr. B. Magdoff of the Boyce Thompson Institute, Yonkers, N.Y. for the diffraction patterns.

of a new reducing group (Table I). The primary product of the reaction was rapidly converted by bromine at pH 5-6 and room temperature to a product with the characteristics of a hexuronic acid, as demonstrated by chromatography and the carbazole reaction (4). After decarboxylation (6) the uronic acid yielded L-arabinose, which was identified by chromatography and with a specific isomerase (7). Further treatment with bromine resulted in a decrease in uronic acid and the parallel appearance of a white crystalline precipitate which has been identified as mucic acid (Table I).

The purified enzyme preparations are active with a number of other galactoside derivatives which possess a free hydroxyl group in the 6-position

TABLE II

Substrates for Polyporus Galactose Oxidase

Substrate tested	Relative activity	Substrate tested	Relative activity
D-galactose	100	α-Methyl-D-galactoside	125
2-deoxy-D-galactose	32	β-Methyl-D-galactoside	340
N-acetyl-D-galactosamine	92	β-Methyl-thio-D-galactoside	91
Dulcitol	0.02	Lactose	2
D-galactonic acid	0.001	Melibiose	80
D-galacturonic acid	0.0001	Melibiitol	70
D-fucose	0.0001	Raffinose	180
L-arabinose	0.0001	Stachyose	610
D-glucose	0.000001	Galactose-1-P	9

The assay system contained 5×10^{-4} M of substrate, 0.1 ml (30 μg protein) of galactose oxidase and 1.8 ml of buffered peroxidase-o-dianisidine system ("Glucostat" preparation of the Worthington Biochemical Co., without glucose oxidase). The total volume was 2.0 ml. The rate of oxidation was followed by reading the optical density at 420 mμ. Negative or poor substrates were assayed at higher concentrations of substrate and the values reported were calculated assuming a first order reaction. The enzyme preparation did not exhibit any galactosidase activity.

(Table II). In the case of melibiitol the reaction product was oxidized with bromine and then hydrolyzed with pectinase. The hydrolyzed product was identified as galacturonic acid by chromatography and decarboxylation to L-arabinose.

On the basis of the above observations, it would appear that the first step in the utilization of galactose by the mold is oxidation to the D-galacto-hexodialdose. Further enzymatic steps in the metabolism of galactose and galactosides are now under investigation. On the basis of growth experiments there is reason to believe that further oxidation to galacturonic acid and decarboxylation to L-arabinose may occur, since the mold grows very well on these substances, but not at all on galactonic acid. Since polymers containing

galactose are excellent substrates, it may be that the natural substrates for the enzyme are plant galactans, rather than the free sugar.

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