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Note

Participation of HO-2 in the cleavage of β-D-galactosides by the β-D-galactosidase from *E. coli*

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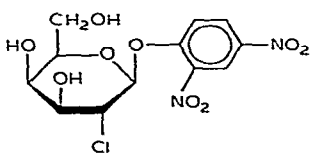
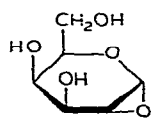
2,4-Dinitrophenyl 2-chloro-2-deoxy-β-D-galactopyranoside (**1**) was shown by Hengstenberg and Wallenfels[1] to be a good competitive inhibitor of β-D-galactosidase (β-D-galactoside galactohydrolase EC 3.2.1.23), but not a substrate. The absence of a hydroxyl group at C-2 in **1** was claimed to be responsible for this lack of reactivity. According to Wallenfels and Weil[2], HO-2 in ordinary β-D-galactosides functions as a participating, neighbouring group during aglycon displacement.



1



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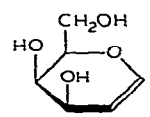


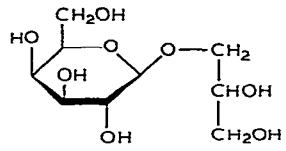
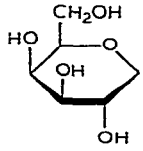
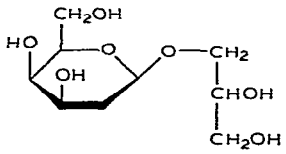
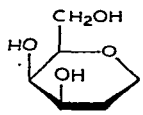
3

The intermediate formation of 1,2-anhydro-D-galactose (**2**) by such a neighbouring-group reaction in enzymic cleavage[2] would agree well with the exceptionally low K_i found[3] for the stereo-analogue D-galactal (**3**). Since the resistance of the glycoside **1** to enzyme-catalyzed hydrolysis could also be due to the strong I-effect imposed by the chlorine substituent adjacent to the anomeric centre[4], the essential role of HO-2 should be checked with a less ambiguous probe than **1**.

Ideal substrates for the elucidation of a neighbouring-group participation in enzymic β-D-galactoside cleavage should be 2-deoxy-β-D-*lyxo*-hexopyranosides. In 2-deoxyglycosides, stabilization of the glycosidic bond by an I-effect (2-hydroxy- or 2-halo-) is non-existent[5]. The relative rates of acid hydrolysis of methyl α-D-glucopyranoside and methyl 2-deoxy-α-D-*arabino*-hexopyranoside are 1:2090. Therefore, enzymic hydrolysis *without* neighbouring-group participation ought to be about equal in rate, if not faster, for a 2-deoxy-β-D-*lyxo*-hexoside compared to an ordinary β-D-galactoside, provided the aglycon is the same. The comparable glycosides 1-deoxyglycerol-1-yl-*1,3*-$^{14}C_2$ β-D-galactopyranoside[6] (**4**) and 1-deoxyglycerol-1-yl-*1,3*-$^{14}C_2$ 2-deoxy-β-D-*lyxo*-hexopyranoside[7] (**5**), synthesized enzymically, were used

TABLE I

DATA ON SUBSTRATES AND INHIBITORS

Substrate	*Relative rate of hydrolysis ($\mu mol.l^{-1}.min^{-1}$)*	*Inhibitor*	K_i(mM)
 4	100	 6	7.8
 5	0.0074	 7	69.6

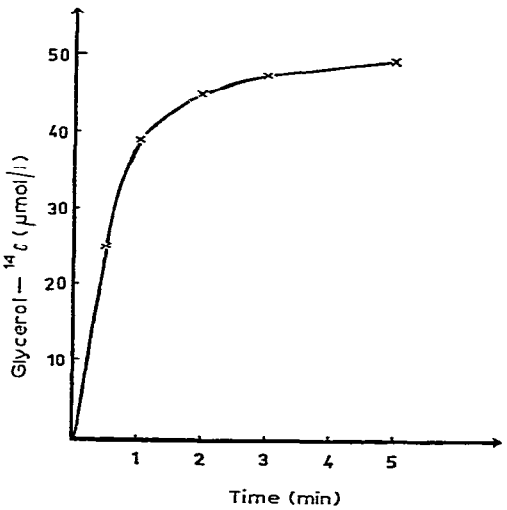


Fig. 1 (left). Enzymic hydrolysis of 1-deoxyglycerol-1-yl-*1,3*-$^{14}C_2$ β-D-galactopyranoside (4) (50μM; 26 mCi/mmol) in 0.1M sodium phosphate buffer (pH 6.8) containing mM $MgCl_2$ at 30° by β-D-galactosidase (6.7 U/ml). Samples (20 μl) were taken at intervals and the enzyme was denatured by heating. Glycerol-*1,3*-$^{14}C_2$ was separated by p.c. (Whatman No. 1 paper; pyridine–1-butanol–water, 4:6:3) and quantified by liquid scintillation counting. β-D-Galactosidase activity was based on the assay described in Ref. 10.

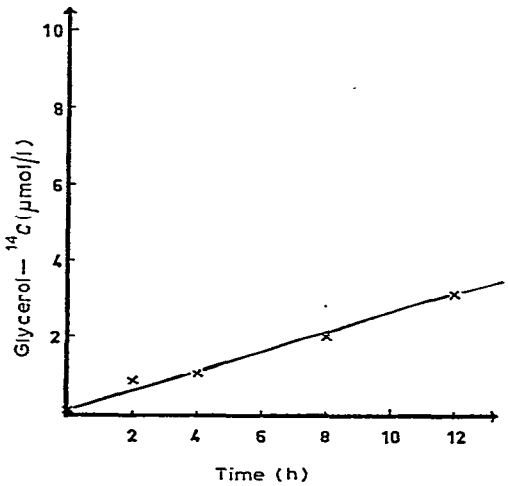


Fig. 2 (right). Enzymic hydrolysis of 1-deoxyglycerol-1-yl-*1,3*-$^{14}C_2$ 2-deoxy-β-D-*lyxo*-hexopyranoside (5) (50μM; 26 mCi/mmol); for conditions, see legend to Fig. 1.

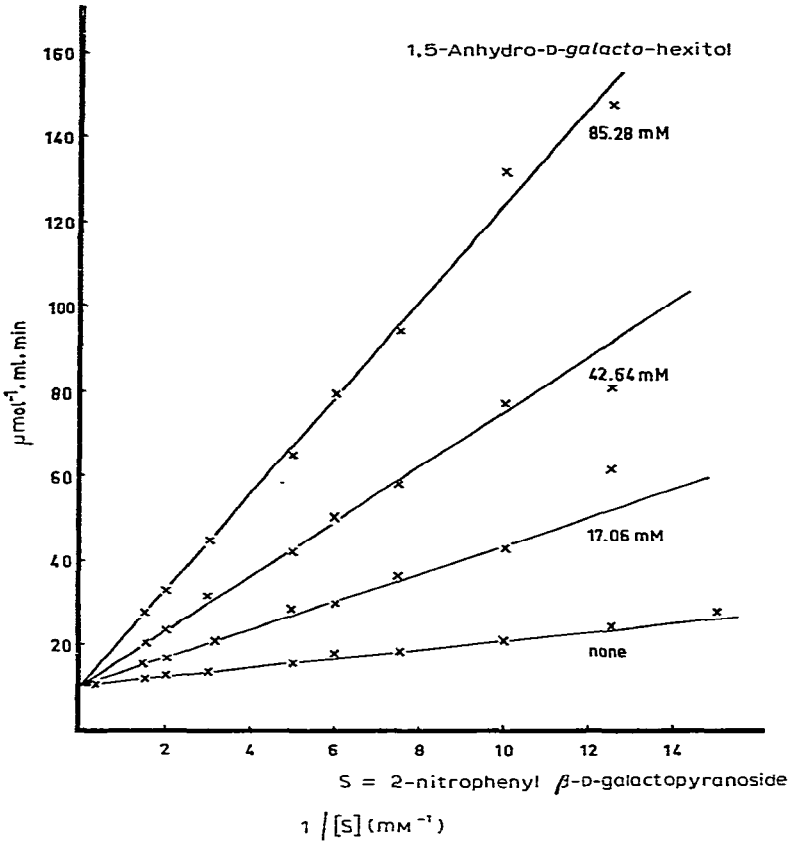


Fig. 3. Determination of the inhibition constant K_i for 1,5-anhydro-D-*galacto*-hexitol (**6**) in 0.05M sodium phosphate buffer (pH 6.8) containing mM $MgCl_2$ at 30°. Each test (1 ml) contained 0.08 U of β-D-galactosidase. The units (U) of enzymic activity relate to the hydrolysis of 2-nitrophenyl β-D-galactopyranoside (2.66mM), which was followed spectrophotometrically at 405 nm. One unit (U) of enzyme is defined as that amount which catalyzes the release of one μmol of 2-nitrophenol per min.

as substrates. The rates of hydrolysis by β-D-galactosidase for **4** and **5** were measured under the same conditions (Table I). The extremely low rate for **5** compared to that for **4** seems to confirm the essential role of HO-2 in the enzyme-catalyzed cleavage of β-D-galactosides.

In order to confirm our proposal of neighbouring-group participation in the actual reaction of glycosylic bond-cleavage, it has to be proved that the low rate of hydrolysis for the 2-deoxy-β-D-*lyxo*-hexoside **5** is not due simply to poor binding. The inhibition constants of the inert inhibitors 1,5-anhydro-D-galactitol[8] (**6**) and 1,5-anhydro-2-deoxy-D-*lyxo*-hexitol[9] (**7**), which can be regarded as pyranosyl models for the corresponding substrates **4** and **5**, are listed in Table I. Although **7** is bound less tightly to the enzyme than **6**, which is quite plausible if HO-2, as well as all of the other hydroxyl groups, has its share in general substrate binding, the difference in

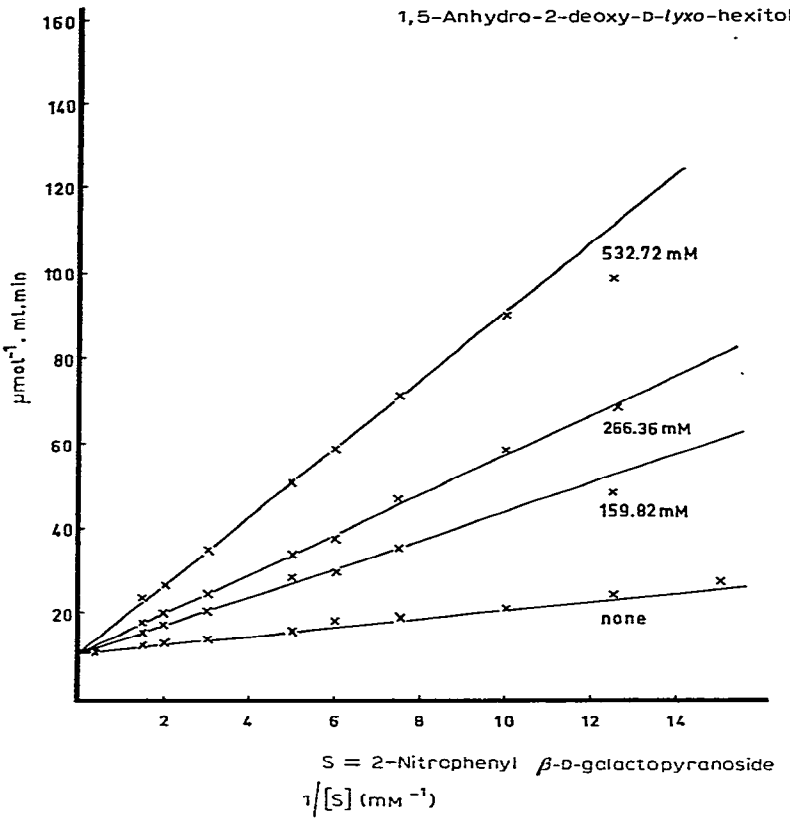


Fig. 4. Determination of the inhibition constant for 1,5-anhydro-2-deoxy-D-*lyxo*-hexitol (7). For conditions, see legend to Fig. 3.

K_i of less than one order of magnitude cannot account for the large difference in rates of hydrolysis for **4** and **5**. Thus, the requirement of a hydroxyl group at C-2 for full effectiveness in enzymic cleavage of β-D-galactosides is clearly established.

Our suggestion of neighbouring-group participation in enzymic hydrolysis of glycosides by β-D-galactosidase from *E. coli* is not incompatible with the results obtained by Sinnott and Souchard[10] on the α-deuterium kinetic isotope effects of this reaction. The participation of HO-2 in the glycosylic bond-breaking step, which causes a rate acceleration, would account for the α-deuterium kinetic isotope effects with "slow reacting" substrates. The participation of HO-2 in the stabilization of an enzyme-bound galactosyl intermediate is also consistent with the kinetic isotope effects assigned to the degalactosylation of the enzyme. The difference lies in our emphasis on an intramolecular process, as opposed to the suggestion by Sinnott and Souchard[10] of a uniquely intermolecular process. If neighbouring-group participation by HO-2 were exclusively operative in β-D-galactosidase action, we should expect full resistance of 1-deoxyglycerol-1-yl 2-deoxy-β-D-*lyxo*-hexopyranoside to enzymic hydrolysis. Since this is not the case, additional contributions, such as general acid–

base catalysis and conformation changes, must be considered. Optimal activity may be reached when all contributions become effective, as already discussed[11].

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