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Note

The enzymic determination of the enantiomers of galactose

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The determination of the enantiomeric configuration of sugars in polysaccharides or glycoconjugates is a problem when both enantiomers are present. Enzymic assays have been used to establish the D configuration of glucose and mannose[1]. Chemical methods involve g.l.c. of the acetylated chiral oct-2-yl glycosides[2,3] and of the trimethylsilylated glycosides obtained after solvolysis of polysaccharides with chiral butan-2-ol[4]. Resolution of derivatives of enantiomers has been achieved by g.l.c. using chiral stationary phases[5,6].

D-Galactose is widely distributed in Nature, but the L form is much less common. L-Galactose was first reported[7] as a constituent of the galactan of the snail *Helix pomatia*, and the content was based on the optical rotation of the hydrolysate. Both enantiomers are present in the storage galactans in the spawn of *Biophalaria glabrata*[8] and four other snails[9], and in polysaccharides isolated from the extracellular mucilages of the unicellular fresh-water alga *Porphyridium aerugineum*[10] and the unicellular marine alga *P. cruentum*[11]. The latter polysaccharides also contain D-glucose, D-xylose, and D-glucuronic acid. The content of the L form, isolated chromatographically from the hydrolysed polysaccharide of *P. aerugineum*, was estimated from the difference between the total reducing power and the amount of the D form determined using D-galactose oxidase. L-Galactose, present in the hydrolysates of polysaccharides isolated from flax seed, corn cob, corn root, and the cell walls of *Acer pseudoplatanus*, was determined[12] by g.l.c. of the trimethylsilyl derivative after oxidation of the D form with galactose dehydrogenase.

The approach now reported involves D-galactose dehydrogenase and L-fucose dehydrogenase for the determination of the proportions of the enantiomers of galactose. D-Galactose dehydrogenase (D-galactose:NAD 1-oxidoreductase, EC 1.1.1.48) was originally described[13] for the oxidation of D-galactose and several related monosaccharides in the presence of NAD and has been used for the quantitation of D-galactose in the absence of L-arabinose, D-fucose, and, to a lesser extent, 2-amino-2-deoxy-D-galactose[14,15].

NAD-dependent L-fucose dehydrogenase (6-deoxy-L-galactose:NAD^+ 1-oxidoreductase EC 1.1.1.122) oxidises L-fucose, L-galactose, and D-arabinose. Therefore, in the absence of L-fucose and D-arabinose, the levels of L-galactose can be measured on the basis of the reaction:

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TABLE I

Content of D- and L-galactose in polysaccharides from various sources

Source	*Enantiomer (%)*				*Ref.*
	This work		*Literature*		
	D	L	D	L	
H. pomatia	78	22	85	15	7
B. glabrata	100	0	99.9	0.1	16
P. aerugineum	70	30	75	25	10
P. cruentum	62	38	67	33	10

L-Galactose + NAD → L-Galactono-1,4-lactone + NADH + H^+.

Although the relative rates of reaction and values of K_m are poorer for L-galactose than for L-fucose, measurement of the initial velocity of the reaction allows L-galactose to be determined readily. A linear relationship between the velocity of the enzymic reaction (A_{340}/min) was observed for the range 0–2.0 μmol of L-galactose.

Examination of hydrolysates with the fucose dehydrogenase system confirmed the presence of L-galactose in the polysaccharides from *H. pomatia*, *P. aerugineum*, and *P. cruentum*, but its absence from that of *B. glabrata* (Table I). Correa *et al.*[8] reported > 36% of L-galactose in the galactan of *B. glabrata*, whereas Bretting *et al.*[9] found only 12.2%, although this was based on the use of D-galactose oxidase and the $[\alpha]_D^{20}$ value of the galactan. More recently, Bretting *et al.*[16] found L-galactose to be absent from *B. glabrata* galactan on the basis of g.l.c. of trifluoroacetylated methyl glycosides, using a chiral stationary phase. An L-galactose content of 12.5–14.3% for the *H. pomatia* galactan has been calculated from the optical rotation of a hydrolysate of the polysaccharide[7,9,17]. Widely divergent DL-ratios have been reported for the galactose in the polysaccharide from *P. cruentum*, namely, 10:90 by Medcalf *et al.*[11] and 67:33 by Percival *et al.*[10].

The results of the study now reported indicate that the proportions of the D and L forms of galactose can be measured using the two enzymic procedures, provided that the action pattern of the enzymes towards other monosaccharides is known. The D-galactose dehydrogenase from *Pseudomonas fluorescens* used in this study will oxidise L-arabinose, D-fucose, and D-galactose, whereas the enzyme from rat liver[12] will not oxidise L-arabinose, but is strongly inhibited by NADH, one of the products of the reaction[18]. The fucose dehydrogenase can be used for the estimation of L-galactose, provided that L-fucose is absent.

EXPERIMENTAL

L-Fucose dehydrogenase from porcine liver was purchased from Sigma, and β-D-galactose dehydrogenase from *P. fluorescens* from Boehringer.

Hydrolysis of polysaccharides. — Samples (~5 mg) were hydrolysed in M H_2SO_4 for 1 h at 100° and the pH of each hydrolysate was adjusted to 8.0 with 2M NaOH. Samples were kept at −10° until assayed.

Enzymic estimations. — *(a)* D-*Galactose.* The galactose dehydrogenase end-point assay involving measurement of the NADH produced at pH 8.6 (ref. 1) was used[14].

(b) L-*Galactose.* The initial absorbance at 340 nm of a mixture of 0.5M Tris buffer (0.75 mL, pH 8.0), NAD (0.1 mL, 10 mg.mL^{-1}), and a solution of L-galactose (either a standard or from a polysaccharide hydrolysate, 0.15 mL) was measured. The reaction was initiated by the addition of L-fucose dehydrogenase (0.02 mL, 0.2 U) and the change in absorbance at 340 nm was measured during 3 min. Using a standard solution of L-galactose, a linear response for the rate of production of NADH was obtained in the range 0.2–2.0 μmol.

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REFERENCES

1 C. J. Brigden and S. G. Wilkinson, *Carbohydr. Res.*, 138 (1985) 267–276.
2 K. Leontein, B. Lindberg, and J. Lonngren, *Carbohydr. Res.*, 62 (1985) 359–362.
3 S. Furn and S. G. Wilkinson, *Carbohydr. Res.*, 139 (1985) 293–297.
4 C. G. J. Gerwig, J. P. Kamerling, and J. F. G. Vliegenthart, *Carbohydr. Res.*, 77 (1979) 1–7.
5 W. A. Konig, P. Mischnicklubbecke, B. Brassat, S. Lutz, and G. Wenz, *Carbohydr. Res.*, 183 (1988) 11–18.
6 W. A. Konig, I. Benecke, and H. Bretting, *Angew. Chem. Int. Ed. Engl.*, 20 (1981) 693–694.
7 D. J. Bell and E. Baldwin, *J. Chem. Soc.*, (1941) 125–131.
8 J. B. C. Correa, A. Dmytraczenko, and J. H. Duarte, *Carbohydr. Res.*, 3 (1967) 445–452.
9 H. Bretting, N. F. Whittaker, E. A. Kabat, K. Konigsmann-Lange, and J. Thiem, *Carbohydr. Res.*, 98 (1981) 213–236.
10 E. E. Percival and R. A. J. Foyle, *Carbohydr. Res.*, 72 (1979) 165–176.
11 D. G. Medcalf, J. R. Scott, J. H. Brannon, G. A. Hemerick, R. L. Cunningham, J. H. Chessen, and J. Shah, *Carbohydr. Res.*, 44 (1975) 87–96.
12 R. M. Roberts and E. Harrer, *Phytochemistry*, 12 (1973) 2679–2682.
13 K. Wallenfels and G. Kurz, *Biochem. Z.*, 335 (1962) 559–572.
14 R. J. Sturgeon, *Methods Carbohydr. Chem.*, 8 (1980) 131–133.
15 J. Melrose and R. J. Sturgeon, *Carbohydr. Res.*, 118 (1983) 247–253.
16 H. Bretting, G. Jacobs, I. Benecke, W. A. Konig, and J. Thiem, *Carbohydr. Res.*, 139 (1985) 225–236.
17 H. J. Horstmann and M. Geldmacher-Malinckrodt, *Hoppe-Seyler's Z. Physiol. Chem.*, 325 (1961) 251–259.
18 P. Cuatrecasas and S. Segal, *J. Biol. Chem.*, 241 (1966) 5910–5918.