

Synthesis and Paper Electrophoresis of the Galactose Monomethyl Ethers

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The mono-*O*-methyl ethers of D-galactose have been synthesised by partial methylation of suitable galactose derivatives and the separation of the mixtures of methyl ethers obtained has been performed on carbon columns. — The M_G -values of the monomethyl ethers and of some other galactose derivatives have been determined.

In his studies on paper electrophoresis of glucose derivatives, Foster¹ found that the M_G -values were chiefly dependent on the position of a substituent and only to a lesser extent upon the nature of this substituent. In connection with an investigation of the arabogalactosan in larch wood², it was therefore of interest to study the migration of the monomethyl ethers of galactose in electrophoresis and to compare them with those of some oligosaccharides, obtained by partial hydrolysis of the polysaccharide.

Each of the four monomethyl ethers of D-galactose, which can exist in pyranosidic form, is known and has been synthesised by unambiguous methods³. The 6-*O*-methyl ether is easily available but the routes leading to the others are more tedious. We therefore prepared them by partial methylation of 4,6-benzylidene- β -methylgalactoside⁴ and 6-trityl- β -methylgalactoside, respectively, and separated the mixtures of galactose methyl ethers obtained on carbon columns, using the technique described by Lindberg and Wickberg⁵. The 3- and 4-*O*-methyl ethers, however, could not be separated by this method, but were easily separated when borate was added to the eluting solvent as described by Barker, Bourne and Theander⁶.

The partial methylations were performed with the modified Purdie methylation method using N,N-dimethylformamide as solvent, as devised by Kuhn *et al.*⁷ for the complete one step methylation of non-reducing sugars. The yield of methoxyl groups, calculated from the added amount of methyl iodide, was 50 % in one experiment and 90 % in another. There is of course a competition for the methyl iodide between the hydroxyl groups of the sugar and the water, present as an impurity in the starting materials and formed during the reaction, and this might explain the difference between these figures.

The monomethyl ethers thus obtained were electrophoretically and chromatographically pure and the melting points and rotations were in good agreement with previously published data. The method of partial methylation and separation on carbon columns of the mixture obtained seems to be of general value for the preparation of less available ethers.

The paper electrophoresis apparatus was similar to that used by Foster⁸. The M_G -values did not differ more than ± 0.02 units from the mean value and no significant influence from differences in the applied voltages or from the use of different papers could be detected. The measurements were performed in a borate buffer of pH 10.

Table 1. M_G -values of some galactose and glucose derivatives.

Substance	Complexes formed	M_G	Substance	Complexes formed	M_G
Galactose	X Y β'	0.93	Glucose	X β' C	1.00
β -Methylgalactoside	Y	0.38	β -Methylglucoside		0.19
2-O-Methylgalactose	Y β'	0.43	2-O-Methylglucose	β'	0.23
3-O-Methylgalactose	X β'	0.63	3-O-Methylglucose	X β' C	0.82
4-O-Methylgalactose	X	0.30	4-O-Methylglucose	X	0.24
6-O-Methylgalactose	X Y	0.86	6-O-Methylglucose	X β' C	0.82
Disaccharide I	—	0.69			
Disaccharide II	—	0.83			

In Table 1 the M_G -values of the galactose monomethyl ethers and some other galactose derivatives are given and compared with the values of the corresponding glucose derivatives, these being taken from Foster's investigation¹. Foster found that the rate of migration of the glucose derivatives in electrophoresis could be explained by the assumption that essentially three types of borate complexes are formed. The first, X, is due to complex formation between the borate and the hydroxyls at C₍₁₎:C₍₂₎ in the α -pyranosidic or α -furanosidic form of the derivative. The two other types are due to complex formation between the borate and two hydroxyls in the β -position in the aldehyde form of the derivative. These hydroxyl groups should be in *cis*-relationship in the Fisher projection formulae (β' C) or one of them should be a primary hydroxyl group (β'). These two possibilities are realised by the hydroxyl groups at C₍₂₎:C₍₄₎ and C₍₄₎:C₍₆₎, respectively, in the glucose molecule, and Foster found that when both alternatives are possible, β' C takes preference over β' . In galactose, there is no possibility of forming a β' C complex, but there is a contribution from a complex between borate and the two hydroxyls at C₍₃₎:C₍₄₎ in the ring forms, here denoted as Y. It is evident from Table 1 that the M_G -values of the galactose derivatives can also be correlated with the structure by application of Foster's theory. The contribution from the Y complexes seems to be of the same order of magnitude as those of X and β' C, and the relatively small contribution from the β' complex is also indicated.

The unexpectedly low migration of 4-O-methylgalactose and 4-O-methylglucose may be explained by the fact that these substances cannot assume a

furanosidic but only a pyranosidic structure, hence the contribution from X complexes might be reduced.

Two disaccharides (I and II) obtained from the arabogalactosan by partial hydrolysis, and which on further hydrolysis yielded galactose only, were also investigated. From previous investigations of this polysaccharide⁹, the disaccharides could be supposed to be 3- and 6- β -galactopyranosyl galactopyranose, respectively, and the observed M_G -values are in good agreement with this assumption.

EXPERIMENTAL

- Chromatography.* Paper: Whatman No. 1.
Solvent: Butanol:ethanol:water 10:3:5.
Developer: Anisidine hydrochloride.
- Electrophoresis.* Papers: Whatman No. 1 and Whatman 3 MM.
Borate buffer: 7.45 g boric acid and 4.0 g sodium hydroxide in 1 000 ml of aqueous solution (pH = 10.0).
Developer: Silver nitrate/sodium ethoxide and, in the case of β -methylgalactoside, periodate/benzidine¹⁰, the paper at first being neutralised with acetic acid.
Voltages: 600–800 volts.

All melting points corrected. All evaporations were performed under reduced pressure.

Fractions obtained in the separations were investigated by paper chromatography, paper electrophoresis and determination of the optical rotation.

Partial methylation of 4,6-benzylidene- β -methylgalactoside. To a rapidly stirred solution of 4,6-benzylidene- β -methylgalactoside (6.2 g, 1 mole) and methyl iodide (2.6 ml, 1.95 mole) in 25 ml of dimethylformamide, silver oxide (10 g, 4 mole) was added in portions during 1 hour, the apparatus and the chemicals being previously carefully dried. The temperature was kept below 20° by external cooling and the stirring was continued for another 11 hours. The reaction mixture was worked up according to Kuhn *et al.*³. The product obtained was then boiled for 4½ hours in 150 ml of acetone and 75 ml of 0.5 N HCl. The benzaldehyde formed was removed by steam distillation under reduced pressure. The acid was removed by filtering through a column of IR 4B and the solution concentrated to a sirup, which was treated for 14 hours in 28 ml of 0.5 N HCl at 100°, in order to remove the glycosidic group. Neutralisation (IR 4B) and concentration yielded a lightcoloured sirup (4.8 g).

Separation of products from partial methylation of 4,6-benzylidene- β -methylgalactoside.

Column: 4.5 × 33 cm. Eluent: 81 0–10% ethanol. Fractions: 26 ml. Amount added: 4.8 g.

Fract. 47–49. Galactose (traces).

› 81–98. 0.57 g of 3-methylgalactose (crystallised when seeded).

› 99–131. 1.51 g of a mixture of 2- and 3-methylgalactose of which 0.79 g of 2-methylgalactose crystallised after seeding; the rest gave after some time fairly pure 3-methylgalactose.

› 132–153. 0.30 g cryst. 2-methylgalactose.

› 218–290. 0.87 g 2,3-dimethylgalactose.

Approximate yields: 35 % 2-methyl-, 40 % 3-methyl- and 25 % 2,3-dimethylgalactose.

Partial methylation of 6-trityl- β -methylgalactoside. 6-Trityl- β -methylgalactoside (28.7 g, 1 mole) was treated in the same manner as described above with methyl iodide (6.2 ml, 1.5 mole) and silver oxide (22 g, 3.7 mole) in dimethylformamide (50 ml). The product obtained was detritylated in 100 ml 0.5 N alcoholic hydrochloric acid for 2 hours at room temperature, concentrated and diluted with water and then filtered and neutralised (IR 4B). The solution was concentrated to a sirup and treated for 12 hours in 50 ml of 0.5 N HCl at 100°. Neutralisation (IR 4B) was followed by decolorisation with carbon in 50 % alcohol. Concentration yielded 14.1 g of a light coloured sirup.

Preliminary separation of products from partial methylation of 6-trityl- β -methylgalactoside.

Column: 6.5 × 46 cm. Eluent: 81 0–15 % ethanol. Fractions 150–100 ml. Amount added: 14.1 g.

- Fract. 22–27. 1.05 g of galactose.
 » 28–31. 1.06 g of 3-methyl- and 4-methylgalactose.
 » 32–33. 1.06 g of 2-methyl-, 3-methyl- and 4-methylgalactose.
 » 34–41. 1.01 g of 2-methylgalactose (cryst.) and 0.71 g of 2-methyl- and 3-methylgalactose (sirup).
 » 42–64. 3.71 g of dimethylgalactoses.

50 % ethanol-eluate. 2.77 g of 2,3,4-trimethylgalactose with small amounts of dimethylgalactoses.

Approximate yields: 10 % galactose, 34 % monomethyl ethers, 32 % dimethyl ethers and 24 % of the trimethyl ether.

Separation of 3-O-methyl- and 4-O-methylgalactose (fract. 28–31 above).

Column: 4.5 × 33 cm. Eluent: 1.5 l water + 4 l 0–4 % ethanol, containing 7.45 g boric acid and 4.0 g sodium hydroxide in 1000 ml. Fractions: 26 ml. Added amount: 1.06 g.

Fract. 124–151. 0.69 g of 3-methylgalactose (cryst.)

» 177–188. 0.07 g of 4-methylgalactose (cryst.)

The fractions were neutralized by adding 2 ml of strong cation exchanger (IR 120) to the fractionating tubes. After filtration and concentration the boric acid was evaporated with methanol.

Fractions 32–33 were resolved in exactly the same manner, yielding in the following order: 0.33 g 3-methyl-, 0.37 g 2-methyl- and 0.36 g 4-methylgalactose (cryst.). The separation of 3-methyl- and 2-methylgalactose was not quite complete, but both fractions crystallised on seeding.

The sirupy part of fractions 34–41 (0.71 g) was separated in the usual manner yielding 0.35 g 3-methyl- and 0.33 g 2-methylgalactose.

The approximate ratio of the monomethyl ethers thus obtained is 2-O-methyl-: 3-O-methyl-: 4-O-methylgalactose = 5:4:1.

The 6-O-methylgalactose, prepared according to Freudenberg and Hixon¹¹, failed to crystallise, even when purified by means of carbon column chromatography. It was therefore characterised as its osazone, prepared in the presence of bisulphite, according to Hamilton¹².

Table 2. Properties of Galactose Derivatives.

Compound	M.p.	$[\alpha]_D^{20}(\text{H}_2\text{O})$	$R_{\text{Galactose}}$	Colour with anisidine-HCl
2-O-methylgalactose	149–151°	+ 50 → + 84°(c=2)	1.50	orange-red
3-O-methylgalactose	145–146.5°	+ 147 → + 106°(c=2)	1.37	light yellow
4-O-methylgalactose	206–208°	+ 54 → + 81°(c=1)	1.37	yellow
6-O-methylgalactose			1.39	brown
—, phenylosazone	200–201°			
Disaccharide I				light yellow
Disaccharide II				brownish yellow

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