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Preliminary communication

A high-yielding, specific method for the chemical derivatization of D-galactose-containing polysaccharides: oxidation with D-galactose oxidase, followed by reductive amination

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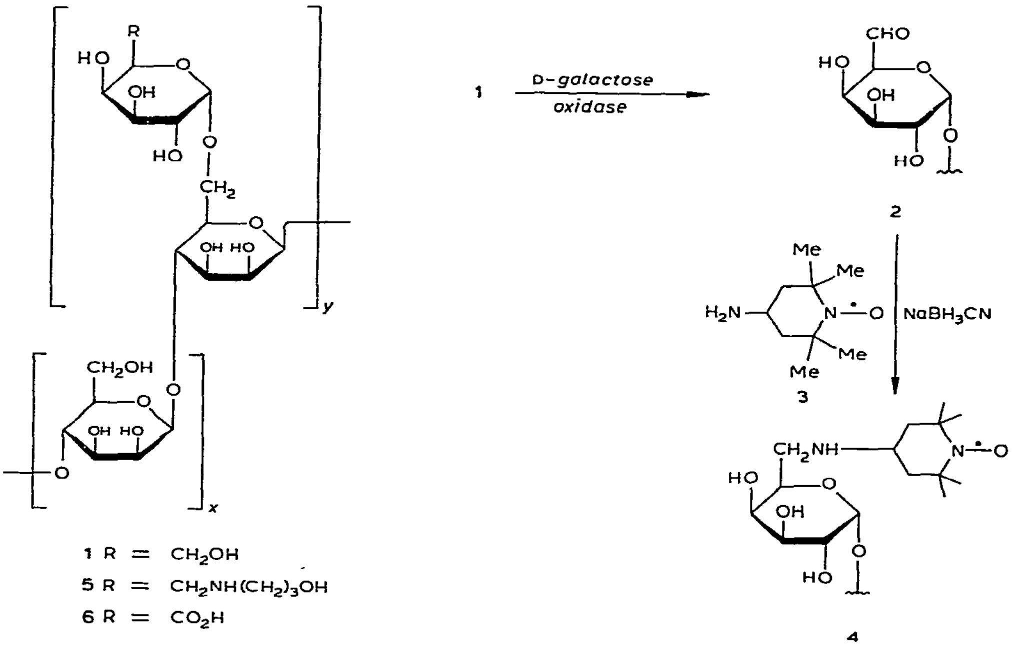
Interest in both understanding the properties of aqueous solutions of naturally occurring polysaccharides and their derivatives[1], and their uses in industry and medicine, is rapidly expanding for a number of reasons. At the same time, the limitations of many of the procedures traditionally used for "graft" derivatization of polysaccharides are becoming increasingly obvious. D-Galacto-D-mannans are a family of polysaccharides which are widely used[2], and we draw attention here to a procedure that results in a high-yielding, specific derivatization of such polymers, exemplified by guaran (**1**). Following a suggestion of Avigad *et al.*[3] and Schlegel *et al.*[4], D-galactose oxidase (EC 1.1.3.9) is used to introduce, at C-5 of the D-galactosyl groups, an aldehyde group whose reductive amination[5], using any primary or secondary amine and sodium cyanoborohydride, affords a polymer bearing a substituent of choice on C-5.

The general sequence of reactions is summarized in Scheme 1, and the following spin-labelling of **1** typifies the conditions used. Pure[6] guaran (60 mg; 120 μmol. equiv. of D-galactose) in 25 mM phosphate buffer (5 mL; pH 7) was treated with D-galactose oxidase (Sigma; 90 units) and catalase[7] (EC 1.11.1.6; 10,500 units) for 24 h, to give **2**. Reductive amination of **2** was performed *in situ* by the addition of aqueous solutions of 4-amino-2,2,6,6-tetramethylpiperidin-1-oxyl (**3**; 92 mg, 540 μmol) and sodium cyanoborohydride (300 mg, 4.4 mmol) during 36 h. Purification of the product (**4**) could be achieved either by extensive dialysis (4 days), or ethanol precipitation followed by careful washing. Although no attempt has yet been made to optimize the reaction conditions fully, the "yields" (based on content of D-galactose) obtained so far are rather encouraging, typically lying between 60 and 70%, as determined by elemental microanalysis, ^{13}C-n.m.r. spectroscopy, or e.s.r., double integration (in the case of **4**).

Without giving an exhaustive compilation here of all of the reactions possible, it is clear that aldehyde **2** is an extremely versatile intermediate and, besides reductive amination (exemplified by the spin-labelling reaction and the formation of the hydroxypropylamine derivative **5**), we simply point out its oxidation by bromine water[8] at pH 6 to give the carboxylic acid **6**, which is itself a versatile intermediate. The wide range of amines that



Scheme 1

successfully undergo reductive amination, together with the interesting changes in the rheological properties of aqueous solutions of the products, compared to those of the parents, encouraged us to pursue these studies further. For example, we have already obtained similar results with locust-bean gum. Although the oxidation procedure described here may not be directly compatible with industrial production, alternative procedures for the large-scale oxidation of D-galactose-containing polymers exist (for example[9], periodate oxidation), and the reductive amination reaction appears to have many inexpensive variants[10].

Novel information concerning the distribution of the nitroxide groups of 4, and, by inference, that of the D-galactosyl groups of guaran itself, was obtained from the dipolar-coupling contribution to the e.s.r. line-width[11]. The mean, nearest-neighbor distance between spins, $\bar{r}$ (derived[12] from the spectral parameter d_1/d at 77 K), was found to be 1.36 nm (±5%), which implies that the D-galactosyl groups are distributed in blocks. This conclusion was supported by the observation that, even at low *overall* levels of spin-labelling, strong dipole—dipole interactions are present for 4, and it is in accord with other findings[13]. The inclusion of e.s.r. and other spectroscopic probes should enable us to study the details of the viscosity of these substances at the molecular level.

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