Carbohydrate Research, 53 (1977) 276–277
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Note

The presence of rhamnose and 3-*O*-methylxylose in the extracellular mucilage from the red alga *Rhodella maculata*

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(Received May 21st, 1976; accepted for publication, June 10th, 1976)

The extracellular mucilage from the microscopic, unicellular, red alga *Rhodella maculata* has been shown[1] to be a sulphated polysaccharide containing mainly xylose and glucuronic acid together with lesser amounts of galactose, glucose, and two carbohydrates (*X* and *Y*) having high chromatographic mobility. We have now isolated *X* and *Y* from a hydrolysate of the mucilage and shown *X* to be rhamnose by its chromatographic mobility and by its retention times on g.l.c. as the Me_3Si ether before and after reduction to the alditol. Sugar *Y*, which stains pink with aniline oxalate, has the chromatographic mobility of 3-*O*-methylxylose. This identity was confirmed by g.l.c. as for *X*, and by conversion into the aldose and alditol acetate derivatives and analysis by g.l.c. and g.l.c.–m.s. Authentic 3-*O*-methylxylose and *Y* gave products having identical retention times and mass-spectrometry patterns after reduction with borodeuteride and labelling at C-1 with deuterium (major peaks at *m/e* 43, 87, 88, 129, 130, 189, and 190). Furthermore, *Y* gave only xylose on demethylation[2], and 2,3,4-tri-*O*-methylxylose after methylation. This is the first report of rhamnose as a constituent of a red-algal polysaccharide, and of 3-*O*-methylxylose in any polysaccharide.

Methylation of the mucilage before and after reduction of the glucuronic acid residues to glucose, with analysis of the derived alditol acetates, confirmed the linkages previously found[1] for xylose and galactose, and showed that the rhamnose is present solely as end groups. The glucose is both 1,2- and 1,4-linked, and both galactose and glucose may occur at branch points 1,3,4- and 1,2,4-linked. There was a two-fold increase in the relative size of the peak (*T* 1.7) from the methylated, reduced material, compared with that from the initial methylated polysaccharide. This peak had the retention time and mass-fragmentation patterns of 3,4,6-tri-*O*-methylhexose and 2,4,6-tri-*O*-methylglucose. Since the fragmentation pattern for 2,4,6-tri-*O*-methylglucose was absent from this peak for the initial polysaccharide

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after methylation, it may be concluded that it is derived from the glucuronic acid, which is therefore 1,3-linked and possibly also 1,2-linked.

Periodate-oxidation studies gave results which were in agreement with the methylation data, and confirmed that a proportion of the xylose, galactose, and glucuronic acid residues are 1,3-linked, and that the rhamnose is probably present as end group since it was vulnerable to periodate oxidation.

EXPERIMENTAL

The analytical methods were as described previously[3]. The *Rhodella* mucilage was hydrolysed with 90% formic acid, and the derived syrup was analysed by paper chromatography. The substances having the same mobility as that of rhamnose (R_{GAL} 1.8) and that of 3-*O*-methylxylose (R_{GAL} 2.2; solvent *A*) were separated by preparative paper chromatography. Both substances were examined by g.l.c. (column *4*), as their Me_3Si ethers before and after reduction to their respective alditols, and the retention times compared with those of authentic rhamnose and 3-*O*-methylxylose run as standards. The suspected 3-*O*-methylxylose was labelled at C-1 with deuterium by reduction with borodeuteride. The product and the original substance were acetylated, and the derived acetates were analysed by g.l.c.–m.s. on columns of 3% of OV225[4] and 3% of OV17[5].

A portion of the mucilage was methylated[1], and a second portion (123 mg) was reduced with sodium borohydride *via* the carbodiimide complex[6], and the derived, neutral polysaccharide, recovered in 72% yield, was methylated[1]. The products were hydrolysed and the methylated sugars converted into the methylated alditol acetates. These were analysed by g.l.c.–m.s.[4,5].

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