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Research Note

Structural determination by means of gas chromatography-mass spectrometry of 3-O-(α-D-glucopyranosyluronic acid)-galactopyranose, an aldobiuronic acid derived from *Porphyridium* sp. polysaccharide

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The structure of a disaccharide, basic constituent, 3-O-(α-D-glucopyranosyl-uronic acid)-galactopyranose, derived from *Porphyridium* sp. polysaccharide was determined by a gas chromatography-mass spectrometry method. The permethylated disaccharide alditol was prepared and analysed by gas chromatography-mass spectrometry (chemical ionization and electron impact). Additional support for the nature of the glycosyl residue was obtained from gas chromatography-mass spectrometry analysis of the corresponding permethylated alditol acetate.

INTRODUCTION

In recent years significant advances have been made in methods for structural investigations of natural oligosaccharides (Kärkkainen, 1970, 1971; Dorland *et al.*, 1978*a,b*; Fournet *et al.*, 1978; Schut *et al.*, 1978; Valent *et al.*, 1980; York *et al.*, 1980; Aman *et al.*, 1981). However, progress in this field is limited by the unavailability of these compounds on the market and by the absence of convenient synthetic methods for such compounds. Hence, the structural elucidation of a naturally occurring oligosaccharide can present a considerable challenge, especially if it originates from red microalgal polysaccharides with potential commercial importance (Arad (Malis), 1988). A characteristic common to all the polysaccharides of the red unicells is the presence of uronic acid residues, which complicate investigations of the primary structure, since the uronic acid glycoside is resistant to hydrolysis (Bemiller, 1967; Jones & Albersheim, 1972). Thus, it is necessary to determine monosaccharide sequences by first producing oligosaccharide fragments by acid-catalysed partial hydrolysis and then analysing them by different methods. It has previously been found by our group (by NMR investigation) that the disaccharide isolated from the cell wall polysaccharides of *Porphyridium* sp., *P. cruentum, P. aerugineum* and *Rhodella reticulata* is identical, constituting a basic building block of these polysaccharides (Geresh *et al.*, 1990).

In this paper we present the structure of the same disaccharide, isolated from *Porphyridium* sp. polysaccharide (ca. 9% glucuronic acid; Arad (Malis), 1988) determined by gas chromotography-mass spectrometry as the permethylated alditol derivative. This method

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might constitute the basis for structural investigation of higher oligosaccharides containing this disaccharide fragment.

EXPERIMENTAL

Isolation of the polysaccharide, acid hydrolysis, and separation of the disaccharide fragment were performed as previously described (Geresh *et al.*, 1990). Glycosyl composition of the disaccharide was determined by the TFA-derivatives method according to Montreuil *et al.* (1986*a*). The reducing disaccharide (200 μg) was converted into the disaccharide alditol with sodium borodeuteride. The alditol was permethylated with methyl iodide in the presence of lithium methylsulphinyl carbanion and analysed by gas chromatography-mass spectrometry [electron impact (EI) and chemical ionization (CI) modes] as previously described (Parente *et al.*, 1985; Montreuil *et al.*, 1986*b*).

The methylated sugars obtained by methanolysis of the permethylated disaccharide alditol (100 μg) with 0·5 M methanol-HCl (1 ml) for 16 h at 80°C were acetylated and analysed by gas chromatography-mass spectrometry according to Fournet *et al.* (1981).

RESULTS AND DISCUSSION

By using a separation procedure elaborated previously (Geresh *et al.*, 1990), we obtained the maximal amount of the acidic disaccharide by hydrolysing the polysaccharide with 2 M trifluoroacetic acid for 2 h at 100°C. The fraction giving the TLC R_f value of 0·29 (Geresh *et al.*, 1990) was freeze-dried and used as necessary in this study.

Methanolysis of the dried disaccharide followed by trifluoroacetylation gave, on gas chromatographic analysis, two major series of peaks in addition to other unidentified minor peaks. The peaks with a higher retention time were identified (by comparison with a standard sample) as originating from glucuronic acid, and the other series showed the same retention time as a standard authentic sample of galactose. The ratio between the two identified components was roughly 1:1.

At a later stage, the disaccharide was reduced with $NaBD_4$ (a method that avoids complications due to the presence of anomers and labels the reducing end; Kärkkäinen, 1970, 1971), methylated, and analysed by gas chromatography-mass spectrometry in the CI and EI modes. The main component of the dissaccharide sample was identified unambiguously as the high-intensity peak eluted at 60 min retention time, but efforts to identify the minor component gave no results. The lack of homogeneity of the dissaccharide sample was also seen in a ^{13}C-NMR spectrum (Geresh *et al.*, 1990), but the minor component could not be characterized.

The CI (ammonia) spectrum of the permethylated disaccharide alditol used to complement the EI-mass spectrometry showed, as expected, a number of prominent ions. The spectrum was dominated by the m/z 503 ion which was attributed to an $[M + NH_4]^+$ ion. The presence of this ion demonstrates that the dissaccharide contains a uronosyl residue and a hexitol residue deuterated at C-1 and that the derivative has a molecular weight of 485. Less abundant ions appeared in the spectrum, but their intensity was low and generally of less structural utility.

The structure of the permethylated disaccharide alditol was then elucidated from diagnostic ions of the EI mass spectrum (Fig. 1), and the primary fragements are illustrated in Scheme 1.

The symbols a-j employed by Kochetkov & Chizhov (1966) are used in the interpretation of the mass spectral fragments. The base peak was evident at m/z 101 and the molecular ion was not present, but information about its molecular weight could be obtained on the basis of ions M-45 (440), M-89 (396), and M-133 (352) formed through primary cleavage of the C-C bond in the alditol moiety. The ions at m/z 440 and m/z 352 were very weak, but the ion at m/z 396 represented more than 40% of the base peak, indicating

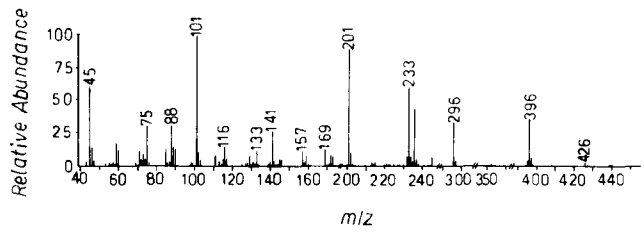
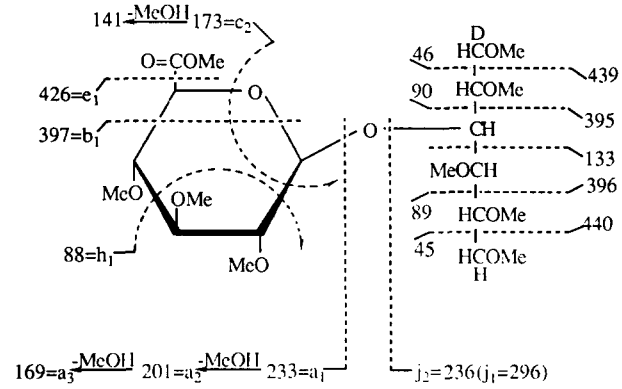


Fig. 1. Electron impact spectrum of the permethylated disaccharide alditol derived from *Porphyridium* sp. polysaccharide.



Scheme 1. Origin of the fragment ions produced during electron impact-mass spectrometry analysis of the permethylated disaccharide alditol derived from *Porphyridium* sp. polysaccharide.

that the cleavage between C4 and C5 was most likely and suggesting a C-3 substitution of the galactitol residue. In addition, the spectrum contained two major series of fragment ions, one derived from the glucuronosyl portion of the molecule and the other from the alditol portion of the molecule.

The presence of the glucuronate residue may be recognized from the ion series of m/z 233, 201 and 169. The fragment ion a_1 at m/z 233 produced by loss of alditol from C-1 subsequently eliminates two molecules of methanol giving rise to a_2 at m/z 201 (MeO group from C-3; Kochetkov & Chizhov, 1966; Gattegno *et al.*, 1981) and to a_3 at m/z 169 (MeO group from C-4).

The corresponding fragments arising from the ionized alditol unit at m/z 236 (j_2), 204 (236–32) and 172 (204-32) are specific for a hexitol containing one deuterium atom (Kärkkäinen, 1970). The presence of these two ions, a_1 and j_2, permitted the indirect estimation of the molecular weight of the disaccharide, which was completely in accord with the CI determination. The j_1 ion, resulting from a complicated fragmentation process (Kochetkov & Chizhov, 1966; McNeil *et al.*, 1982) and acting as a marker for the C-3 substituent of the non-reducing glycosyl residue, is present as a prominent peak at m/z 296. In addition, the h_1 ion at m/z 88 confirms the presence of an O-methyl group at C-2 and C-3 of the non-reducing glycosyl residue. The high-mass ion of low intensity at m/z 426 is thought to be formed by elimination of a carboxy group such as $COOH_3$ and to be an e_1 ion. Its usual fragmentation pathway gives rise to a weak ion at m/z 394 (e_2) and to an intense ion at m/z 141 (e_3). The high intensity of the ion at m/z 141 can better be explained in terms of the latter pathway, i.e. elimination of methanol from a c_2^2 ion at m/z 173. The parent ion of the b series, the b_1 ion, is identified as a low-intensity mass ion at m/z 397.

The position of the glycosidic linkage to the alditol can be identified in terms of the ions produced by cleavage of the alditol chain. The abundance ratio of the m/z 395 to m/z 396 ions was about 0·15. According to the method developed by Albersheim's group (Aman *et al.*, 1981) for discriminating between 3- and 4-linked alditols, such a ratio confirms the presence of a 3-linked hexitol. In addition, the ion at m/z 133, containing three carbon atoms of the alditol chain, demonstrates that the alditol unit is glycosylated in the C-3 position (Kärkkäinen, 1970).

Additional evidence for the point of attachment of the non-reducing residue was obtained by methanolysis of the permethylated disaccharide alditol and acetylation of the newly formed hydroxyl group (which will be borne only by an aglycon), followed by gas chromatography-mass spectrometry (EI) analysis of methylated galactitol acetate. Thus, the acetyl group marks the point of attachment of the uronosyl residue to the alditol moiety. As can be seen from the spectrum

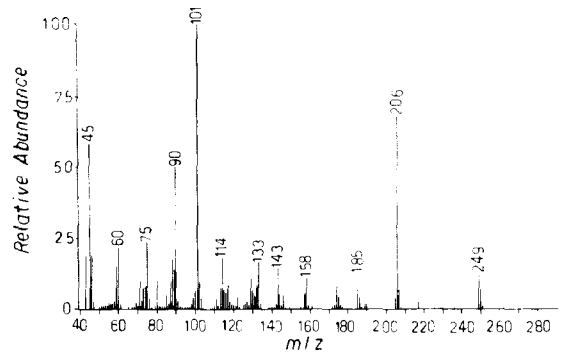


Fig. 2. Electron impact spectrum of the methylated alditol acetate obtained by methanolysis and acetylation of the permethylated disaccharide alditol derived from *Porphyridium* sp. polysaccharide.

(Fig. 2), the molecular ion is not traced, but the ions at m/z 249, 250, 205, 206, 133, 89, 90 and 45, 46 are consistent with primary cleavage of the alditol chain. Their fragmentation consists principally of elimination of acetic acid or ketene (ions of m/z 207, 190, 189, 146 and 142) or of elimination of methanol (ions of m/z 175, 174, 158, 157, 143, 101, 100).

The most favourable position for backbone cleavage is between the C-4 and C-5 carbon atoms (two adjacent methoxy groups), leading to the high intensity m/z 206 ion containing an O-acetyl group at C-3 and a deuterium atom at C-1. As was expected, the ions at m/z 133 and m/z 101 (133-32) are still present after derivatization, and their presence confirms that the alditol moiety bears an acetyl group on C-3.

All the findings confirmed the major component of the disaccharide derived from *Porphyridium* sp. to be 3-O-(glucopyranosyluronic acid)-galactopyranose, which is completely in accord with its NMR structural determination (Jaseja *et al.*, 1989) and with the structure of the aldobiouronic acid isolated from *R. reticulata*, *P. cruentum* and *P. aerugineum* (Jaseja *et al.*, 1989; Geresh *et al.*, 1990). Since the anomeric nature of the glycosidic linkage and the configuration of the glucuronic acid and galactose residues cannot be obtained from the mass spectrum, alternative methods (Jaseja *et al.*, 1989; Geresh *et al.*, 1990) were used for ascertaining this information.

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