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# Note

## An arabinoglucuronomannoglycan from the leaves of *Ornithogalum thyrsoides*

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Several species of *Ornithogalum* (Hyacinthaceae, Asparagales)[1] are indigenous to the Southern and Western Cape regions of South Africa, and are cultivated for the cut-flower market here and abroad. The most common species, *O. thyrsoides*, has been studied with a view to isolating the pathogen Ornithogalum mosaic virus[2]. Isolation procedures were complicated by the mucilaginous nature of the plant sap. The structure of the major polysaccharide associated with the mucilage, which was known to be degraded by a hemicellulase (from *Aspergillus niger*), is now described.

The water-soluble acidic polysaccharide from the leaves of *O. thyrsoides*, isolated from an aqueous extract by precipitation with acetone, was dialysed and purified by way of its cetyltrimethylammonium complex[3]. The polysaccharide (*A*) gave highly viscous aqueous solutions and had $[\alpha]_D$ $-25°$, the total uronic acid content was ~39% by weight[4], and the constituent neutral sugars were Ara and Man in the molar ratio ~5:7. Combustion analysis indicated that no protein was present. Determination of the sugar composition by methanolysis of *A*, reduction of the resulting methyl esters of the uronic acid glycosides with $NaBD_4$, glycoside hydrolysis, and g.l.c.–m.s. of the derived alditol acetates[5] showed that GlcA was the only uronic acid present.

Methylation analysis of *A* indicated that all of the Ara*f* was non-reducing terminal and that the Man*p* was 2-linked. Similar analysis of lithium aluminium deuteride (LAD)-reduced, methylated *A* (*RMA*) and re-methylated *RMA* (*MRMA*) showed that ~25% of the GlcA was 4-linked and that the remainder was 3,4-disubstituted.

Mild hydrolysis of *A* (20mM trifluoroacetic acid, 100°, 8 h) removed most of the Ara, to yield a degraded product (*B*). The facile release of Ara indicated that the sugar was furanoid. The $[\alpha]_D$ value of the released Ara showed it to be L, and the change in $[\alpha]_D$ from $-25°$ for *A* to $+7°$ for *B* showed that the Ara*f* residues were *α*. The uronic acid content (~55% by weight) indicated approximately equimolar proportions for Man and GlcA, in addition to a small amount of Ara, in *B*. Methylation analysis of *B*,

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involving LAD-reduction and subsequent re-methylation, revealed that all of the Man was 2-linked and almost all of the GlcA was 4-linked. In addition to the little 3,4-disubstituted GlcA, there was a corresponding amount of residual, terminal Ara*f*. This result implies that the Ara*f* groups were 3-linked to GlcA in *A*. Therefore, *A* must consist of a main chain of 2-linked Man and 4-linked GlcA, to which Ara is appended in the form of side groups. Such a main chain could be of the glucuronomannoglycan type found in naturally occurring polysaccharides[6], wherein the sugar and uronic acid occur as alternating residues.

Aqueous solutions of *B* were considerably less viscous than those of *A*, thereby facilitating $^{1}$H- and $^{13}$C-n.m.r. spectroscopy. In the $^{1}$H-n.m.r. spectrum of *B*, the signal at $\delta$ 5.39 (bs) was attributed to H-1 of α-D-Man*p*, and that at $\delta$ 4.51 (d, *J* 6.7 Hz), with an integral value similar to that of the peak at 5.39, to H-1 of β-D-Glc*p*A. These assignments were in agreement with those reported by Percival *et al.*[7] for a glucuronomannoglycan derived from a polysaccharide isolated from the brown seaweed *Lessonia nigrescens*. However, the signal at $\delta$ 4.15 (bs) was assigned to H-2 (not H-3) of D-Man*p* on the basis of a COSY experiment. Furthermore, the signal at $\delta$ 3.42 (dd, *J* 7 and 8 Hz) was assigned to H-2 of D-Glc*p*A. The integral values of the H-1 signals of Man and GlcA indicated that the two sugars occurred in equal proportions.

The $^{13}$C-n.m.r. spectrum of *B* contained 12 major peaks (*cf.* ref. 7), the chemical shifts appearing slightly upfield (by ~2 p.p.m.) of those reported in the literature. C-1 of β-D-Glc*p*A resonated at 102.44 p.p.m. and the peak due to C-1 of α-D-Man*p* was at 99.41 p.p.m. The signals at 61.16 and 172.93 p.p.m. were attributed to C-6 of Man and of GlcA, respectively.

Evidence for the alternation of the constituent sugars in *B* was provided by partial hydrolysis, which generated members of the homologous series →4)-[GlcA-(1→2)-Man]$_n$-(1. The first two members of the series were available to us (courtesy of Mr. P. F. K. Eagles) as products of the partial hydrolysis of the gum exudate of *Hakea sericea* and were used as markers in the p.c. of hydrolysates of *B*. The appropriate function of $R_F$ (Bate-Smith and Westall[8]) for the acidic products released, when plotted against d.p., gave the linear relationship expected of an homologous series. Mild hydrolysis of *B* (0.5M trifluoroacetic acid, 100°, 4 h) gave components which appeared to be the monomer, dimer, and trimer according to their mobilities in p.c. [solvent (*d*)], but no higher oligomer could be detected. However, hydrolysis with 0.2M trifluoroacetic acid for 4 h at 100° released the first eight members of the homologous series [p.c., solvent (*f*)]. The latter conditions were adopted on a preparative scale, the duration of hydrolysis being extended to 6 h. Resolution of the products was less satisfactory by preparative p.c. From 240 mg of *B*, the following components (expected value of *n*, yield in brackets) were isolated: $n = 1$ (8 mg), 2 (34 mg), 3 (20 mg), 4 (24 mg), 5 (5 mg), and $>5$ (102 mg). P.c. of the first five components indicated that, for $n = 1$–3, they were homogeneous, whereas they were mixtures for $n = 4$ and 5. Acid hydrolysis (0.5M trifluoroacetic acid, 100°, 20 h) of the fraction with $n > 5$ gave mannose and glucuronic acid (glucurone) in similar proportions together with the aldobiouronic acid ($n = 1$); no neutral disaccharide was detected at any stage of the hydrolysis of *B*.

Identification of components with $n = 2$ and 3 followed from the $^1$H-n.m.r. data. In the region for anomeric protons, the dimer gave signals at $\delta$ 5.44 ($J$ 2 Hz), 5.30 ($J$ 2 Hz), 4.94 (s), 4.57 ($J$ 8 Hz), and 4.55 ($J$ 8 Hz), with integration ratios of 1.0:0.8:0.2:1.0:1.0. These signals were assigned to in-chain α-D-Man*p*, reducing end α-D-Man*p* and β-D-Man*p*, and end-group and in-chain β-D-Glc*p*A, respectively, in accord with those reported[9] for the dimeric species. The suspected trimer gave the following signals for anomeric protons: $\delta$ 5.41 (s), 5.29 ($J$ 2 Hz), 4.94 (s), 4.58 ($J$ 8 Hz), and 4.54 ($J$ 8 Hz) with integration ratios of 2.0:0.8:0.2:1.0:2.0. These results were as expected and it was concluded that *B* consists of a chain of alternating units of 2-linked α-D-Man*p* and 4-linked β-D-Glc*p*A.

Thus, polysaccharide *A* is a conventional glucuronomannoglycan, which has ~75% of the GlcA 3-substituted with L-Ara*f* groups. The structure resembles those of mucilaginous polysaccharides isolated from *Drosera capensis*[10] and *D. binata*[11], and of a polysaccharide from suspension-cultured cells of *Nicotiana tabacum*[12]. A unique feature is the attachment of side groups to GlcA, but not to Man.

## EXPERIMENTAL

*Isolation and purification of polysaccharide* A. — Leaves from *Ornithogalum thyrsoides*, harvested in November 1988, were homogenized in a Waring Blendor with distilled water, and the homogenate was pressed through muslin cloth in order to isolate the water-soluble material. Polysaccharide, precipitated by the addition of acetone (1 vol.), was re-dissolved and dialysed against water for 5 days. The non-dialysable portion, which had become cloudy, was centrifuged, and the supernatant solution was freeze-dried. The product, which contained Man, Ara, GlcA, and Gal residues, was freed of Gal by precipitation[3] with Cetavlon, as described by Mabusela and Stephen[13] with the exception that 3M NaCl was used instead of 2M. From 35 g of dry leaves, the yield of purified polysaccharide *A* was ~1.0 g (Found: C, 45.0; H, 5.9; N, 0.5%).

*General procedures.* — Analytical methods have been described[13–15]; in addition, solvent (*f*), 1-butanol–acetic acid–water (8:5:7) was used. The $^{13}$C-n.m.r. spectrum of *B* was measured at 70° and the COSY experiment was conducted at 80°; all measurements for *B* were on a Varian VXR-200 spectrometer. The oligouronic acids were examined on a Bruker WH 90 instrument.

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