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Note

A novel β-D-(1→2)-linked D-mannopyranan from *Crithidia deanei*†

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The polysaccharides from a number of protozoa inhabiting the gut of insects have been isolated and examined in this laboratory as part of a program whereby polysaccharides of nonpathogenic, insect flagellates are compared, from a chemical and antigenic viewpoint, with those of *Trypanosoma cruzi* (Chagas' disease). The latter preparation is known to give, in part, mannose on hydrolysis[1,2], but its chemical structure is not clear, as Alves and Colli[3] found that the epimastigote form[4] of *T. cruzi* agglutinates with concanavalin A (con A), whereas the trypomastigote forms do not. Later work by Chiari *et al.*[5] indicated that both forms react, perhaps because of the presence of α-D-mannopyranoside or α-D-glucopyranoside residues. During the course of our survey, *Crithidia deanei*, a trypanosomatid of the reduviid *Zelus leucogrammus*[6], was examined, and found to contain a mannan as the only polysaccharide component.

The polysaccharide of *C. deanei* was isolated in yields of 6% by extraction during 6 h with hot, aqueous alkali, which decomposed the protein and most of the nucleic acids. After precipitation with ethanol, the rest of the latter was removed by passage through a column of mixed, ion-exchange resins. Cells of *C. deanei* grown on a sucrose medium provided polysaccharide containing glucose, mannose, and rhamnose in the ratios of 1:2.8:1, whereas a proline medium, which is likely to be more akin to the content of the insect gut[7,8], gave rise to cells containing polysaccharide having a simpler composition; only a mannan was formed, whose ^{13}C-nuclear magnetic resonance (n.m.r.) spectrum contained 6 signals, at δ_c 103.0, 81.1, 77.8, 73.7, 69.3, and 62.6, consistent with a linear structure having one type of linkage. Signals at δ_c 69.3 and 62.6 corresponded to those for C-4 and C-6, and these had the same chemical shift as those of these atoms in the anomers of methyl mannopyranoside[9], which is consistent with 2- or 3-*O*-substitution. The former structure was indicated, because a Smith degradation using strong, hydrolytic conditions provided glycerol only, and a methylation acetylation experiment gave rise to a 3,4,6-tri-*O*-methylmannitol triacetate, characterized by g.l.c.–m.s. β-Glycosidic

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linkages were indicated, as the specific rotation (−50°) corresponded to β-[10], rather than to α-linkages[11]; also, the ^{1}H-n.m.r. spectrum contained a single H-1 signal, at δ 5.5, indicative of β-linkages, as it is at higher field than signals arising from the α configuration[12]. Furthermore, the ^{13}C-n.m.r. spectrum contained signals at δ_c 103.0 (C-1) and 81.1 (substituted -O-C-2), similar to those of β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-, and differing from those of a linear, α-D-(1→2)-linked mannopyranose structure having δ_c 102.3 (C-1) and 80.0 (substituted-O-C-2)[13].

The β-D-(1→2)-linked mannopyranan structure does not appear to have been reported previously. The polymer presumably arises from the protozoa, rather than from a bacterial endosymbiont[14], as a related organism, *Crithidia fasciculata*, under certain cultural conditions, gives a somewhat contaminated mannan showing predominant, ^{13}C-n.m.r. signals having similar chemical-shifts[15]. 2-*O*-Substituted β-D-mannopyranose structures have been reported in mannans of certain yeasts, but only in side chains of structures having α-D-(1→6)-linked mannopyranose main-chains[13]. Such structures and the *C. deanei* mannan do not precipitate[16] with con A, which is specific for α-D-mannopyranosyl residues having free, 3-, 4-, and 6-hydroxyl groups, and are not degraded by exo-α-D-mannosidase[17].

EXPERIMENTAL

Culture conditions. — *Crithidia deanei*, originally isolated from *Zelus leucogrammus*[6], was kindly supplied by Prof. Isaac Roitman of the University of Brasilia. Cells were grown in two different media, with sucrose (medium A), or proline (medium B) as the carbon source. These contained (g/l): sucrose or proline, 20; yeast extract, 3; trypticase 3; KCl, 20; folic acid, 0.02; hemin, 0.01 (dissolved in M NaOH), and the pH was finally adjusted to 7.0.

Large-scale preparations were conducted with 20-liter batches in a 25-liter New Brunswick Fermentor for 48 h at 28°, with agitation (100 r.p.m.) and aeration (8 liter.min^{-1}).

Isolation of the polysaccharides. — Cells were isolated from a large-scale preparation by centrifugation, and washed three times with physiological saline. They were extracted with 6% aqueous KOH (200 ml) for 6 h at 100°, the solution was made neutral with AcOH, the suspension was centrifuged, and the supernatant liquor was added to EtOH (3 vol./vol.). The precipitate was isolated, dissolved in H_2O, and passed through a column of mixed-bed, ion-exchange resins to remove nucleic acids. The eluate was concentrated to a small volume; this was acidified with AcOH, the suspension centrifuged to remove the insoluble material, and the polysaccharide was precipitated by addition of an excess of ethanol to the supernatant liquor. Medium A and medium B both gave 2.7 g (dry weight) of cells, from which 0.15 g of polysaccharide was obtained.

Properties of the polysaccharides. — The polysaccharide isolated from cells grown in medium B had $[\alpha]_D^{25}$ −50° (*c* 0.3, H_2O), and hydrolysis (0.5M H_2SO_4) for 18 h at 100° gave mannose, identified by paper chromatography[18] and by g.l.c.

of the derived alditol acetate[19]. The D enantiomer was indicated, as the material in the hydrolyzate was oxidized by commercial D-glucose oxidase, as revealed by paper chromatography.

The ^{13}C-n.m.r. spectrum of a solution of the mannan in D_2O at 70° under previously described conditions[20] gave 6 signals, at δ_c 103.0, 81.1, 77.8, 73.7, 69.3, and 62.6 (relative to external Me_4Si). The p.m.r. spectrum of a solution of the polymer in 99.97% D_2O, maintained at 70°, contained an H-1 signal at δ 5.5 (relative to external Me_4Si).

Methylation of the mannan, followed by hydrolysis, reduction with $NaBH_4$, and acetylation, gave a 3,4,6-tri-*O*-methylmannitol acetate, characterized by its retention time and e.i. pattern in g.l.c.–m.s.[21]. A Smith degradation incorporating strong hydrolytic conditions[22] gave glycerol only.

No precipitate was observed for con A (5 mg/ml) with the polysaccharide (2–5 mg/ml), conditions which give a precipitate with the mannan of bakers' yeast, which contains α-D-(1→2)-linked, D-mannopyranosyl side-chains. The exo-α-D-mannosidase[23] of *Arthrobacter* GJM-1 gave no mannose after digestion with it for 24 h.

Polysaccharide of cells grown in medium A. — This contained mannose, glucose, and rhamnose in the ratios of 1:2.8:1, as shown, following hydrolysis, by g.l.c. of the derived alditol acetates.

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