Structural Studies of the Extracellular Polysaccharide
Produced by the Diatom Chaetoceros curvisetus Cleve

BERIT SMESTAD, a ARNE HAUG b and SVERR MYKLESTAD b

a Institute of Pharmacy, University of Oslo, P.O. Box 1068, Blindern, Oslo 3, Norway and b Institute of
Marine Biochemistry, University of Trondheim, N-7034 Trondheim-NTH, Norway

Methylation studies and partial acid hydrolysis
have been carried out on the extracellular poly-
saccharide produced by the diatom Chaetoceros
curvisetus. The polysaccharide contains the
sugars galactose, rhamnose, and fucose and is
partially sulfated. The presence of fucose in both
furanose and pyranose forms within one mole-
cule is reported for the first time. The
fucofuranose is present as end groups, 1,2-
linked and as branch points. Fucopyranose is
mainly present as branch points in the poly-
saccharide, some being 1,3-linked, and a small
fraction as end groups. The main part of rham-
bose is 1,2-linked and galactose is mainly 1,3-
linked.

Several diatoms are known to produce extracel-
lar polysaccharides. The production of extracel-
lar polysaccharides produced by species of
the Chaetoceros family, has been studied
and three members of this family were shown to
excrete significant amounts of polysaccharide
into the surrounding medium when being in
the stationary growth phase. The three species
were C. affinis (clone CH1), C. curvisetus (CH24),
and C. decipiens (CH40).

These polysaccharides were all sulfated and
contained the sugars galactose, rhamnose, and
fucose in different proportions. Structural stud-
ies on the polysaccharide produced by C.
affinis showed that this polysaccharide was
highly branched and had a complex structure.
Methylation studies showed that the polysac-
charide produced by C. decipiens had a very
similar structure, while the polysaccharide
produced by C. curvisetus was different. The
present paper describes structural studies of
this polysaccharide.

RESULTS AND DISCUSSION

The cultivation of the diatom C. curvisetus
and isolation of the extracellular polysaccharide
was performed as for C. affinis.

The polysaccharide (CH24) had \( [\alpha]_D = -68^\circ \),
a carbohydrate content of 80 %, \(-\text{SO}_4\text{Na} \) was
6.9 % determined by the method of Antono-
poulos. Potentiometric titration gave an
analogous weight of 950, which, if the only
anion present is sulfate, was calculated to
correspond to approximately 10 % \(-\text{SO}_4\text{Na} \).

SubJECTED to free boundary electrophoresis
at pH 2 and pH 7, the polysaccharide moved as
a single, anionic compound, with approximately
the same mobility at both pH values, also
indicating that the anion is sulfate (or another
strong acid).

Complete acid hydrolysis of the polysaccha-
ride and analysis by GLC of the derived alditol
acetates showed the presence of rhamnose,
fucose, and galactose in the proportions 0.3 to
3.5 to 1.0. No other sugars were detected. The
configuration of the sugars has not been de-
termined due to shortage of material.

Preliminary methylation experiments, com-
combined with analysis of the derived partially
methylated alditol acetates by GLC-MS, indicated
that some of the fucose present as end
groups was in the furanose form. It is well
known that end groups in furanose form are
readily released from the rest of the polysac-
charide under weak acidic conditions. In the
extracellular polysaccharide produced by C.
affinis (CH1) rhamnopyranose is responsible
for the main part of the non reducing end
groups. CH1 and CH24 polysaccharides were
Table 1. Composition of polysaccharide before and after partial hydrolysis.

<table>
<thead>
<tr>
<th>Carbohydrate content (mg)</th>
<th>Sugar ratio Rhamnose</th>
<th>Fucose</th>
<th>Galactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH24</td>
<td>19</td>
<td>0.3</td>
<td>3.5</td>
</tr>
<tr>
<td>1 h</td>
<td>10</td>
<td>0.3</td>
<td>2.3</td>
</tr>
<tr>
<td>3 h</td>
<td>7.5</td>
<td>0.2</td>
<td>1.6</td>
</tr>
</tbody>
</table>

both subjected to weak acid hydrolysis (see Experimental). The hydrolysates of both polysaccharides were dialysed, and the dialysates tested for carbohydrate. The dialysate from CH1 polysaccharide contained no carbohydrate, while that from CH24 polysaccharide contained considerable amounts. When analysed by TLC, and GLC after conversion to alditol acetates, no other monosaccharide than fucose was found in the dialysate.

The starting material and the products after 1 and 3 h of hydrolysis, were all analysed for sugar ratios by GLC and for carbohydrate contents by the phenol-sulfuric acid method, using as standards solutions containing the sugars in appropriate ratios (Table 1). It can clearly be seen from Table 1 that fucose is lost during this hydrolysis, and the fact that it is readily released indicates that it is present in the furanose form.

The three polysaccharide samples were subjected to methylation, hydrolysed and converted into the partially methylated alditol acetates. These were all analysed by combined GLC-MS (col. OV-225). The identifications were thus confirmed both by their retention times (T) and mass-spectra (Table 2). The compounds with T-values 0.56, 0.96 and 1.33, gave a MS-fragmentation pattern different from those derived from partially methylated alditol acetates originally present in pyranose form. The compound with T = 0.56 gave as the main fragments m/e 59, 117, and 175 (Fig. 1), which previously has been shown to derive from the alditol acetate corresponding to 2,3,5-tri-O-methylfucose. That with T = 0.96 gave as main fragments m/e 59, 175, and 189 (Fig. 1). These can only be obtained from the alditol acetate corresponding to 3,5-di-O-methylfucose. The compound with T = 1.33 gave as the main fragment m/e = 59 (Fig. 1), indicating that the compound is the alditol acetate corresponding to 5-O-methylfucose.

The presence of these three methylated products shows that fucose in the furanose form is not only present as end groups, but also as part of the chain and as branch points. The presence of these forms in a polysaccharide

Table 2. GLC analysis of the methylated alditol acetates.

<table>
<thead>
<tr>
<th>Retention times * (T)</th>
<th>Column OV-225</th>
<th>Weights calculated from GLC (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4-Tri-O-methylrhamnose</td>
<td>0.47</td>
<td>0.2</td>
</tr>
<tr>
<td>2,3,5-Tri-O-methylfucose</td>
<td>0.56</td>
<td>2.9</td>
</tr>
<tr>
<td>2,3,4-Tri-O-methylfucose</td>
<td>0.60</td>
<td>tr</td>
</tr>
<tr>
<td>3,4-Di-O-methylrhamnose</td>
<td>0.88</td>
<td>1.0</td>
</tr>
<tr>
<td>3,5-Di-O-methylfucose</td>
<td>0.96</td>
<td>4.0</td>
</tr>
<tr>
<td>2,4-Di-O-methylfucose</td>
<td>1.04</td>
<td>1.8</td>
</tr>
<tr>
<td>2,3,4,6-Tetra-O-methylgalactose</td>
<td>1.15</td>
<td>0.3</td>
</tr>
<tr>
<td>5-O-Methylfucose</td>
<td>1.33</td>
<td>1.8</td>
</tr>
<tr>
<td>2-O-Methylfucose</td>
<td>1.41</td>
<td>1.1</td>
</tr>
<tr>
<td>4-O-Methylfucose</td>
<td>1.71</td>
<td>2.9</td>
</tr>
<tr>
<td>2,4,6-Tri-O-methylgalactose</td>
<td>1.92</td>
<td>1.5</td>
</tr>
<tr>
<td>4,6-Di-O-methylgalactose</td>
<td>2.95</td>
<td>1.3</td>
</tr>
</tbody>
</table>

* Relative to 2,3,4,6-tetra-O-methyl-D-glucitol 1,5-disacinate.
of the fucose residues lost were linked to fuco-
pyranose residues being branch points; the
main part has probably been linked to fucose in
the 2-position, as the weight of 4-O-methyl-
fucose has dropped most. 2-O-Methylfucose can
be derived from fucose present both in the
pyranose and furanose form. There is a con-
siderable loss of 2-O-methylfucose during the
first period of the hydrolysis (Table 2). This
indicates that some of the fucose present as
2-O-methylfucose in the methylated starting
material is present as furanose in the polysac-
charide.

The ratio between rhamnose present as end
groups to that present as part of the chain
increases from 0.2:1 to 1:1 as hydrolysis time
increases. From this it can be deduced that
some of the fucose lost was linked to rhamnose
at C-2, which then gives rise to end groups when
the fucose residues disappear. The changes in
the amounts of the different methylated galac-
tose derivatives are relatively small. The main
being in 4,6-di-O-methylgalactose, indicating
that some half-estersulfate and/or fucose at-
tached to galactose is released during hydrolysis.

The results above show that the extracellular
polysaccharide produced by the diatom *C. curvistus* is a highly branched polysaccharide
of a complex structure. Fucose is present both
in furanose and pyranose forms, the former
being responsible for the main part of the end
groups. Fucofuranose is also present as part
of chains and as branch points. Fuco pyranose
appears to be responsible for the main part of
the branch points of the polysaccharide. Some
of the fucose appears to be linked to rhamnose
at C-2. Rhamnose and galactose are responsible
for a small part of the end groups. Galactose
is mainly present at the inner part of the mole-
cule.

**EXPERIMENTAL**

*Weak acid hydrolysis.* The polysaccharide was
dissolved (1%) in 0.01 N sulfuric acid. The
mixture was heated at 100 °C and aliquots
withdrawn after 1 and 3 h. The hydrolysates
were, after cooling, neutralized with sodium
hydrogencarbonate. The hydrolysates were
freeze dried after dialysis.

Carbohydrate contents and sugar ratios were
measured after all the above-mentioned steps.
Other experimental details were as previously
described.4

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REFERENCES

3. Myklestad, S. *To be published.*
5. Smestad, B. *Unpublished results.*

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