Sulphated Polysaccharides of Brown Seaweeds
*Cystosira compressa, Fucus virsoides, and Dyciopteris membranacea*

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Sulphated polysaccharides have been isolated from three kinds of brown algae—*Fucus virsoides, Cystosira compressa,* and *Dyciopteris membranacea.* The specimens of the algae were collected during three different seasons. Extraction of sulphated polysaccharides was carried out using different media and at different temperatures. Extractive fractions were precipitated by successive adding of ethanol containing magnesium chloride. The purity of single fractions was checked by electrophoresis on cellulose acetate. Following total acid hydrolysis, the composition of each single fraction was studied by gas chromatography. Xylose, manose, galactose and α-L-fucose were identified in various proportions. Low concentrations of glucose were identified in a smaller number of fractions. Uronic acids, sulphate, and proteins occur in quite variable amounts. So, the investigated algae synthesize a wide range of sulphated polysaccharides which can be separated from the complex mixture. Seasonal variation in the composition of the fractions are of minor importance.

INTRODUCTION

Sulphated polysaccharides from brown seaweeds have been studied extensively since 1913.¹ ² The isolated polysaccharides differ in composition and physical and chemical properties.³ ⁷ They contain various proportions of monosaccharides, uronic acids, and sulfates. The proportion of protein is small but always present.

In this paper, we report an extensive study of three kinds of Adriatic brown seaweeds:⁸ ⁹ *Fucus virsoides, Cystosira compressa,* and *Dyciopteris membranacea.* It should be noted that this is the first investigation of brown algae in the Adriatic with respect to their sulphated polysaccharides. Food reserves and structural polysaccharides have not been studied by us.

*Fucus virsoides* is an Adriatic endemic seaweed and grows abundantly in places where water salinity or temperature are decreased. *Cystosira compressa* and *Dyciopteris membranacea* grow uninterruptedly over the whole year.
EXPERIMENTAL

Material

The algae were harvested at the promontory of Marjan (Split, Croatia) in March, July and November of 1981, and 1982. The algae were washed in tap water to remove impurities, then air-dried at room temperature. For each extraction, 45 g of dry powder-ground algae were used.

Methods

Extraction of Polysaccharides

Extractions were carried out according to Scheme I.\textsuperscript{10,11}

The various fractions were obtained by precipitation with ethanol after dialysis, concentration and freeze-drying. Further subfractions were obtained after addition of MgCl\textsubscript{2} or CPC and ethanol according to the procedure of Larsen.\textsuperscript{12-15}

Further Fractionation

To 1 vol. of 1% extract solution, the same volume of 0.1 mol / l MgCl\textsubscript{2} and then ethanol (2 vol.) were added. The precipitate was separated by centrifugation, dissolved in distilled water, dialyzed against distilled water (48 hours), frozen, and lyophilized. This was the so-called insoluble fraction marked as F(insol.). Ethanol was removed from the supernatant in vacuo (up to 40 °C). The remainder was dialyzed against distilled water (48 hours), frozen, and lyophilized. This was the so-called soluble fraction, marked as F(sol.). A further fractionation of this fraction took place after the separation of the neutral component, carried out by adding 2.5% cetylpyridinium-chloride to a 2% solution of this fraction. The suspension was allowed to stand

\begin{center}
\begin{tikzpicture}

\node {45 g powdered dry specimen} ;
\node [below] {Water (20 hrs, room temperature)} ;
\node [below] {Extract} ;
\node [below] {2% CaCl\textsubscript{2}, (2 extractions, 5 hrs each, room temperature)} ;
\node [below] {Extracts combined} ;
\node [below] {pH 2.0 and 2.5, (2 extractions, 15 hrs each, room temperature)} ;
\node [below] {Extracts combined} ;
\node [below] {3% Na\textsubscript{2}CO\textsubscript{3}, (4 times, 4 hrs each, 50 °C)} ;
\node [below] {Extracts combined} ;
\node [below] {Soluble} ;
\node [below] {Insoluble} ;
\node [below] {Rest} ;
\end{tikzpicture}
\end{center}

Scheme I. Extraction of sulphated polysaccharides from seaweeds. The extracts in acid media were subjected to fractionation by ethanol.
overnight at room temperature. The precipitate was separated by centrifugation, then dissolved in 4 mol/l NaCl at 37 °C. Then, ethanol (3.5 to 4 vol.) was added and left at room temperature for 5 hours. The precipitate was separated by centrifugation, dissolved in distilled water, dialyzed against distilled water (48 hours), frozen, and lyophilized. This was, in fact, the soluble fraction freed from neutral components. It was again fractionated, by taking 1 vol. of 1% fraction solution and adding 0.1 mol/l MgCl₂ (0.35 vol.) and ethanol (1 vol.). The precipitate was separated by centrifugation, dissolved in distilled water, dialyzed against distilled water, frozen, and lyophilized. This was fraction F₁(sol.). As long as the formation of the precipitate continued, ethanol, in various volumes, was added to the supernatant and successive fractions collected.

Each fraction was separated by centrifugation, dissolved in distilled water, dialyzed against distilled water, frozen, and lyophilized. Further fractions were given the marks F₂, F₃ and F₄, and subfractions were designated (F₁)F₁, (F₁)F₂ or (F₂)F₁, (F₂)F₂; in brackets are the fractions which had to be further subfractionated and which gave F₁, F₂ and so on subfraction.

The insoluble fraction was fractionated by taking 1% solution of this fraction (1 vol.) and adding the same volume of 0.05 mol/l MgCl₂ and also ethanol (1.5 vol.). The precipitate was separated, dissolved in distilled water, dialyzed, frozen, and lyophilized, and fraction F₁ obtained. By adding ethanol (1 vol.) to the supernatant, fractions F₂, F₃ and eventually F₄ were prepared in the same way, following the method of Larsen, slightly modified in some steps.

The extract obtained with 3% sodium carbonate was fractionated following dialysis and lyophilization. Fractionation was carried out after Larsen, slightly as described above. Fractions F₁ and F₂ were obtained. If further fractionation proved necessary, a greater volume of ethanol was added.

Chemical Methods

All reagents were pro analysi, purchased from Kemika, Zagreb. The purity and homogeneity of individual polysaccharides were determined by cellulose-acetate electrophoresis using a modified method of Seno. The separation of neutral from sulphated components was carried out by the method of Scott. Proteins were determined according to Lowry. Sulfates and carboxyl groups were assayed after Scott. Assay of 1-fucose was carried out calorimetrically. Neutral sugars were determined after acid hydrolysis, reduction and formation of alditol acetates. The mixtures were analyzed by gas-liquid chromatography (using a HP 5840A GC) on a column of 3% ECNS-M.

RESULTS AND DISCUSSION

Analysis of the extracts and fractions shows that there are differences between individual algae and differences between fractions from the same alga, depending up on the extraction conditions. All extracts contain mixtures of heteropolysaccharides and contain different proportions of monosaccharides, sulfates, uronic acids, and proteins.

Tables I and II show the composition of aqueous and CaCl₂ extracts.

In all the three algae studied, the amount of sulfates was markedly higher than the amount of uronic acids.

Table III gives the composition of the fractions of the algae extracted in acid media (pH 2.0 and 2.5). In the majority of fractions, the proportion of sulphates exceeded that of uronic acids. In all fractions, proteins were found only in traces. The content of monosaccharides in individual fractions differed considerably.

Table IV shows the composition of fractions isolated from algae by extraction with 3% sodium carbonate.

The fractions presented in Tables I, II, III and IV are the results of an extensive analysis of the three kinds of algae studied. The findings indicate certain peculiari-
ties of brown algae which have not been observed in previous studies. This relates to the contribution of fucose in the formation of individual polysaccharides. Other results are generally in good agreement with most of the previous findings. This study and a number of previous studies\textsuperscript{12-14} have shown that in brown algal fuco-
dian contains, in addition to fucose, also xylose, galactose and mannose which are not impurities but participants in the formation of these polysaccharides. This special-
ly relates to polysaccharides obtained in pure state. It can be generally stated that proportions of monosaccharides in different fractions vary considerably. This has been corrobอated by this study. One further confirmation comes from ascophyl-
lan obtained from the alga Ascophyllum nodosum\textsuperscript{15} and from sargassan obtained from the alga Sargassum linofolium.\textsuperscript{10} Ascophyllan has a high percentage of xylose, while in sargassan five monosaccharides (galactose, glucose, mannose, xylose and fucose) are present, with fucose having the smallest share. Fractions from brown Mediterra-
nean algae possess all five monosaccharides in different proportions, which is not always the case of the algae from the northern seas.

Fucose was the dominant sugar in fractions from all the three algae. According to the published data, the contribution of fucose in fractions from a neutral medium was expected to be smaller. The statement of Larsen\textsuperscript{15} that the amount of fucose in

\begin{table}
\centering
\caption{Composition of the water extract from algae}
\begin{tabular}{lcccccccc}
\hline
Alga & Yield\textsuperscript{a} (%) & Sulphates\textsuperscript{b} & Uronic acids\textsuperscript{b} & Proteins\textsuperscript{b} & Fucose\textsuperscript{b} & Distribution of neutral sugars\textsuperscript{c} (%) \\
& & & & & & Fuc & Xyl & Man & Gal & Glc \\
\hline
Fucus viridosides & 3.2 & 15.9 & 8.2 & 7.4 & 19.1 & 34.5 & 35.1 & 3.4 & 7.3 & 19.0 \\
Cystosira compressa & 2.9 & 21.3 & 11.2 & 2.5 & 39.4 & 73.7 & 13.1 & 0.6 & 12.6 & 0 \\
Dyctiopteris membranaeae & 1.5 & 19.3 & 12.7 & 4.5 & 29.1 & 50.5 & 38.9 & 0 & 10.5 & 0 \\
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\begin{table}
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\caption{Composition of the extracts obtained by 2\% CaCl\textsubscript{2}}
\begin{tabular}{lcccccccc}
\hline
Alga & Yield\textsuperscript{a} (%) & Sulphates\textsuperscript{b} & Uronic acids\textsuperscript{b} & Proteins\textsuperscript{b} & Fucose\textsuperscript{b} & Distribution of neutral sugars\textsuperscript{c} (%) \\
& & & & & & Fuc & Xyl & Man & Gal & Glc \\
\hline
Fucus viridosides & 2.8 & 15.6 & 9.2 & 5.2 & 19.5 & 28.4 & 34.2 & 4.5 & 9.7 & 23.1 \\
Cystosira compressa & 23.5 & 15.8 & 6.7 & 1.3 & 32.1 & 62.9 & 8.9 & 0 & 26.4 & 1.7 \\
Dyctiopteris membranaeae & 15.7 & 12.3 & 13.5 & 1.1 & 4.7 & 8.6 & 60.2 & 5.4 & 4.3 & 21.5 \\
\hline
\end{tabular}
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<th>Sulphates&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Uronic acids&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Proteins&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Fucose&lt;sup&gt;b&lt;/sup&gt;</th>
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<sup>a</sup> Percentage of the previous fraction

<sup>b</sup> Percentage relative to the lyophilized fraction

<sup>c</sup> Calculated from gas chromatograms, taking the total area under peaks as 100%
TABLE IV
Composition of fractions from the extracts obtained by 3% Na₂CO₃. Numbering of fractions explained in the text.

<table>
<thead>
<tr>
<th>Alga</th>
<th>Fraction</th>
<th>Yield&lt;br&gt; (%)</th>
<th>Sulphates&lt;br&gt; (%)</th>
<th>Uronic acids&lt;br&gt; (%)</th>
<th>Proteins&lt;br&gt; (%)</th>
<th>Fucose&lt;br&gt; (%)</th>
<th>Distribution of neutral sugars&lt;br&gt; (%)</th>
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* Percentage of the previous fraction
* Percentage relative to the lyophilized fraction
* Calculated from gas chromatograms, taking the total area under peaks as 100%

Individual soluble fractions increase with increased acidity has not been confirmed by the present results. The reason might be that the respective algae were first extracted in two media, which led to fractions containing a high proportion of fucose.

In conclusion, it may be stated that brown algae synthesize a wide spectrum of polysaccharides whose basic component does not have to be fucose but some other monosaccharides (xylose, galactose, glucose and even mannose). The composition of individual fractions depends on fractionation procedures.

REFERENCES
SULPHATED POLYSACCHARIDES OF BROWN ALGAE

SAŽETAK

Sulfatirani polysaharidi smedih algi Cystosira compressa, Fucus virsoides, i Dyctiopteris membranacea

Ante Pelivan i Aleksandar Lutkic

Iz smedih algi Fucus virsoides, Cystosira compressa i Dyctiopteris membranacea, ubranih u tri godišnja doba, ekstrahirani su sulfatirani polysaharidi u različitim medijima i pri različitim temperaturama. Ekstrahirane frakcije dobivene su uzastopnim taloženjem otopinom MgCl₂ u etanolu, a čistoća pripravaka bila je provjeravana elektroforezom na celuloznom acetatu. Nakon kiselo hidrolize frakcija određene su s pomoću plinske kromatografije količine pojedinih monosaharida: ksilaze, manoze, galaktoze i α-L-fukoze. U nekim su frakcijama nađene i male količine glukoze. Uronske kiseline, sulfat i proteini dolaze u vrlo različitim količinama. Proučavane alge proizvode veliku porodicu sulfatiranih polysaharida koji se mogu izdvojiti iz kompleksne smjese. Sezonske varijacije nemaju znatnijeg utjecaja na sastav pripravaka.