

COMPARATIVE STUDIES ON THE CARBOHYDRATE COMPOSITION OF  
MARINE MACROALGAE

An Annotated Bibliography

Submitted To:

SERI-DOE

Robert W. Mack, Ph.D.  
Jackson State University  
Subcontract No. XK-3-03149-1

February 1984

ISOLATION AND PARTIAL CHARACTERIZATION OF A XYLOGLUCAN FROM THE CELL WALLS  
OF Phaseolus coccineus

Malcolm A. O'Neill and Robert R. Selvendran

Carbohydrate Research, 11 (1983) 239-255.

Cell-wall material from Phaseolus coccineus was fractionated by successive extraction with aqueous inorganic solvents. From the 4M KOH-soluble fraction, a polymer composed of L-arabinose (4.2%), L-fucose (6.0%), D-galactose (9.3%), D-xylose (34.1%), and D-glucose (46.4%) was isolated and purified by ion-exchange and cellulose column chromatography; the product was homogenous by moving boundary electrophoresis and ultracentrifugation. The  $S^{20,w}$  and  $D_{obs}$  were 2.92 S and  $1.7 \times 10^{-7}$ , respectively, and the molecular weight was  $\sim 110,000$ . Methylation analysis suggested a (1  $\rightarrow$  4)-linked glucan backbone with  $\sim 3$  out of 4 glucosyl residues substituted through O-6 with xylose or oligosaccharides terminating in galactose, fucose, and arabinose. Limited acetolysis gave several di- and tri-saccharide derivatives of which four [d-Galp-(1  $\rightarrow$  2)-D-Xylp, D-Glcp-(1  $\rightarrow$  4)-D-Glcp, L-Fucp-(1  $\rightarrow$  2)-D-Galp-(1  $\rightarrow$  2)-D-Sylp, and D-Glcp-(1  $\rightarrow$  4)-D-Glcp-(1  $\rightarrow$  4)-D-Glcp] were tentatively identified. Specific glycosidases were used to determine the configuration of the glycosidic linkages. The backbone was shown to be a (1  $\rightarrow$  4)- $\beta$ -D-glucan and the L-fucosyl groups were  $\alpha$ . Neither  $\alpha$ - or  $\beta$ -D-galactosidase removed D-galactose. The structure of the xyloglucan is discussed in relation to other cell-wall polymers, especially cellulose.

This study is highly significant because it describes procedures for cell-wall polysaccharide extraction, fractionation and characterization.

METHANOLYSIS STUDIES OF CARBOHYDRATES, USING H.P.L.C.

Norman W.H. Cheetham and Padmini Sirimanne

Carbohydrate Research, 112(1983)1-10

An h.p.l.c. system that separates carbohydrates as their methyl glycosides has been used to study the products obtained on treatment of various carbohydrates with methanolic hydrogen chloride. Results are presented for the monosaccharide composition of several polysaccharides, lactone and ester formation during the treatment of D-glucuronic acid, and relative rates of glycosidation vs. esterification during the treatment of D-galacturonic acid.

THE AGAR-TYPE POLYSACCHARIDE FROM THE RED ALGAE, Gracilaria secundata

Donald J. Brasch, Chaw T. Chuan, and Lourence D. Melton

Carbohydrate Research, 115 (1983) 191-198.

Aqueous extraction of the red alga Gracilaria secundata gives a low-sulfated, agar-type polysaccharide which gels strongly. The structure of the polysaccharide has been investigated by methylation, partial hydrolysis of the permethylated agar, enzymic oxidation, and  $^{13}\text{C}$ -n.m.r. spectroscopy. The agar is mainly composed of the familiar (1 $\rightarrow$ 3)-linked  $\beta$ -D-galactosyl residues and (1 $\rightarrow$ 4)-linked 3,6-anhydro-xpL-galactosyl residues, but important variations occur. The distribution of 6-O-methyl-D-galactosyl residues is considered to be in "blocks". Preparative paper-chromatography was effective in separation of the components of the polysaccharide hydrolysate proceeding identification by mass spectrometry.

PURIFICATION AND CHARACTERIZATION OF AGAR FROM Digenea simplex

Mohamed M. El-Sayed

Carbohydrate Research, 118 (1983) 119-126

The agar of Digenea simplex was isolated in 26.12% yield. The agarose fraction (19.59%) was purified by anion-exchange chromatography on DEAE-Sephadex A50. Acid hydrolysis of the agar isolated gave galactose, 3,6-anhydrogalactose, 6-O-methylgalactose, glucose and xylose. Acid hydrolysis of the agarose fraction afforded galactose, 3,6-anhydrogalactose, and 6-O-methylgalactose. The content of  $\text{SO}_4^{2-}$  was lowered from 2.33 to 0.07% by fractionation. The molecular weight and rheological properties of the agarose isolated were studied.

A SIMPLE AND RAPID PREPARATION OF ALDITOL ACETATES FOR MONOSACCHARIDE ANALYSIS

Anthony B. Blankney, Philip J. Harris, Robert J. Henry, and Bruce A. Stone

Carbohydrate Research, 113(1983) 291-299

A simple and rapid method is described for the preparation of alditol acetates from monosaccharides. It can be performed in a single tube without transfers or evaporations. Monosaccharides are reduced with sodium borohydride in dimethyl sulphoxide and the resulting alditols acetylated using 1-methylimidazole as the catalyst. Removal of borate is unnecessary and acetylation is complete in 10 min. at the room temperature. Monosaccharides are quantitatively reduced and acetylate by this procedure. The alditol acetates are completely separated by glass-capillary, gas-liquid chromatography on Silar 10C. The method has been applied to the analysis of monosaccharides in acid hydrolysates of a plant cell-wall.

THE POLYSACCHARIDES OF THE BROWN SEAWEED, Turbinaria murrayana

Mohamed M. El-Sayed

Carbohydrate Research, 110 (1982) 277-282

Polysaccharides of the brown seaweed Turbinaria murrayana were isolated. Laminaran (3.2%) was isolated from the hot-water extract of the algae by using ion-exchange chromatography. Fucans (2.1%) were isolated from the hot-water extract, as well (4.7%) as from the extract of the algae with dilute acid. Acid hydrolysis of the isolated fucans revealed glucose, mannose, fucose, glucuronic acid, xylose, and galactose. Alginic acid (22.6%) was separated and reduced to a neutral polysaccharide. The polysaccharides isolated were analyzed by methylation and Smith degradation.

THE DETERMINATION OF THE URONIC ACID COMPOSITION OF ALGINATES BY ANION-EXCHANGE LIQUID CHROMATOGRAPHY

Peter Gacesa, Alison Squire, and Peter J. Winterburn

Carbohydrate Research, 118(1983)1-8

An isocratic, anion-exchange, liquid chromatography system has been developed to separate and quantify the uronic acids (D-mannuronic and L-guluronic acids) that are present in acid hydrolysates of alginates. The sensitivity of the technique will allow 10  $\mu$ g of uronic acid to be detected. The method has the advantages of speed (typical 140  $\mu$ g of hydrolysate), while retaining accuracy of traditional methods.

A GAS-CHROMATOGRAPHIC METHOD FOR IDENTIFICATION OF THE REDUCING UNITS OF  
DISACCHARIDES VIA REDUCTION OF THE METHOXIMES WITH BORANE

Higuinaldo Jose Chaves Das Neves, Ernst Bayer, Gunter Blos, and Hartmut Frank  
Carbohydrate Research, 99(1982) 70-74

For disaccharides, the identity of the products obtained after methanolysis of the modified molecule reveals the sequence of the monosaccharides. The derivative of the reducing monosaccharide affords a single peak in g.l.c., which is well-separated from those for the methyl glycosides arising from the non-reducing moiety.

Reduction of methoximes with borane affords a stable amine-borane complex from which the free amine can be liberated by heating with M methanolic hydrogen chloride, a treatment which simultaneously cleaves the glycosidic bonds.

The method reported can be used with quantities in the picomole range and offers a simple and sensitive method for determining the identity of reducing-terminal sugars and is also compatible with methylation analysis. Also, the high yields of the derivatisation steps coupled with the high sensitivity of glass-capillary g.l.c. render the method useful for the structural analysis of carbohydrates available in only minute quantities.

THE GALACTAN SULFATE FROM THE EDIBLE, RED ALGA Porphyra columbina

Donald L. Brasch, Hi Mui Chang, Chaw T. Chuan, and Laurence D. Melton  
Carbohydrate Research, 97(1981) 113-125

A galactan sulfate has been isolated from the seaweed Porphyra columbina, and its structure established by a combination of methylation, methanolysis, treatment with alkali followed by methylation, and <sup>13</sup>C-n.m.r. spectroscopy. The polysaccharide belongs to the porphyran class, and consists of 3-linked -D-galactose residues and 4-linked α-L-galactosyl residues. 3,6-Anhydro-L-galactose and L-galactose 6-sulfate residues total approximately half of the sugar units, the other half being made up of D-galactose and 6-O-methyl-D-galactose residues. Some evidence is presented that suggests that the galactan sulfate does not have a completely alternating structure.

## TOTAL CHARACTERIZATION OF POLYSACCHARIDES BY GAS-LIQUID CHROMATOGRAPHY

Susan H. Turner and Robert Cherniak

Carbohydrate Research, 95(1981) 137-144

The g.l.c. procedures presented consist of a consolidation and modification of existing methods that allow the analysis of glycoses, hexosamines, Smith-degradation products, and O-acyl content on a single, stationary phase by the selection of simple, derivatization techniques and appropriate, temperature parameters.

The procedures described herein have numerous advantages over other methods generally used for carbohydrate analysis. Per-(trimethylsilyl)ation results in the formation of as many as four discrete products, which arise from the  $\alpha$ - and  $\beta$ -pyranose and  $\alpha$ - and  $\beta$ -furanose forms of the aldoses investigated. Additionally, the analytical procedures using alditol acetates eliminate the problem of multiple products and alditol acetates are stable.

## IDENTIFICATION OF ALDOSES BY USE OF THEIR PERACETYLATED ALDONONITRILE DERIVATES: A G.L.C.-M.S. APPROACH

Fred R. Seymour, Edward C. M. Chen, and Stephen H. Bishop

Carbohydrate Research, 73(1979) 19-45

The g.l.c. retention-time and detector responses have been examined for peracetylated aldonitrile derivatives from aldoses. Correlations have been made between changes in g.l.c. retention-times and changes in the stereochemistry and functional groups of the parent aldose. The mass spectra [electron impact (e.i.), ammonia chemical ionization (c.i.), and methane c.i.] for these g.l.c. peaks were recorded. C.i.-mass spectrometry (m.s.) indicated the molecular weights of the derivatives, and the number of aldehyde and alcohol groups in the parent aldose. E.i.-m.s. indicated the nature and position of functional groups present in the parent aldose. Aldoses containing acetamido, amino, deoxy, and thio substituents were studied.

# MARINE MACROALGAE AS FOODS FOR FISHES: AN EVALUATION OF POTENTIAL FOOD QUALITY

W. Linn Montgomery and Shelby D. Gerking

Environmental Biology Fishes Vol. 5, No. 2, pp.143-153, 1980

A revitalized view of feeding by herbivorous marine fishes is sought through two questions. First, what characteristics of major taxa of algae identify them as predictably high or low quality foods? Second, are marine algae valuable foods for fishes which do not mechanically disrupt cell walls and do not harbor specialized enzymes or microbes capable of lysing cell walls? Energy, ash and nutrient content of 16 species of marine algae were employed to assess food quality of fleshy red, green, brown and calcareous red algae. On the basis of ash, calories, total protein and total lipid content, fleshy algae should be superior to calcareous algae as foods for fishes; in addition, green algae should be superior to brown algae and brown algae superior to red algae. When the probable digestibility of storage and extracellular carbohydrates is considered, green and red algae are predicted superior to brown algae as food. Two species of damselfishes (Pomacentridae) from the Gulf of California, Eupomacentrus rectifraenum and Microspathodon dorsalis, eat red and green algae and ignore brown and calcareous algae. They feed, therefore, in a fashion consistent with predictions based only on algal chemistry. These fishes absorb at least 20-24% of the biomass, 57-67% of the protein, 46-56% of the lipid and 37-44% of the carbohydrate contained in algae eaten in the wild. Since these damselfishes do not masticate their food, it appears that herbivorous fishes can digest major fractions of algal nutrients without mechanical destruction of algal cells.

This study is significant because it provides information relative to the chemical composition of three divisions of marine algae. More specifically, the collective averages of all three divisions (green, brown and red) of fleshy macroalgae were approximately 3% lipid, 8% protein, 54% carbohydrate and 34% ash.

STRUCTURAL STUDIES OF THE CAPSULAR POLYSACCHARIDE OF Klebsiella TYPE 33

Bengt Lindberg, Frank Lindh, Jorgen Lonngren, and Wolfgang Nimmich

Carbohydrate Research, 70(1979)135-144

The structure of the capsular polysaccharide from Klebsiella type 33 has been investigated. Methylation analysis, various specific degradations, graded hydrolysis with acid, and n.m.r. spectroscopy were the principal methods used. It is concluded that the polysaccharide is composed of pentasaccharide repeating-units. The D-galactopyranosyl group, with pyruvic acid linked as a ketal to O-3 and O-4, was degraded on treatment of the fully methylated polysaccharide with strong base. It is proposed that methyl pyruvate is eliminated, in an E2 type of reaction.

CHANGES IN CARBOHYDRATE LEVELS IN RED KIDNEY BEAN (Phaseolus vulgaris L.) EXPOSED TO SULPHUR DIOXIDE

M. J. Koziol

Journal of Experimental Botany, Vol. 29, No. 112, pp. 1037-1043, 1978

Red kidney bean (Phaseolus vulgaris L.) was exposed to various concentrations of SO<sub>2</sub> for 24 hr (16 h light/8 h dark photoperiod) with continuous monitoring of photosynthetic and respiratory activity. Plants were harvested at the end of the dark period into samples of mature and immature leaf tissue, stems, and roots for determination of sugar and starch levels.

In all tissue samples the levels of total sugars were increased by exposure to the lower concentrations of SO<sub>2</sub>, but decreased by the higher concentrations. Starch levels in leaves followed by similar trend. Increase in sugar and starch levels preceded symptoms of visible injury. Decreasing rates of photosynthesis were correlated with increasing rates of respiration, the occurrence of visible injury, and the depletion of sugar and starch levels.

This paper is of moderate significance in that it describes procedures for the extraction and colorimetric assay of total sugars and starch only.

CELLULAR AND EXTRACELLULAR POLYSACCHARIDES OF THE BLUEGREEN ALGA, NOSTOC

Veela B. Mehta and B. S. Vaidya

Journal of Experimental Botany, Vol. 29, No. 113, pp. 1423-1430, 1978

The carbohydrate content of various cellular fractions of the blue-green alga, Nostoc, was studied as a function of age of the culture. The production of extracellular and intracellular polysaccharides was higher in actively growing cultures. Mannose and glucose were the main components of cell wall polysaccharides. Glucosamine and diamino-pimelic acid were also detected in the cell walls. The kinetics of incorporation of radioactivity from sodium [<sup>14</sup>C] bicarbonate showed that the extracellular polysaccharides were labelled within 10 min. of incubation suggesting the active exudation of polysaccharides by this alga. The selective excretion of polysaccharides by the alga Nostoc is also suggested.

This investigation provides a possible method for extraction and fractionation of seaweed polysaccharides. Methodology for quantitation of polysaccharide components is also described.

CARBOHYDRATES OF THE SEAWEEDS, Desmarestia ligulata and D. firma

George E. Carlberg, Elizabeth Percival, and M. Anisur Rhana

Biochemistry, 1978, Vol. 17, pp. 1289-1292

Crystalline mannitol and some oligosaccharides were separated from ethanolic extracts of Desmarestia ligulata and D. firma. Laminaran, "fucans" and alginic acid were also isolated from both species. The laminaran from D. ligulata comprised both M- and G-chains but no M-chains were found in the laminaran from D. firma. In both species the amount of 'fucan' was small, particularly in D. firma. Both 'fucans' contained glucuronic acid, galactose, xylose and fucose and that from D. ligulata also contained mannose. After sequential extraction of D. ligulata with water, acid and alkali evidence was obtained for the presence of cellulose, a uronan, and protein in the residual material.

## THE CARBOHYDRATES OF THE GREEN SEaweEDS

George Erik Carlberg and Elizabeth Percival

Carbohydrate Research, 57 (1977) 223-234

Urospora wormskioldii and Codiolum pusillum are different life forms of this arctic alga. They both metabolize D-glucose, D-fructose, sucrose, myo-inositol, glyceric acid, and malto-oligosaccharides. In Codiolum, 1,3-linked D-glucose and b-rhamnose oligosaccharides were also present. The major polysaccharide extracted by water from both forms is a polydisperse, sulphated glucuronoxylorhamnan. Polysaccharides containing 1,3-, 1,4-, and tripyl linked D-glucose residues were also isolated from the aqueous extracts. Pure amylopectin-type polysaccharides were isolated from acid extracts of both forms of the weed. The major difference between the two forms was the presence in Codiolum of a sulphated (1→4)-linked β-D-mannan branched at C-6 and sulphated at C-2. The similarities and differences of the carbohydrates with those of Urospora penicilliformis and other green seaweeds are discussed.

Protocols are described for sequential ethanolic, aqueous and acidic extractions. Respective fractions were subsequently characterized by gas liquid chromatography - mass spectroscopy.

## A NEW METHOD FOR THE ANALYSIS OF LAMINARINS AND FOR PREPARATIVE-SCALE FRACTIONATION OF THEIR COMPONENTS

J. Roger Stark

Carbohydrate Research, 47 (1976) 176-178

DEAE-Sephadex (A-50)-molybdate column was used to fractionate laminarins into mannitol-terminated chains and glucose-terminated chains in an undegraded form. After the column was packed, 10-200 mg of laminarin was loaded on to the column and eluted with water followed by 0.25 M sodium chloride. Assays for total carbohydrate were carried out by the phenol-sulfuric acid method and paper chromatograms. Similar results were obtained using a DEAE-cellulose-molybdate column.

STRUCTURAL INVESTIGATIONS OF THE EXTRACELLULAR POLYSACCHARIDES ELABORATED  
BY Beijerinckia mobilis

Avril A. Cooke and Elizabeth Percival

Carbohydrate Research, 43(1975)117-132

The extracellular mucilage from Beijerinckia mobilis, a member of the Azotobacteriaceae, after removal of contaminating protein, was separated into a neutral polysaccharide (N-2, 10%); a neutral, dialysable fraction (N-1, 5%), consisting of glucose and oligosaccharides containing glucose, arabinose, and rhamnose; and an acidic polysaccharide (85%). N-2 (mol. wt, 1900) was highly branched and comprised glucopyranose, mannopyranose, and arabinofuranose residues (1:1:1). The various linkages were determined. The acid fraction was a polymer of high molecular weight composed of L-guluronic acid (65%), D-glucose (15%), and D-glycero-D-mannoheptose (20%), together with acetic and pyruvic acids. From the results of methylation, periodate oxidation, and partial hydrolysis, a branched molecule with a backbone of guluronic acid and heptose, and side chains of glucose and guluronic acid is proposed. Pyruvic acid was found to be acetal-linked to 25% of the heptose residues. The similarities between this polysaccharide and that from the related species Azotobacter indicum are discussed.

It appears that carbohydrate polymer structure may be deduced by utilizing infrared spectrophotometry and gas chromatography - mass spectroscopy.

GAS-LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY OF METHLATED AND DEUTERIMETHY-  
LATED PER-O-ACETYLALDONONITRILES FROM D-MANNOSE

Fred R. Seymour, Ronald D. Plattner, and Morey E. Soldki

Carbohydrate Research, 44(1975)181-198

Peracetylated aldonitriles of the tetra-, tri-, and di-methyl ethers of D-mannopyranose were separated by gas-liquid chromatography, and analyzed by mass spectrometry. Through introduction of deuterio-methyl ether groups, various fragmentations constituting the mass spectra were identified and related to the parent methylated sugar structures. Also identified were several characteristic series of fragment ions that are common on two or more methylated D-mannopyranosides. As expected, mass spectra of the D-mannose derivatives were identical to those previously observed for p-glucose methylated in the same positions. Distinctive mass spectra were also recorded for all additional di-O-methyl-D-mannose derivatives. This information permits use of peracetylated aldonitrile derivatives in methylation-fragmentation analysis of aldohexans.

CARBOHYDRATES OF THE BROWN SEAWEEDS. Part III. Desmarestia aculeata

Elizabeth Percival and Margaret Young

Carbohydrate Research, 32 (1974) 195-201

Mannitol, sucrose, and laminitol have been isolated from ethanolic extracts of the brown seaweed Desmarestia aculeata and characterized. Rhamnose, sedoheptulose, glucose, fructose, and 2-O-methyl- and 3-O-methyl-fucose have been identified by their chromatographic mobilities and g.l.c. retention times. Laminarin, alginic acid, and 'fucans' were isolated also and characterized. The laminarin contained 1.7% of mannitol end-groups, and the fucans a relatively high proportion of galactose which was present as end-group and (1 → 3)-linked units.

CARBOHYDRATES OF THE BROWN SEAWEEDS Himantalia lorea, Bifurcaria bifurcata,  
AND Padina pavonia

A. Jabbar Mian and Elizabeth Percival

Carbohydrate Research, 26 (1973) 133-146

Mannitol constitutes the major carbohydrate of low molecular weight in the brown seaweeds named in the title; glucose is also present in Himantalia, and glucose and myo-inositol were detected in Bifurcaria. Laminarin, alginic acid, "fucans", and cellulose were separated and characterised from each of the species investigated. The "fucans", which were present in five sequential extracts obtained with different extractants, comprised variable proportions of fucose, xylose, glucuronic acid, galactose (traces), and half-ester sulphate. Fractionation on DE-cellulose led to the isolation of highly sulphated materials having a high content of sulphate, and polysaccharides with proportions of sugars and sulphate between these two extremes. It is concluded that all three seaweeds synthesize a wide spectrum of these polysaccharides.

P.M.R. SPECTROSCOPY OF DIMETHYL ETHERS OF D-GALACTOPYRANOSE AND ITS DERIVATIVES

E.B. Rathbone, A. M. Stephen, and K.G.R. Pachler

Carbohydrate Research, 21 (1972) 73-81

P.m.r. parameters (determined at 100 MHz for solutions in deuterium oxide) are presented for di-O-methyl derivatives of D-galactopyranose (ten), methyl D-galactopyranoside (ten), and galactitol (five). The effects, on the methoxyl and anomeric-proton chemical-shifts, of anomeric change, methylation of neighboring hydroxyl groups, and change in configuration of adjacent carbon atoms bearing hydroxyl or methoxyl groups (other than at C-1) are discussed.

This paper describes an alternative spectroscopic method for studying carbohydrate derivatives.

## THE AGAR POLYSACCHARIDES OF GRACILARIA SPECIES

M. Duckworth, K. C. Hong, and W. Yaphe

Carbohydrate Research, 18 (1971) 1-9

Red algae polysaccharides from Gracilaria debilis, G. compressa, G. foliifera, G. domingensis, G. damaecornis and G. ferox have been evaluated as sources of agar. Chemical and enzymic analyses, coupled with fractionation of the agars on DEAE Sephadex A-50, have shown the differences in the series of related polysaccharides which constitute different agars. Of these agars, only G. debilis agar has a high gel-strength. The gel strength of the agars from G. debilis, G. compressa, and G. foliifera are increased by alkaline treatment. Possible reasons for the differences in gel strengths of the agars are discussed.

## THE STRUCTURE OF AGAR

M. Duckworth and W. Yaphe

Carbohydrate Research, 16 (1971) 435-445

Agar consist of a spectrum of polysaccharides with three extremes in structure, namely, neutral agarose, pyruvate agarose having little sulphation, and a sulphated galactan. Components close to these three extremes of structure were degraded with the purified, extracellular agarase from Pseudomonas atlantica. Characterization of the products of hydrolysis indicates that the masking of the base repeating unit with 4,6-0-(1-carboxyethylidene)-D-galactose in place of D-galactose occurs in regions of the molecule low in sulphate content. Two new oligosaccharides containing 4,6-0-(1-carboxyethylidene)-D-galactose but no sulphate are described, namely, 4<sup>3</sup>,6<sup>3</sup>-0-(1-carboxyethylidene)neoagarotetraose and 4<sup>5</sup>,6<sup>5</sup>-0-(1-carboxyethylidene)neoagarohexose.

GAS-LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY OF ALDONONITRILE ACETATES  
AND PARTIALLY METHYLATED ALDONONITRILE ACETATES

B.A. Dmitrien, L.V. Backinowsky, O.S. Chizhov, B.M. Zolotarev and  
N.K. Kochetkov

Carbohydrate Research, 19 (1971) 432-435

The parent monosaccharides were treated with hydroxylamine hydrochloride in dry pyridine followed by acetic anhydride, however, it was found that the time required for preparation of aldononitrile acetates could be decreased to 15 min. with excellent separations by gas-liquid chromatography on polyester stationary phases. The fragmentation of the molecules of partially methylated aldononitrile acetates is analogous to that described for methylated alditol acetates. Although none of the derivatives yields the molecular ion, all gave ions at  $m/e$  M-73 and M-98, corresponding to the loss of  $-CH_2OAc$  and  $-CHOAcCN$  moieties, respectively. The base peak was  $m/e$  43( $CH_3CO^+$ ) and the prominent peaks were  $m/e$  157 (217-60), 145(289-60-2X42), 115(157-42), and 103(145-42) derived from fragments which do not carry the nitrile group and none contain ions from the C-1--C-2(98) and C-1--C-2--C-3(170) moieties.

CHEMICAL HETEROGENEITY OF THE AGAR FROM Gelidium amansii

K. Izumi

Carbohydrate Research, 17 (1971) 227-230

Agar was extracted from Gelidium amansii harvested on the coast of the Izu Peninsula. Fractionation was performed on Dowex-1 and eluted successively with water, ammonium formate, and ammonium salicylate solutions at pH 7.0. Fraction I contained very small differences in proportions of the neutral sugars, and the molar ratio of anhydrogalactose to galactose was approximately unity. Fraction II-IV showed remarkably difference among the fractions. In comparing the compositions of the agar fraction separated by this method versus the established method, agarose by the established method appears to be a mixture of fractions I and II obtained in this method, and agaropectin in a mixture of fractions III and IV.

THE ACTIVE CARBOHYDRATE METABOLITES OF THE BROWN SEAWEED, Fucus vesiculosus

E. J. Bourne, Pamela Brush, and Elizabeth Percival

Carbohydrate Research, 9 (1969) 415-422.

Biosynthetic studies with  $\text{Na}_2^{14}\text{CO}_3$  on Fucus vesiculosus by Bidwell have shown that D-mannitol is the main respiratory substrate, but that a proportion of the radioactivity is incorporated into fucoidin, alginic acid, alkali-soluble alcohol-soluble material, and into the insoluble residue remaining after the removal of the other materials by acid and alkali extraction. The quantity of seaweed used in the biosynthetic studies prevented a complete investigation of the various materials. Large-scale extraction, under the conditions used in the above studies, has now revealed that the "fucoidin" is indeed a mixture of fucoidin and laminarin, and that the "alginic acid" is contaminated with a sulphated glucuronoxylfucan which has been found in this genus for the first time. The alkali-soluble, alcohol-soluble material is a partially degraded portion of this glucuronoxylfucan. Further extraction of the insoluble residue gives additional, crude glucuronoxylfucan and an acid-insoluble and an acid-soluble glucan are separated by extraction with 6N alkali after mild treatment with chlorite. Tentative evidence is advanced for the presence of (1 3)- and (1 4)-linked D-glucose units in these glucans and in the final residue.

POLYSACCHARIDES ELABORATED BY Polyporus Fomentarius (FR.) AND Polyporus Igniarius (FR.)

Hakan BJORNDAL and Bengt LINDBERG

Carbohydrate Research, 10 (1969) 79085

The fruit bodies of the fungi Polyporus fomentarius and Polyporus igniarius have been examined for polysaccharide content. They both contain a mannofucogalactan and a glucuronoglucan. Investigation of the neutral polysaccharides has shown that they have a similar structure, consisting of a backbone of  $\alpha$ -(1 $\rightarrow$ 6)-linked D-galactopyranosyl residues, of which 30 to 40% are substituted in the 2-position by L-fucopyranosyl residues, 3-O-x-D-mannopyranosyl-L-fucopyranosyl residues, or D-galactopyranosyl residues.

Procedures outlined in this study of polysaccharides of fungi may be of extrapolated application to the study of algae carbohydrates.

STRUCTURAL STUDIES ON THE O-SPECIFIC SIDE-CHAINS OF THE CELL-WALL  
LIPOPOLYSACCHARIDE FROM Salmonella Typhimurim 395 MS

Carl Gustaf Hellerqvist, Bengt Lindberg, and Sigfrid Svensson

Carbohydrate Research, 8(1968) 43-55

The structure of the O-specific side-chains of the cell-wall lipopolysaccharide of Salmonella typhimurium 395 MS has been investigated. Methylation analyses of the original lipopolysaccharide, of the material obtained on mild hydrolysis of the lipopolysaccharide with acid, and of a product obtained by acetalation of all of the, free hydroxyl groups in the lipopolysaccharide, followed by alkaline deacetylation, have provided the essential information in this study. The mixtures of sugars obtained on acid hydrolysis of the different methylated products were analysed, as alditol acetates, by g.l.c.-mass spectrometry. As a result of these studies, a detailed structure of the repeating unit of these side-chains is presented.

Protocols for the isolation and complete characterization of lipopolysaccharides, as described in this study, may be similarly applicable to the study of algae.

SUBSTRATE SPECIFICITY OF D-GALACTOSE OXIDASE

Robert A. Schlegel, Claire M. Gerbeck, and Rex Montgomery

Carbohydrate Research, 7 (1968) 193-199

The rate of oxidation of methyl ethers of D-galactose and 2-amino 2-deoxy-D-galactose, and of oligosaccharides and polysaccharides containing D-galactosyl residues having a free hydroxyl group at C-6, has been followed by various procedures that depend either upon the hydrogen peroxide or the aldehyde groups produces, or upon the unoxidized D-galactose residues remaining in the reaction mixture. Derivatives of D-galactose having substituents on the hydroxyl group at C-4 are not oxidized. 2-Amino-2-deoxy-D-galactose residues having glycosyl substituents at C-3 are not oxidized by the enzyme, and therefore, neither are chondroitin 4-sulfate nor dermatan sulfate. No completely satisfactory procedure was found for following the oxidation reaction to termination, which, in none of the cases studied, was 100% complete.

GLUCURONOXYLOFUCAN, A CELL-WALL COMPONENT OF Ascophyllum nodosum. Part I

Elizabeth Percival

Carbohydrate Research, 7 (1968) 272-283.

A sulphated glucuronoxylfucan containing L-fucose (49%), D-xylose (10%), and D-glucuronic acid (11%) has been extracted from the cell-walls of Ascophyllum nodosum, after removal from the weed of laminaran, fucoidan, and the major part of the alginic acid. Partial hydrolysis of the extract led to the characterisation of 3-O-( $\beta$ -D-glucopyranosyluronic acid)-L-fucose as a major structural feature of the molecule, and to the separation of small quantities of 3-O- $\beta$ -D-sylopyranosyl-L-fucose and 4-O-x-L-fucopyranosyl-D-sylose. From the results of alkali treatment and mild methanolysis studies, deductions are made concerning the site of the sulphate groups. Characterisation of the fragments found in the hydrolysates, after periodate oxidation, reduction, and hydrolysis of the initial polysaccharide, the degraded polysaccharide recovered after partial hydrolysis, the alkali-treated polysaccharide, and the degraded material recovered after methanolysis, indicates that at least some of the glucuronic acid residues are (1 $\rightarrow$ 4)-linked, that some of the fucose residues are vulnerable to periodate, and that the molecule is branched with end-group and (1 $\rightarrow$ 4)-linked xylose residues situated near the periphery of the molecule.

THE CONSTITUTION OF LAMINARIN. PART V. THE LOCATION OF 1,6-GLUCOSIDIC LINKAGES

W.D. Annan, Sir Edmund Hirst and D. J. Manners

J. Chem. Soc. (1965) 885

Structural analysis of insoluble laminarin, from Laminaria hyperborea by periodate oxidation and methylation, indicates the presence of 1,6-inter-chain linkages rather than 1,6-inter-residue linkages.

Since a sample of soluble lamminarin, from L. saccharina, contained more inter-chain linkages than insoluble laminarin, it is suggested that the differences in solubility are due to differences in degree of branching.

Methodology for analysis of laminarin, as presented in the context of this paper, would appear to be applicable for several algal species.

## QUANTITATIVE DETERMINATION OF SUGARS ON PAPER CHROMATOGRAMS

Curtis M. Wilson

Analytical Chemistry, 31, No. 7, (1959) 1199-1201

Quantitative paper chromatography allows individual determination of mixed sugars in small amounts. The method described is a simple adaptation of the aniline hydrogen phthalate method for the detection of sugars. The colored spots are eluted from the paper and the concentration of sugar is determined by spectrophotometry. The absorbance of the solution is proportional to the concentration of sugar. The coefficient of variation of a single measurement is less than 2%. Aldohexoses, aldopentoses, and rhamnose have been determined.

## COLORIMETRIC METHOD FOR DETERMINATION OF SUGARS AND RELATED SUBSTANCES

Michel Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and Fred Smith

Analytical Chemistry, 28 (1956) 350-356

Simple sugars, oligosaccharides, polysaccharides, and their derivatives, including the methyl ethers with free or potentially free reducing groups, give an orange-yellow color when treated with phenol and concentrated sulfuric acid. The reaction is sensitive and the color is stable. By use of this phenol-sulfuric acid reaction, a method has been developed to determine submicro amounts of sugars and related substances. In conjunction with paper partition chromatography the method is useful for the determination of the composition of polysaccharides and their methyl derivatives.

## REAGENT FOR DIFFERENTIATION OF L,4-AND 1,6-LINKED GLUCOSACCHARIDES

Sigmund Schwimmen and Arthur Revenue

Science, 123 (1956) 543-544

A mixture of aniline, diphenylamine and phosphoric acid were used to detect oligosaccharide. It was found that those saccharides in which the carbon-4 hydroxyl proximal to the reducing end of the molecule was combined in glucosyl linkage yielded blue to purple colors, whereas, those saccharides in which this hydroxyl was uncombined yielded slate, green or yellow spots. All compounds tested showed an absorption peak in the 610 to 640 nm and 520 to 540 nm regions. The presence of a minimum in the region of 460 nm seemed to be characteristic of the isomaltose series, whereas only the substances containing an alpha 1,4-linkage exhibited a minimum in the region of 440 nm. Oligosaccharide spots obtained with this reagent are readily amenable to direct spectrophotometric examination.

## SPRAY REAGENT FOR THE DETECTION OF CARBOHYDRATES

R. V. Lemieux and H. F. Bauer

Analytical Chemistry, Vol. 26, No. 5, 1954, 920-921

A slightly alkaline (pH 7.2) aqueous solution of periodate and permanganate was used to detect sugars and their derivatives on filter paper after separation by chromatography. The presence of reducing substance on the paper results in the formation of a greenish-yellow spot. One of the advantages of this method is that in alkaline medium periodate is able to regenerate the permanganate from the reduced state formed on its oxidation of an organic compound. This regeneration stops once the pH drops to about pH 6.