Carrageenans. Part IX.[1] Methylation Analysis of Galactan Sulphates from *Furcellaria fastigiata, Gigartina canaliculata, Gigartina chamissoi, Gigartina atropurpurea, Ahnfeltia durvillaei, Gymnogongrus furcellatus, Eucheuma isiforme, Eucheuma uncinatum, Aghardhiella tenera, Pachymenia hymantophora,* and *Gloiopeltis cervicornis*. Structure of ξ-Carrageenan

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In continuation of the search for polysaccharide structures with which to develop conformational analysis, the sixteen polysaccharides for which a preliminary characterization was described in the preceding paper have been investigated by the methylation method. The earlier conclusions are confirmed and extended, with few qualifications. Amongst the structural variations in the κ and ι-carrageenans are undersulphation on C(4) of the 3-linked residue and on C(6) of the 4-linked residue. A new polysaccharide type is discovered in certain *Gigartina* species and the (as yet hypothetical) idealized form of it is named ξ-carrageenan. It is the polymer of D-galactose 2-sulphate in which the glycosidic linkages are 1,4 and 1,3, probably arranged alternately with β- and α-configurations respectvely

Two polysaccharides emerge with exceptional promise for conformational analysis, namely an agarose sulphate from *Gloiopeltis furcata* and a possible ξ-carrageenan from *Gigartina atropurpurea*.

THIS paper describes a continuation and consolidation of the investigation of sixteen galactan sulphates described in the preceding paper,[1] in which we analysed directly for the proportions and the sequence of certain types of structural units. The results could satisfactorily be explained in terms of existing concepts of the structures of agars and carrageenans, if certain assumptions were made. The polysaccharides could be classified in four main groups and are listed under these headings in the Table.

The assumptions in the earlier work have now been checked by methylation analysis, with surprising results for some polysaccharides. Since we cannot distinguish between the sites of sulphation and glycosidic substitution by this method, we interpret the methylation products in terms of structural units that normally occur in carrageenans. This is always supported by i.r. spectroscopy,[2] which can distinguish clearly between the different sulphate esters.[3] Main conclusions are listed in the Table, and detailed results are given in the Experimental section. Comparison of the gas chromatograms shown in Figures 1—4 illustrates how readily the main structural types are distinguished by methylation analysis. Because 6-sulphate and 2,6-disulphate residues would often be lost by elimination in the alkaline conditions of methylation and may even escape detection, the estimates of these residues (Table) are from earlier measurements.[1]

We shall discuss the results for each polysaccharide, using the information and the general framework that was outlined before.

Agarose sulphate: the polysaccharide from Gloiopeltis cervicornis. This polysaccharide contains even more sulphate ester than porphyran,[4] which is the other known member of the agarose family with an appreciable

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[1] Part VIII, C. J. Lawson, D. A. Rees, D. J. Stancioff, and N. F. Stanley, preceding paper.

[2] D. J. Stancioff and N. F. Stanley, in ' Proceedings of the VIth International Seaweed Symposium,' ed. R. Margalef, Subsecretaria de la Marina Mercante—Direccion General de Pesca Maritima Madrid, 1969, p. 595.

[3] N. S. Anderson, T. C. S. Dolan, A. Penman, D. A. Rees, G. P. Mueller, D. J. Stancioff, and N. F. Stanley, *J. Chem. Soc.* (*C*), 1968, 602.

[4] D. A. Rees, *J. Chem. Soc.*, 1961, 5168.

amount. The sulphate is also differently placed since the structure consists, to a first approximation, of alternate 3-linked D-galactose 6-sulphate and 4-linked 3,6-anhydro-L-galactose residues (I). A small proportion of the former are without sulphate and some of the latter carry 2-sulphate.

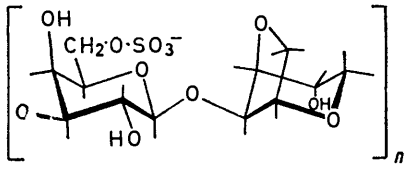
fractionation a small amount of dextrorotatory polysaccharide having relatively little 3,6-anhydride. However, their sample contained a significant minor proportion of 6-*O*-methyl-D-galactose residues and some 2-*O*-methyl-3,6-anhydro-L-galactose, and they did not propose the presence of any 4-linked L-galactose 6-

Methylation analysis of algal polysaccharides

Sample no.	Algal species [a]	Polysaccharide fraction	Conclusions from methylation evidence, about structural units and their proportions [b]: 3-Linked residues		4-Linked residues		Conclusions from other evidence [c]	
Agarose sulphate;								
1	*Gloiopeltis cervicornis*	Whole	β-D-Galactopyranose 6-sulphate β-D-Galactopyranose	(45) (5)	3,6-Anhydro-α-L-galactopyranose 3,6-Anhydro-α-L-galactopyranose 2-sulphate	(35) (6)	4-Linked α-D-galactopyranose 2,6-disulphate	(9)
κ-Carrageenans and ι-carrageenans								
2	*Furcellaria fastigiata*	κ	β-D-Galactopyranose 4-sulphate β-D-Galactopyranose Branching units?	(18) (28) (4)	3,6-Anhydro-α-D-galactopyranose	(46)	4-Linked α-D-galactopyranose 6-sulphate	(4)
3	*Gigartina canaliculata*	κ	β-D-Galactopyranose 4-sulphate β-D-Galactopyranose	(40) (10)	3,6-Anhydro-α-D-galactopyranose 3,6-Anhydro-α-D-galactopyranose 2-sulphate	(34) (9)	4-Linked α-D-galactopyranose 6-sulphate and perhaps 2,6-disulphate	(7)
4	*Gigartina chamissoi*	κ	β-D-Galactopyranose 4-sulphate	50)	3,6-Anhydro-α-D-galactopytanose 3,6-Anhydro-α-D-galactopyranose 2-sulphate	(34) (8)	4-Linked α-D-galactopyranose 2,6-disulphate	(8)
5	*Eucheuma cottonii*	Whole	β-D-Galactopyranose 4-sulphate	(50)	3,6-Anhydro-α-D-galactopyranose	(43)	4-Linked α-D-galactopyranose 6-sulphate	(7)
6	*Eucheuma spinosum*	Whole	β-D-Galactopyranose 4-sulphate	(50)	3,6-Anhydro-α-D-galactopyranose 2-sulphate	(40)	4-Linked α-D-galactopyranose 2,6-disulphate	(10)
7	*Eucheuma uncinatum*	Whole	β-D-Galactopyranose 4-sulphate β-D-Galactopyranose	(45) (5)	3,6-Anhydro-α-D-galactopyranose 2-sulphate	(37)	4-Linked α-D-galactopyranose 6-sulphate and 2,6-disulphate	(5) (8)
Deviant ι-carrageenans								
8	*Eucheuma isiforme*	Whole	β-D-Galactopyranose 4-sulphate	(50)	3,6-Anhydro-α-D-galactopyranose 3,6-Anhydro-α-D-galactopyranose 2-sulphate	(7) (27)	4-Linked α-D-galactopyranose 2,6-disulphate (9); 4-linked α-D-galactopyranose 2-sulphate	(7)
9	*Ahnfeltia durvillaei*	Whole	β-D-Galactopyranose 4-sulphate β-D-Galactopyranose	(45) (5)	3,6-Anhydro-α-D-galactopyranose 3,6-Anhydro-α-D-galactopyranose 2-sulphate	(24) (12)	4-Linked α-D-galactopyranose 2,6-disulphate 4-Linked α-D-galactopyranose 2-sulphate	(7) (7)
10	*Aghardhiella tenera*	Whole	β-D-Galactopyranose 4-sulphate	(50)	3,6-Anhydro-α-D-galactopyranose 2-sulphate	(33)	4-Linked α-D-galactopyranose 6-sulphate 4-Linked α-D-galactopyranose 2-sulphate	(7) (10)
11	*Gymnogongrus furcellatus*	Whole	β-D-Galactopyranose 4-sulphate β-D-Galactopyranose	(45) (5)	3,6-Anhydro-α-D-galactopyranose 3,6-Anhydro-α-D-galactopyranose 2-sulphate	(13) (25)	4-Linked α-D-galactopyranose 2-sulphate	(12)
λ-Carrageenans and related polysaccharides; ξ-carrageenans								
12	*Furcellaria fastigata*	λ	β-D-Galactopyranose 4-sulphate β-D-Galactopyranose Branching units?	(15) (30) (5)	α-D-Galactopyranose 6-sulphate 3,6-Anhydro-α-D-galactopyranose α-D-Galactopyranose	(44) (6) (Trace)		
13	*Gigartina canaliculata*	λ	β-D-Galactopyranose 2-sulphate	(50)	α-D-Galactopyranose 2-sulphate	(36)	4-Linked α-D-galactopyranose 2,6-disulphate 3,6-Anhydro-α-D-galactopyranose 2-sulphate	(6) (8)
14	*Gigartina chamissoi*	λ	β-D-Galactopyranose 2-sulphate	(50)	α-D-Galactopyranose 2-sulphate	(36)	4-Linked α-D-galactopyranose 2,6-disulphate 3,6-Anhydro-α-D-galactopyranose 2-sulphate 4-Linked α-D-galactopyranose 6-sulphate	(4) (10) (2)
15	*Gigartina atropurpurea*	Whole	β-D-Galactopyranose 2-sulphate β-D-Galactopyranose 4-sulphate	(40) (10)	α-D-Galactopyranose 2-sulphate	(26)	4-Linked α-D-galactopyranose 2,6-disulphate 3,6-Anhydro-α-D-galactopyranose and perhaps its 2-sulphate	(10) (12)
16	*Pachymenia himantophora*	Whole	β-D-Galactopyranose 2-sulphate β-D-Galactopyranose 4-sulphate	(30) (20)	α-D-Galactopyranose 3,6-Anhydro-α-D-galactopyranose	(43) (4)	α-D-Galactopyranose 6-sulphate	(2)

[a] For sources of algae, see Part VIII. [b] Configurations of glycosidic linkages are assigned on evidence given elsewhere.[1] The derivation of the relative proportions o residue types, is made on the assumption (for which some justification exists [1]) that the total proportions of 1,3- and 1,4-linkages are equal.[1] [c] This other evidence is chiefly that given in Part VIII.[1]

Very similar conclusions have been reached independently by Hirase and Watanabe [5] who worked with



(I) Idealized structure of agarose sulphate. For other details, see text and Table

material from the closely related species, *Gloiopeltis furcata*. They were able, in addition, to separate by sulphate. They were unable to show the presence of 3,6-anhydride 2-sulphate by methylation analysis, although this structural unit was inferred from the total sulphate ester content.

This new polysaccharide structure is proving useful for conformation studies because it crystallizes for *X*-ray diffraction and shows a conformation transition in aqueous solution.[6]

κ-Carrageenans and ι-carrageenans: fractions from F. fastigiata, G. canaliculata, G. chamissoi, *and whole polysaccharide extracts from* E. cottonii, E. spinosum, E. uncinatum. All these are alternating 1,3- and 1,4-linked D-galactans with large variation in types of 4-linked

[5] S. Hirase and K. Watanabe, in ' Proceedings of the VIIth International Seaweed Symposium,' University of Tokyo Press, Tokyo, 1973, p. 451.

[6] I. C. M. Dea, E. J. Handson, R. Moorhouse, and D. A. Rees, in preparation.

residue, as expected. In *E. spinosum* and *E. uncinatum* this residue is mainly 3,6-anhydrogalactopyranose 2-sulphate, and therefore the polysaccharides are ι-carrageenans. In *F. fastigiata*, the two *Gigartina* species, and *E. cottonii*, most of the anhydride is nonsulphated and the polysaccharides are therefore to be classed as κ-carrageenans. The distinction between the two groups is not absolute because some 3,6-anhydride 2-sulphate does exist in the *Gigartina* species, as indeed is quite common in κ-carrageenans.[3,7,8]

Other 4-linked residues which occur in these polysaccharides are galactose 6-sulphate and/or galactose 2,6-disulphate. Their total proportion is always less than that of the anhydride residues and the polysaccharides are therefore regarded as κ- and ι-respectively, rather than μ-and ν-carrageenans. More surprising is the significant amount of nonsulphated galactose in two polysaccharides which are otherwise so close to κ-carrageenan (*i.e. G. canaliculata* polysaccharide) or ι-carrageenan (*i.e. E. uncinatum* polysaccharide). It was known already that *Furcellaria* polysaccharide is undersulphated on the 3-linked residues.[9] Galactose 4-sulphate is of course the most abundant 3-linked residue in all polysaccharides except that from *Furcellaria*, as was assumed in the preliminary classification.[1]

Deviant ι-carrageenans: polysaccharides from E. isiforme, A. durvillaei, A. tenera, G. furcellatus. These polysaccharides are classed as ' deviant ' because they fail to give a consecutive carrabiose content of 100% after modification with alkaline borohydride.[1] This is attributed to the presence of ' kinking residues ' without 6-sulphate, possibly 4-linked galactose 2-sulphate residues. These were not revealed by methylation analysis but the evidence for ξ-carrageenans (see later), in which they occur in larger amounts, would suggest that their detection by this method is relatively insensitive because of side reactions such as epoxide formation under the alkaline conditions.

These polysaccharides are otherwise similar to those of the preceding group, again with some examples of incomplete 4-sulphation (*A. durvillaei* and *G. furcellatus*). Except for the product from *A. durvillaei*, they are all heavily 2-sulphated on the 4-linked residues and therefore correspond to the ι rather than the κ end of the spectrum.

λ-Carrageenan and related polysaccharides: ξ-carrageenan. Fractions from F. fastigiata, G. canaliculata, *and* G. chamissoi, *and whole polysaccharides from* G. atropurpurea *and* P. himantophora. As we noted before,[1] this group may include all polysaccharide types that do not precipitate with potassium chloride, or, in modern terms,[10] which do not have the substitution and stereochemistry to allow K^+-induced double helix formation. We shall show that a number of different structures are indeed present.

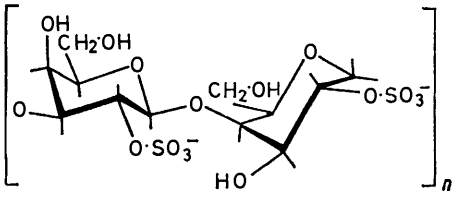
The product from *F. fastigiata* is a new type of μ-carrageenan; a high proportion of the 3-linked galactose residues are without sulphate. It is distinct from λ-carrageenan in that the sulphate that is present on these residues is on C(4) rather than C(2). The products of methylation and hydrolysis of both fractions from *F. fastigiata* (*i.e.* samples 2 and 12 in the Table), included 2,4-di-*O*-methylgalactose, which could indicate, among other possibilities, that some 3-linked galactose residues also act as branching points through C(6). This interpretation would be consistent with the results of an earlier comparison by methylation analysis of the whole polysaccharide before and after desulphation.[11] In this μ-carrageenan from *F. fastigiata*, the 4-linked residues are mostly galactose 6-sulphate residues. It is likely to be the biological precursor of the corresponding κ-fraction (*cf.* ref. 12), especially since it has a similar extent of 4-sulphation (Table).

The three *Gigartina* fractions in this group are λ-like in containing 3-linked galactose 2-sulphate but they show a structural feature that has not been encountered in carrageenans before. Among their hydrolysis products was a di-*O*-methylgalactose that was different from all the known isomers. By a process of elimination it could be identified as 3,6-di-*O*-methylgalactose and this was confirmed by synthesis.[13] Because the i.r. spectrum shows the absence of 4-sulphate [2] and a branched structure would be difficult to reconcile with the other products of methylation and hydrolysis, the 3,6-di-*O*-methyl ether is believed to have arisen from 4-linked galactose 2-sulphate residues.

If the 3- and 4-linked residues are arranged alternately in the usual way, the yields of 4,6- and 3,6-di-*O*-methylgalactoses should be equal and if, as expected, the flame ionization detector has the same sensitivity to both, the peak areas on the gas chromatogram should therefore be equal. However, this is not so (Figure 1) and as we have already mentioned the discrepancy is attributed to alkaline elimination to form the 2,3-epoxide which may then perhaps be converted into other products. 4-Linked residues of the more usual types are also present in smaller amounts, such as galactose 2,6-disulphate and 3,6-anhydrogalactose or its 2-sulphate (Table). The polysaccharide from *G. atropurpurea* is especially interesting because the consecutive carrabiose content is 100% after treatment with alkaline borohydride,[1] which proves that the conventional 4-linked residues are all grouped together in the structure. Possibly these are present in separate polysaccharides of the usual μ and λ types, the former of which would also contain the small proportion of galactose 4-sulphate. This is especially likely because the polysaccharide is an unfractionated extract. The major component of the mixture would then be a distinct new species (II) which

[7] N. S. Anderson, T. C. S. Dolan, and D. A. Rees, *J. Chem. Soc.* (*C*), 1968, 596.
[8] S. Hirase and K. Watanabe, *Bull. Chem. Soc. Japan*, 1972, **45**, 1839.
[9] T. J. Painter, *Canad. J. Chem.*, 1960, **38**, 112.
[10] N. S. Anderson, J. W. Campbell, M. M. Harding, D. A. Rees, and J. W. B. Samuel, *J. Mol. Biol.*, 1969, **45**, 85.
[11] M. J. Clancy, K. Walsh, T. Dillon, and P. S. O'Colla, *Proc. Roy. Dublin Soc.*, 1960, *A*, **1**, 197.
[12] C. J. Lawson and D. A. Rees, *Nature*, 1970, **227**, 392.
[13] A. Penman and D. A. Rees, following paper.

is a polymer of galactose 2-sulphate residues linked alternatively α-1,4 and β-1,3. By continuation of the usual nomenclature,[14] this is named ξ-carrageenan. It differs from λ-carrageenan [15] in having no 6-sulphation of the 4-linked residues but complete 2-sulphate of the 3-linked residues. This component should be purified for conformation studies, to explore further the stereochemical response of the carrageenan backbone to changed substitution. However, it is also possible that the polysaccharide from *G. atropurpurea* is a heterogeneous block copolymer with ξ-like blocks and λ-like blocks, in admixture with some contaminating κ-like carrageenan.



(II) ξ-Carrageenan

The polysaccharides from *G. canaliculata* and *G. chamissoi* are also composed largely of 1,4- and 1,3-linked galactose 2-sulphate residues. After alkaline elimination of 6-sulphate, most but not all carrabiose residues are consecutive [1] and therefore it is possible that the main polysaccharide component, although largely ξ-like, has some λ-character. λ-Carrageenan could also occur as a minor component, but not μ-carrageenan because galactose 4-sulphate residues are absent.

Yet another polysaccharide type is the product from *Pachymenia himantophora*. It contains comparable proportions of 3- and 4-linked residues (Table), as expected for a polysaccharide of the carrageenan type but it is unusual in that most of the latter are non-sulphated. Further comment must await an investigation of polysaccharide homogeneity.

EXPERIMENTAL

General.—Samples of methylated polysaccharides (10—20 mg) were methanolysed for g.l.c. by heating in sealed tubes on a boiling water-bath for 16 h with methanolic hydrogen chloride [2·3%; prepared by addition of acetyl chloride (4·5 ml) to dry methanol (100 ml); 2 ml]. Each solution was neutralized with silver carbonate, filtered, and concentrated to a syrup. A Pye Argon Chromatograph was used with ^{90}Sr detector and 4 ft columns. Unless stated otherwise, the liquid phase was polyethylene glycol adipate (10% on GasChrom P), and the column temperature was 175° with a gas flow rate of 80 ml min^{-1}. Retention times (t_R) are expressed relative to the faster-moving of the two peaks of methyl 2,3,4,6-tetra-*O*-methyl-αβ-glucopyranoside.

Complete hydrolysis of methylated polysaccharides (10—20 mg) for paper chromatography was performed by heating with aqueous formic acid (45%; 2 ml) on a boiling water-bath for 16 h. Formic acid was removed by evaporation and repeated distillation of water from the residue, in a rotary evaporator. The following four systems were always used for analysis, with Whatman no. 1 paper and *p*-anisidine hydrochloride spray: (*a*) butan-1-ol–ethanol–water (4 : 1 : 5; upper phase), (*b*) ammonia (*d* 0·880)–water–ethyl methyl ketone (1 : 17 : 200), using double development (2 × 4 h), (*c*) the same solvent as (*b*) but with different conditions for double development (2 × 8 h), (*d*) benzene–ethanol–water (169 : 47 : 15), using triple development (2 × 6 h, 1 × 8 h).

Selected methylated polysaccharides were oxidatively hydrolysed for examination of the 3,6-anhydrogalactonic acid derivatives by paper chromatography, as described elsewhere.[3,7] Best separations were achieved when the solvent was less than a week old. The relative mobilities were: 3,6-anhydrogalactonic acid, 0·69; 2,6-di-*O*-methylgalactonic acid, 0·82; 3,6-anhydro-2-*O*-methylgalactonic acid, 1·00; 3,6-anhydro-2,5-di-*O*-methylgalactonic acid, 1·40; 3,6-anhydro-2,4-di-*O*-methylgalactonic acid, 1·56.

Methylation Method.—Each polysaccharide (1 g) was dissolved in water (100 ml) and cooled in ice. Dimethyl sulphate (10 ml) and sodium hydroxide (30% w/v; 30 ml), were added simultaneously over 6 h with vigorous stirring under nitrogen, followed by further stirring under nitrogen for 16 h. The addition was repeated four times at room temperature. The solution was dialysed and then concentrated to 100 ml, and the entire cycle was repeated twice. Methylation was taken to be complete if no galactose was present in the hydrolysate. Most polysaccharides reached this stage after three methylation cycles; a few required a further two cycles and some were suspected of being undermethylated even after repeated cycles.

The product was finally isolated by dialysis and freeze-drying.

Hydrolysis and Methanolysis Products of the Methylated Polysaccharides.—The main conclusions are listed in the Table. The detailed evidence was as follows.

Gloiopeltis cervicornis. Hydrolysis followed by paper chromatography showed 2,4,6-tri-*O*-methylgalactose (trace), 2,4-di-*O*-methylgalactose (++++), 2,6-di-*O*-methylgalactose (trace), mixed mono-*O*-methylgalactoses (+). Oxidative hydrolysis followed by paper chromatography showed 3,6-anhydro-2-*O*-methylgalactonic acid as the major component together with 3,6-anhydrogalactonic acid and unidentified components (presumably *O*-methygalactonic acids) having relative mobilities 0·55 and 0·27. The approximate ratio of concentration was 10 : 2 : 1 : trace. Methanolysis and g.l.c. (Figure 1) showed tetra-*O*-methylgalactosides (t_R 1·71; trace), 2,3,6-tri-*O*-methylgalactosides (t_R 2·89; weak), 2,4,6-tri-*O*-methylgalactosides (t_R 3·56, 4·12; medium), 3,6-anhydro-2-*O*-methylgalactose derivatives (t_R 6·10, 7·35, 16·00; strong), 2,6-di-*O*-methylgalactosides [t_R 8·43, (10·00), 11·12, (14·0); trace], 2,4-di-*O*-methylgalactosides (t_R 13·45, 15·45; strong).

Furcellaria fastigiata, κ-fraction. Hydrolysis followed by paper chromatography showed 2,4,6-tri-*O*-methylgalactose (++++), 2,6-di-*O*-methylgalactose (++++), 2,4-di-*O*-methylgalactose (+), mono-*O*-methylgalactose(s), trace. Oxidative hydrolysis gave 3,6-anhydro-2-*O*-methylgalactonic acid with no trace of 3,6-anhydrogalactonic acid. An additional product was detected in about 10% concentration, having relative mobility 1·19 (2,4,6-tri-*O*-methylgalactonic acid?). Methanolysis and g.l.c. (Figure 2)

[14] D. A. Rees, *Adv. Carbohydrate Chem. Biochem.*, 1969, **24**, 267.
[15] T. C. S. Dolan and D. A. Rees, *J. Chem. Soc.*, 1965, 3534.

showed tetra-*O*-methylgalactosides (t_R 1·67; trace), 2,3,6-tri-*O*-methylgalactosides (t_R 2·76; trace), 2,4,6-tri-*O*-methylgalactosides (t_R 3·66, 4·17; strong), 3,6-anhydro-2-*O*-methylgalactose derivatives (t_R 6·15, 7·35, 16·45; strong), 2,6-di-*O*-methylgalactosides (t_R 8·43, 10·29, 11·69, 14·40;

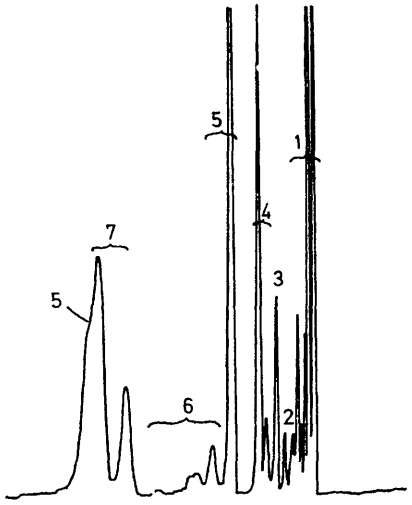


FIGURE 1 G.l.c. of the methanolysis products of the methylated polysaccharide from *Gloiopeltis cervicornis*. For conditions, see text. Peaks are assigned as follows: peaks 1, solvent and degradation products; peak 2, 2,3,4,6-tetra-*O*-methylgalactosides; peak 3, 2,3,6-tri-*O*-methylgalactosides; peaks 4, mixture of 2,3,6- and 2,4,6-tri-*O*-methylgalactosides; peaks 5, 3,6-anhydro-2-*O*-methylgalactose derivatives; peaks 6, 2,6-di-*O*-methylgalactosides; peaks 7, 2,4-di-*O*-methylgalactosides

medium), 2,4-di-*O*-methylgalactosides [t_R 13·61 (15·52); weak].

Gigartina canaliculata, κ-fraction. Hydrolysis gave 2,4,6-tri-*O*-methylgalactose (trace), 2,6-di-*O*-methylgalactose (++++), and mixed mono-*O*-methyl-galactoses (small amounts). Oxidative hydrolysis gave 3,6-anhydrogalactonic acid and its 2-*O*-methyl ether in the approximate ratio 1 : 4. Methanolysis gave tetra-*O*-methylgalactosides (t_R 1·75, trace), 2,3,6-tri-*O*-methylgalactosides (t_R 2·91, trace), 2,4,6-tri-*O*-methylgalactosides (t_R 3·66, 4·18; medium), 3,6-anhydro-2-*O*-methylgalactose derivatives (t_R 6·17, 7·54, 16·72; strong), and 2,6-di-*O*-methylgalactosides (t_R 8·43, 10·25, 11·60, 14·90; strong).

Gigartina chamissoi, κ-fraction. Hydrolysis gave 2,4,6-tri-*O*-methylgalactose (slight traces only), 2,6-di-*O*-methylgalactose (++++), and mono-*O*-methylgalactoses) (slight traces only). Oxidative hydrolysis showed 3,6-anhydrogalactonic acid and its 2-*O*-methyl ether in the approximate ratio 1 : 4. Methanolysis gave tetra-*O*-methylgalactosides (t_R 1·79; trace). 2,4,6-tri-*O*-methylgalactosides (t_R 3·61, 4·12; weak), 3,6-anhydro-2-*O*-methylgalactose derivatives (t_R 6·16, 7·46, 16·49; strong), 2,6-di-*O*-methylgalactosides (t_R 8·43, 10·22, 11·68, 14·71; strong).

Eucheuma cottonii and *Eucheuma spinosum*. The evidence for the methylated polysaccharides from these sources has been published elsewhere.[16]

Eucheuma uncinatum. The products of hydrolysis, oxidative hydrolysis, and methanolysis could be distinguished from the corresponding products from *G. canaliculata* only in their relative proportions; the proportion of 2,4,6-tri-*O*-methylgalactose derivatives was somewhat diminished, and no 3,6-anhydro-2-*O*-methylgalactose derivatives were detected although 3,6-anhydrogalactonic acid was present in abundance after oxidative hydrolysis.

Eucheuma isiforme. The products of hydrolysis, oxidative hydrolysis, and methanolysis could be distinguished from the corresponding products from *G. chamissoi* only in that they contained a higher proportion of 3,6-anhydrogalactose derivatives relative to the 2-*O*-methyl ether.

Ahnfeltia durvillaei. Again the products were qualitatively indistinguishable from those from *Gigartina chamissoi* (κ-fraction). However, the quantities of 2,4,6-tri-*O*-methylgalactose derivatives were now significant, and the relative proportions of the two 3,6-anhydride types were different as indicated in the Table.

Aghardhiella tenera. The products were identical with those from *Gigartina canaliculata*, except that the amounts of 2,4,6-tri-*O*-methylgalactose and 3,6-anhydro-2-*O*-methylgalactose derivatives were very small.

Gymnogongrus furcellatus. The products were closely similar to those from *Ahnfeltia durvillaei*, but with a change in relative proportions of the two anhydride types.

Furcellaria fastigiata, λ-fraction. Hydrolysis gave 2,4,6-tri-*O*-methylgalactose (+++), 2,6-di-*O*-methylgalactose (++), 2,3-di-*O*-methylgalactose (+), 2,4-di-*O*-methylgalactose (trace), and mono-*O*-methylgalactose(s) (trace). Oxidative hydrolysis showed 3,6-anhydro-2-*O*-methylgalactonic acid with other products that were not identified but presumably arose from *O*-methylgalactoses. Methanolysis

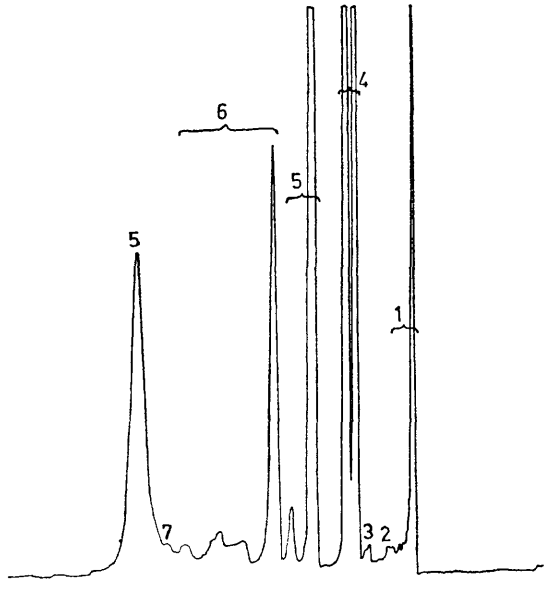


FIGURE 2 G.l.c. of the methanolysis products of the methylated κ-fraction from *Furcellaria fastigiata*. For conditions see text. Peaks were assigned as follows: peaks 1, solvent and degradation products; peak 2, 2,3,4,6-tetra-*O*-methylgalactosides; peak 3, 2,3,6-tri-*O*-methylgalactoside; peaks 4, 2,4,6-tri-*O*-methylgalactosides; peak 5, 3,6-anhydro-2-*O*-methylgalactose derivatives; peaks 6, 2,6-di-*O*-methylgalactosides; peak 7, 2,4-di-*O*-methylgalactosides

and g.l.c. (neopentyl glycol adipate as liquid phase, 3% on GasChrom P; column temperature 175°) showed tetra-*O*-methylgalactosides (t_R 1·33; weak), 2,3,6-tri-*O*-methylgalactosides (t_R 2·48; very weak), 2,4,6-tri-*O*-methyl-

[16] N. S. Anderson, T. C. S. Dolan, and D. A. Rees, *J.C.S. Perkin I*, 1973, 2173.

galactosides (t_R 2·80, 3·14; strong), 3,6-anhydro-2-*O*-methylgalactose derivatives (t_R 4·27, 4·83, 10·47; weak), 2,6-di-*O*-methylgalactosides (t_R 5·52, 6·05, 6·88, 9·16;

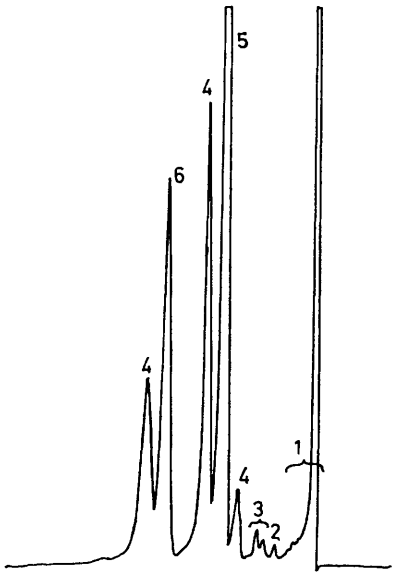


FIGURE 3 G.l.c. of the methanolysis products of the methylated λ-fraction from *Gigartina canaliculata*. The conditions were as given in text except that the liquid phase is neopentyl glycol adipate (3% on GasChrom P). Peaks were assigned as follows: peaks 1, solvent and degradation products; peak 2, 2,3,6-tri-*O*-methylgalactosides; peak 3, mixture of 2,3,6- and 2,4,6-tri-*O*-methylgalactosides; peaks 4, 3,6-di-*O*-methylgalactosides; peak 5, 4,6-di-*O*-methylgalactosides; peak 6, 4,6- and 3,6-di-*O*-methylgalactosides

medium), 2,3-di-*O*-methylgalactose (t_R 5·52, 6·05, 7·64; weak), 2,4-di-*O*-methylgalactosides (t_R 8·19, 9·15; trace).

Gigartina canaliculata, λ-fraction. Hydrolysis gave mono-*O*-methylgalactose (++), galactose (+), and 4,6- and/or 3,6-di-*O*-methylgalactose (++++). The two dimethyl ethers are indistinguishable, both in mobility and colour reaction on paper chromatograms. Methanolysis and g.l.c. (Figure 3) showed tetra-*O*-methylgalactosides (t_R 1·65; trace), 2,3,6-tri-*O*-methylgalactosides (t_R 2·84; trace), 2,4,6-tri-*O*-methylgalactosides (t_R 3·68, 4·16; trace), 3,6-di-*O*-methylgalactosides (t_R 5·83, 9·26, 13·40; medium), 4,6-di-*O*-methylgalactosides (t_R 7·45, 13·40; strong).

Gigartina chamissoi, λ-fraction. The evidence was identical with that for *G. canaliculata* with the minor exception that traces of 3,6-anhydro-2-*O*-methylgalactose derivatives were detected after methanolysis.

Gigartina atropurpurea. Hydrolysis gave 2,3,6-tri-*O*-methylgalactose (very small traces), 2,4,6-tri-*O*-methylgalactose (very small traces), 3,6- and/or 4,6-di-*O*-methylgalactose (++++), 2,6-di-*O*-methylgalactose (++), mono-*O*-methylgalactose, and galactose (+). Methanolysis gave tetra-*O*-methylgalactosides (t_R 1·73; trace) 2,3,6-tri-*O*-methylgalactosides (t_R 2·92; trace), 2,4,6-tri-*O*-methylgalactosides (t_R 3·84, 4·19; trace), 3,6-di-*O*-methylgalactosides (t_R 5·85, 9·15, 13·29; medium), 4,6-di-*O*-methylgalactosides (t_R 7·44, 13·29; strong), and 2,6-di-*O*-methylgalactosides [t_R 8·43, (10·29), 11·69, 14·40; weak].

Pachymenia himantophora. Hydrolysis gave tetra-*O*-methylgalactose (trace), 2,3,6-tri-*O*-methylgalactose (++++), 2,4,6-tri-*O*-methylgalactose (trace), 2,6-di-*O*-methylgalactose (+++), 4,6- and/or 3,6-di-*O*-methylgalactose (++), mono-*O*-methylgalactose(s) (++), and galactose (++). Methanolysis and g.l.c. (Figure 4) gave tetra-*O*-methylgalactosides (t_R 1·72; trace), 2,3,6-tri-*O*-methylgalactosides (t_R 2·90, 3·50, 3·82, 4·23; strong), 3,6-anhydro-2-*O*-methylgalactose derivatives (t_R 6·16, 7·52,

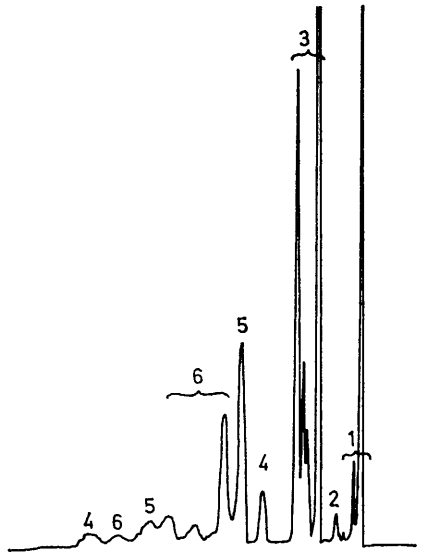


FIGURE 4 G.l.c. of the methanolysis products of the methylated polysaccharide from *Pachymenia himantophora*. For conditions, see text. Peaks are assigned as follows: peaks 1, solvent and degradation products; peak 2, 2,3,4,6-tetra-*O*-methylgalactosides; peaks 3, 2,3,6-tri-*O*-methylgalactosides; peaks 4, 3,6-anhydro-2-*O*-methylgalactose derivatives; peaks 5, 4,6-di-*O*-methylgalactosides; peaks 6, 2,6-di-*O*-methylgalactosides

16·66; weak), 4,6-di-*O*-methylgalactosides (t_R 7·45, 12·98; medium), 2,6-di-*O*-methylgalactosides (t_R 8·43, 10·25, 11·77, 14·91; medium).

We thank Marine Colloids Inc., Rockland, Maine, U.S.A., for financial support, and their research staff for discussions.

[3/527 *Received*, 12*th March*, 1973]