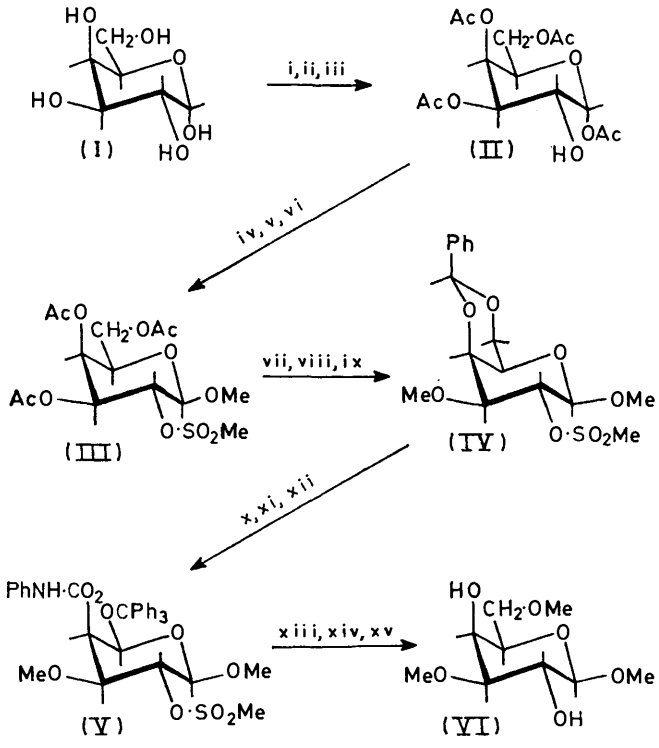
Carrageenans. Part X.[1] Synthesis of 3,6-Di-*O*-Methyl-D-galactose, a New Sugar from the Methylation Analysis of Polysaccharides related to ξ-Carrageenan

By **Alistair Penman** † and **David A. Rees,*** † Chemistry Department, The University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ

3,6-Di-*O*-methyl-D-galactose, which had not previously been identified from natural or synthetic sources, has now been synthesised and shown to be identical with a product from the methylation analysis of polysaccharides related to ξ-carrageenan. All the possible methyl esters of D-galactopyranose are therefore now known.

ALL the possible partially methylated derivatives of D-galactopyranose were characterised 20 years ago[2] except 3,6-di-*O*-methylgalactose. One reason why this sugar has remained unknown is that there is only one report of its possible occurrence in the hydrolysate of a methylated polysaccharide,[3] and the evidence for this is both tentative and relatively recent. A second reason is that selective 3-*O*-substitution in the galactose series is difficult to achieve by classical methods. Our synthesis was long and cumbersome (see Scheme) and was adopted only after several more direct routes had failed. Nevertheless, the yield at each stage was good and the desired compound was obtained without complications. Thus 1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose was prepared by hydrolysis of acetobromogalactose with simultaneous $O(2) \longrightarrow O(1)$ acetate migration and then the free hydroxy-group was blocked by methylsulphonylation. Conversion into the methyl β-glycoside was followed by de-*O*-acetylation and treatment with benzaldehyde dimethyl acetal in the presence of a trace of acid, to give the compound in which only O(3) was unsubstituted and hence the first methyl ether could be introduced. Successive tritylation, phenylcarbamoylation, and detritylation then exposed O(6) for the second methylation, after which the blocking groups were removed to give methyl 3,6-di-*O*-methyl-β-D-galactopyranoside.



SCHEME Reagents: i, Ac_2O, $HClO_4$; ii, PBr_3; iii, AcONa, H_2O; iv, $MeSO_2Cl$, pyridine; v, HBr, AcOH; vi, MeOH, Ag_2CO_3; vii, MeOH, H^+; viii, $PhCH(OMe)_2$, H^+; ix, MeI, Ag_2O; x, H^+, H_2O; xi, Ph_3CCl, pyridine; xii, PhNCO, pyridine; xiii, H_3O^+; xiv Me_3I, Ag_2O; xv, $LiAlH_4$

The structure was confirmed by n.m.r. and elemental analysis, and the fact that the glycoside was not itself oxidised by periodate but was hydrolysed to a sugar which was oxidised to methoxyacetaldehyde and other products. Finally, complete methylation gave a methyl 2,3,4,6-tetra-*O*-methylgalactopyranoside.

The free sugar, 3,6-di-*O*-methyl-D-galactose, was obtained in crystalline condition and the physical constants are recorded. It is very similar to 4,6-di-*O*-methylgalactose in its mobility and colour reactions on paper chromatograms but is clearly distinguished by methanolysis and g.l.c. (Figure). These methanolysis products were shown by g.l.c.–mass spectroscopy to be present also after methanolysis of methylated carrageenans from *Gigartina atropurpurea*, *Gigartina chamissoi*, and *Gigartina canaliculata*. For reasons given earlier,[1] these polysaccharides are considered to be related to ξ-carrageenan.

EXPERIMENTAL

M.p.s were measured on a Kofler hot-stage apparatus. Optical rotation measurements were made with a Perkin-Elmer 141 Automatic Polarimeter (10 cm cells). N.m.r. spectra were recorded with Perkin-Elmer R10 (60 Mz) and Varian HA100 (100 MHz) spectrometers. Internal standards were tetramethylsilane for solutions in organic solvents and t-butyl alcohol in deuterium oxide. Elemental analyses were by Alfred Bernhardt, Elbach uber Engelskerchen, West Germany. T.l.c. was performed on micro plates coated with silica gel (Kieselgel, Merck) and spots were developed with *p*-anisaldehyde–sulphuric acid spray.[4]

Unless stated otherwise, gas chromatography was performed with a Pye 104 gas chromatograph [5 ft column; polyethylene glycol (10% on 100—120 mesh Celite) as

† *Present address*: Unilever Research, Colworth/Welwyn Laboratory, Colworth House, Sharnbrook, Bedford.

[1] Part IX, A. Penman and D. A. Rees, preceding paper.
[2] D. J. Bell, *Adv. Carbohydrate Chem.*, 1951, **6**, 11.
[3] C. V. N. Rao, D. Choudhury, and P. Baghi, *Canad. J. Chem.*, 1961, **39**, 375.
[4] E. Stahl and U. Kaltenbach, *J. Chromatog.*, 1961, **5**, 451.

liquid phase]. The column temperature was 200° with a carrier gas (helium) flow rate of 45 ml min^{-1}. The column effluent was split between the flame ionisation detector and the A.E.I. MS12 mass spectrometer.

Methyl 3,4,6-*Tri*-O-*acetyl*-2-O-*methylsulphonyl*-β-D-*galactopyranoside*.—This was obtained [5] by way of 1,3,4,6-tetra-*O*-acetyl-α-D-galactose (II) and 1,3,4,6-tetra-*O*-acetyl-2-*O*-methylsulphonyl-α-D-galactose, by conversion of the latter compound into the glycosyl bromide with hydrogen bromide in acetic acid and then treatment with silver carbonate in methanol.

Methyl 2-O-*Methylsulphonyl*-β-D-*galactopyranoside*.—The product from the previous stage (73 g) was refluxed with methanolic hydrogen chloride (0·3%; 1 l) for 16 h. After neutralisation with sodium hydrogen carbonate, filtration, and evaporation, the syrupy *methyl* 2-O-*methylsulphonyl*-β-D-*galactopyranoside* (45·8 g, 91·9%) was crystallised from ethanol; m.p. 145—146°, $[\alpha]_D$ −19·9° (*c* 1·6 in pyridine) (Found: C, 35·45; H, 5·94; S, 11·6. $C_8H_{16}O_7S$ requires C, 35·3; H, 5·9; S, 11·75%).

To check that anomerisation had not occurred during deacetylation, part of the product (5 mg) was heated in a sealed tube at 100° with pyridine–acetic anhydride (1 : 1 v/v; 1 ml) for 1 h. The solution was cooled, diluted with water, and extracted with chloroform. A sample of the chloroform layer was analysed by g.l.c. [Pye Argon Chromatograph; 4 ft SE52 column (3% on Gas Chrom P) at 200°]. This showed a single sharp peak, identical with that from the starting material. To check the anomeric configuration [since the Königs–Knorr synthesis does not necessarily lead to the β-anomer when there is a non-participating neighbouring group on C(2) [6]], a sample (10 mg) was dissolved in sodium hydroxide (2N; 0·1 ml) and left at room temperature (16 h). After addition of solid carbon dioxide, then ethanol (5 ml), the solution was filtered and compared by chromatography with methyl 2,3-anhydro-α- and β-talopyranosides, kindly supplied by Prof. J. G. Buchanan. The product was identified with the β-anomer, and well separated from the α-, proving that the 2-*O*-methylsulphonylglycoside had the β-configuration.

Methyl 4,6-O-*Benzylidene*-2-O-*methylsulphonyl*-β-D-*galactopyranoside*.—The product from the previous stage (45 g) in redistilled tetrahydrofuran (1 l) was mixed with toluene-*p*-sulphonic acid (80 mg) and benzaldehyde dimethyl acetal (80 ml). The solution was slowly distilled at atmospheric pressure until the volume was 100 ml (30 min), and neutralised with sodium hydrogen carbonate, filtered, then evaporated to dryness. The syrupy *methyl* 4,6-O-*benzylidene*-2-O-*methylsulphonyl*-β-D-*galactopyranoside* (43·2 g, 72·7%) was crystallised from ethanol; m.p. 183—185°, $[\alpha]_D$ +20·2° (*c* 1·2 in pyridine) (Found: C, 50·25; H, 5·9; S, 8·5; OMe, 8·4. $C_{15}H_{20}O_8S$ requires C, 50·0; H, 5·55; S, 8·9; OMe, 8·6%).

Methyl 4,6-O-*Benzylidene*-3-O-*methyl*-2-O-*methylsulphonyl* β-D-*galactopyranoside* (IV).—The product from the previous stage (42 g), in *NN*-dimethylformamide (390 ml), was methylated with methyl iodide (130 ml) and silver oxide (75 g) by stirring in ice for 2 h then at room temperature for 4 days, anhydrous conditions being maintained throughout. The mixture was diluted with chloroform, filtered, and washed with sodium cyanide solution (1% in water). The cyanide solution was back-extracted with chloroform (five times) then the combined chloroform layers were washed with water and dried (Na_2SO_4). *Methyl* 4,6-O-*benzylidene*-3-O-*methyl*-2-O-*methylsulphonyl*-β-D-*galactopyranoside* (28·4 g, 63·5%) was obtained by evaporation to dryness and crystallisation from ethanol; m.p. 182—184°, $[\alpha]_D$ +30·2° (*c* 1·26 in $CHCl_3$) (Found: C, 51·2; H, 6·15; S, 8·6; OMe, 16·8. $C_{16}H_{22}O_8S$ requires C, 51·3; H, 5·9; S, 8·55; OMe, 16·55%).

Methyl 3-O-*Methyl*-2-O-*methylsulphonyl*-β-D-*galactopyranoside*.—The product from the previous stage (27·5 g) in aqueous formic acid (40% v/v; 675 ml) was heated on a boiling water-bath for 30 min, then cooled, and benzaldehyde was removed by extraction (thrice) with benzene. The aqueous layer was evaporated to dryness and water was distilled from the residue repeatedly to remove formic acid, *methyl* 3-O-*methyl*-2-O-*methylsulphonyl*-β-D-*galactopyranoside* (15·5 g, 71·1%) was finally obtained by crystallisation from ethanol; m.p. 161—163°, $[\alpha]_D$ −5·14° (*c* 1·44 in Me_2CO) (Found: C, 37·8; H, 6·2; S, 11·35; OMe, 21·3. $C_9H_{18}O_8S$ requires C, 37·75; H, 6·3; S, 11·2; OMe, 21·7%).

Part of the product (100 mg) was converted into the acetate, which was crystalline; τ ($CDCl_3$) 6·40 (3H, s) and 6·53 (3H, s) (OMe), 6·88 (3H, s, $MeO{\cdot}SO_2$), and 7·83 (3H, s) and 7·90 (3H, s) (OAc).

Methyl 3-O-*Methyl*-2-O-*methylsulphonyl*-6-O-*triphenylmethyl*-β-D-*galactopyranoside*.—The pyridine for this experiment was redistilled and kept over potassium hydroxide; immediately before use it was redistilled again from phosphorus pentoxide.

The product from the preceding stage (14·96 g) in pyridine (200 ml) was mixed with chlorotriphenylmethane (24·4 g) and kept at room temperature for 16 h before pouring into a large volume of iced water to precipitate *methyl* 3-O-*methyl*-2-O-*methylsulphonyl*-6-O-*triphenylmethyl*-β-D-*galactopyranoside*, which was filtered off, washed thoroughly with water then ether, and crystallised from ethanol (yield 8·18 g, 29·8%); m.p. 92—94°, $[\alpha]_D$ −18·92° (*c* 1·96 in $CHCl_3$) (Found: C, 63·9; H, 6·25; S, 6·45; OMe, 11·7. $C_{28}H_{32}O_8S$ requires C, 63·65; H, 6·05; S, 6·05; OMe, 11·75%), τ (CCl_4) 2·5—2·9 (15H, m, Ph_3C), 6·53 (3H, s) and 6·59 (3H, s) (OMe), and 7·09 (3H, s, $MeO{\cdot}SO_2$).

Methyl 3-O-*Methyl*-2-O-*methylsulphonyl*-4-O-*phenylcarbamoyl*-6-O-*triphenylmethyl*-β-D-*galactopyranoside* (V).—The product from the previous stage (7·1 g), in pyridine (80 ml; prepared as above), was heated at 100° for 2 h with phenyl isocyanate (3·3 ml). *Methyl*-3-O-*methyl*-2-O-*methylsulphonyl*-4-O-*phenylcarbamoyl*-6-O-*triphenylmethyl*-β-D-*galactopyranoside* separated on pouring into water and was filtered off and recrystallised from ethanol–acetone (yield 6·90 g, 78·0%); m.p. 214—215°, $[\alpha]_D$ −13·4° (*c* 2·18 in $CHCl_3$) (Found: C, 64·75; H, 5·85; N, 2·3; S, 5·15; OMe, 9·75. $C_{35}H_{37}NO_9S$ requires C, 64·9; H, 5·7; N, 2·16; S, 4·95; OMe, 9·55%), τ ($CDCl_3$) 2·42—2·83 (20H, m, aromatic), 6·44 (3H, s) and 6·50 (3H, s) (OMe), and 6·95 (3H, s, $MeO{\cdot}SO_2$).

Methyl 3-O-*Methyl*-2-O-*methylsulphonyl*-4-O-*phenylcarbamoyl*-β-D-*galactopyranoside*.—The product from the previous stage (6·22 g) in chloroform (60 ml) was mixed with methanol (180 ml) and acetyl chloride (1·2 ml). The solution was heated on a boiling water-bath for 5 min, cooled, then neutralised with silver carbonate, filtered, and evaporated to dryness. The residue was dissolved in benzene and applied to a Florisil (synthetic silica gel) column (40 × 3 cm diam.), and eluted with benzene to remove chlorotriphenylmethane. Elution with benzene–methanol (9 : 1) and evaporation to dryness gave syrupy

[5] B. Helferich and J. Zirner, *Chem. Ber.*, 1962, **95**, 2604.
[6] M. L. Wolfrom, A. Thompson, and D. R. Lineback, *J. Org. Chem.*, 1963, **28**, 860.

methyl 3-O-*methyl*-2-O-*methylsulphonyl*-4-O-*phenylcarbamoyl*-β-D-*galactopyranoside*, which was crystallised from ethanol (yield 3·71 g, 81·9%); m.p. 142—143°, $[\alpha]_D$ +45·8° (*c* 1·98 in $CHCl_3$) (Found: C, 47·7; H, 6·1; N, 3·6; S, 8·25; OMe, 14·9. $C_{16}H_{23}NO_9S$ requires C, 47·4; H, 5·7; N, 3·45; S, 7·9; OMe, 15·31%), τ ($CDCl_3$) 2·52—3·04 (5H, m, *Ph*NHCO), 6·43 (3H, s) and 6·54 (3H, s) (OMe), and 6·91 (3H, s, MeO·SO_2).

Methyl 3,6-*Di*-O-*methyl*-4-O-[*methyl*(*phenyl*)*carbamoyl*]-2-*methylsulphonyl*-β-D-*galactopyranoside*.—The product from the previous stage (3·06 g) in *NN*-dimethylformamide (30 ml) was methylated with methyl iodide (12 ml) and silver oxide (12 g) by shaking in ice for 2 h then at room temperature for 4 days. The mixture was diluted with chloroform, filtered, and washed with sodium cyanide solution (1% in water). The cyanide solution was back-extracted with chloroform (five times), and the combined chloroform layers were washed with water and dried (Na_2SO_4). Evaporation to dryness and remethylation by the same procedure gave syrupy *methyl* 3,6-*di*-O-*methyl*-4-O-[*methyl*(*phenyl*)*carbamoyl*]-2-O-*methylsulphonyl*-β-D-*galactopyranoside*, which resisted crystallisation (yield 2·90 g, 88·7%), $[\alpha]_D$ +54·7° (*c* 0·55 in Me_2CO) (Found: C, 50·15; H, 6·35; N, 3·2; S, 6·95; OMe, 22·05. $C_{18}H_{27}NO_9S$ requires C, 50·0; H, 6·25; N, 3·25; S, 7·35; OMe, 21·3%), τ ($CDCl_3$) 2·58—2·82 (5H, m, NPh), 6·50 (3H, s), 6·63 (3H, s), and 6·66 (3H, s) (OMe), 6·56br (3H, s, NMe), and 6·92 (3H, s, MeO·SO_2).

Methyl 3,6-*Di*-O-*methyl*-β-D-*galactopyranoside* (VI).—The product from the previous stage (2·73 g) in redistilled tetrahydrofuran (300 ml) was refluxed with lithium aluminium hydride (5 g) for 3 h. Ethyl acetate (40 ml) and water (10 ml) were added to remove the excess of reagent and the tetrahydrofuran was removed by evaporation. Sulphuric acid (1·0N; 150 ml) was added and aniline was removed by extraction (thrice) with benzene. The solution was treated with Amberlite IR 120 (H^+) and IR 45 (OH^-) resins and evaporated to give syrupy *methyl* 3,6-*di*-O-*methyl*-β-D-*galactopyranoside*, which could not be crystallised (yield 1·27 g, 90·7%), $[\alpha]_D$ −11·48° (*c* 1·86 in Me_2CO) (Found: C, 47·65; H, 9·05; OMe, 40·7. $C_9H_{18}O_6$ requires C, 48·6; H, 8·1; OMe, 41·8%), τ ($CDCl_3$) 6·46 (3H, s), 6·53 (3H, s), and 6·61 (3H, s) (OMe).

(a) *Methylation*. Part of the product (10 mg) in *NN*-dimethylformamide (3 ml) was shaken for 16 h with methyl iodide (1 ml) and silver oxide (0·7 g). After dilution with chloroform, filtration, and evaporation, the residue was methanolysed and examined by g.l.c. as described in Part IX.[1] This showed methyl 2,3,4,6-tetra-*O*-methylgalactosides as the main products with traces of 2,3,6-tri-*O*-methylgalactosides.

(b) *Periodate oxidation*. The mixed methyl glycosides of 2,4,6-tri-*O*-methylgalactose and 4,6-di-*O*-methylgalactose were used as internal standards. A mixed solution with synthetic β-glycoside in chloroform was examined by g.l.c. (as in Part IX [1]) to measure the ratios of peak heights. The solution was evaporated and the residue was dissolved in aqueous periodic acid (0·1M; 20 ml) and left for 2 days, then neutralised exactly with saturated aqueous barium hydroxide, centrifuged, and evaporated to dryness. The residue was dissolved in chloroform and examined by g.l.c. The 4,6-di-*O*-methylgalactoside peaks had disappeared completely, whereas the relative intensities of 2,4,6-tri-*O*-methylgalactoside and synthetic β-glycoside peaks were not changed.

(c) *Periodate oxidation after hydrolysis*. The β-glycoside (50 mg) in aqueous formic acid (45% v/v; 4 ml) was heated on a boiling water-bath (16 h) then cooled and evaporated. Water was distilled repeatedly from the residue to remove formic acid. Finally the residue was dissolved in water (5 ml), sodium periodiate (1 g) was added, and the solution was adjusted to pH 7·3 with saturated sodium hydrogen carbonate solution. After 24 h in the dark the solution was acidified with hydrochloric acid (1·0N) before addition of hydrochloric acid (1·0N; 10 ml) and aqueous sodium arsenite (20%; 20 ml). Distillation *in vacuo* and addition of 2,4-dinitrophenylhydrazine (saturated solution in 2N-hydrochloric acid; 100 ml) to the distillate gave methoxyacetaldehyde 2,4-dinitrophenylhydrazone, which was collected on a sintered glass filter and crystallised from ethanol; m.p. and mixed m.p. with authentic compound (prepared by oxidation of 6-*O*-methylgalactose [7]) 115—120°, τ ($CDCl_3$) −0·6br (1H, s), 0·89 (1H, d, *J* 3 Hz), 1·65 (1H, q, *J* 3 and 10 Hz), 2·05 (1H, d, *J* 10 Hz), 2·42 (1H, t, *J* 5 Hz), 5·78 (2H, d, *J* 5 Hz), 5·78 (2H, d, *J* 5 Hz), and 6·57 (3H, s).

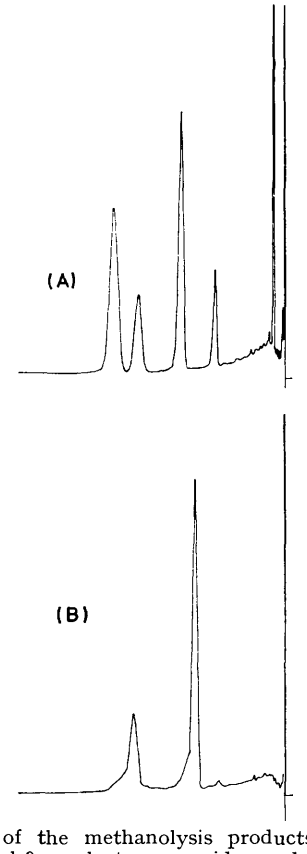


FIGURE G.l.c. of the methanolysis products (A) of methyl 3,6-di-*O*-methyl-β-D-galactopyranoside and (B) of 4,6-di-*O*-methyl-D-galactose. For conditions, see text

3,6-*Di*-O-*methyl*-D-*galactose*.—The β-glycoside (0·20 g) in sulphuric acid (2N; 10 ml) was heated at 100° for 16 h, neutralised with saturated barium hydroxide solution, and treated with Amberlite IR 120 (H^+) and IR 45 (OH^-) resins. Paper chromatography (by the methods of Part IX [1]) showed di-*O*-methyl- and a mono-*O*-methyl-galactose in the approximate ratio of 10 : 1. The di-*O*-methylgalactose was purified by chromatography on paper sheets to give a syrup (75 mg) which slowly crystallised (needles).

[7] J. R. Nunn and M. M. von Holdt, *J. Chem. Soc.*, 1957, 1094.

Recrystallisation from ethanol–water–acetone gave 3,6-*di*-O-*methyl*-D-*galactose*, m.p. 101—102°, $[\alpha]_D$ +93·2° (*c* 1·1 in H_2O) (Found: C, 45·8; H, 7·6. $C_8H_{16}O_6$ requires C, 46·15; H, 7·7%).

G.l.c. and mass spectrometry of methyl 3,6- and 4,6-di-*O*-methylgalactosides

Peak	Relative abundances in the spectrum of: 3,6-di-*O*-methylgalactoside of t_R [a]:				4,6-di-*O*-methylgalactoside of t_R [a]:	
m/e	4·62	6·82	9·62	11·26	6·32	10·28
45	47	25	20	65	56	48
57	—	6	8			
59	12	7	11	10	10	
60						14
71	27	12	12	19	100	100
74	15	100	100	30	77	45
75	35	40	45	45	21	10
87	100	15	10	100	73	35
101					22	18
102					33	25
115	15			15		
147	15	5	14	15		

[a] t_R = retention time on g.l.c. relative to the faster-moving of the methyl 2,3,4,6-tetra-*O*-methylglucosides

Identification of 3,6-*Di*-O-*methyl*-D-*galactosides in the Methanolysis Products of Methylated* ξ-*Carrageenans*.—A sample of the β-glycoside (5 mg) was heated with methanolic hydrogen chloride (2·3% w/v; 100° for 16 h) and the products were analysed by g.l.c.–mass spectroscopy to give four peaks (Figure) with the retention times and mass spectra shown in the Table. Results are given for 4,6-di-*O*-methylgalactosides for comparison.

The methylated ' λ-fractions ' from *Gigartina canaliculata* and *Gigartina chamissoi*, and the methylated polysaccharide from *Gigartina atropurpurea*, were examined in the same way. The retention times and mass spectral data for the peaks assigned to 3,6-di-*O*-methylgalactosides were identical with those given in the Table.

We thank Marine Colloids, Inc., for a grant, and Mr. L. Mindt and Dr. T. A. Bryce for help and advice.

[3/528 *Received*, 12*th March*, 1973]