34. *Red-seaweed Polysaccharides. Part I.* Gracilaria confervoides.

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Partial acid hydrolysis of the agar from *Gracilaria confervoides* followed by reduction of the hydrolysate with sodium borohydride yielded 3 : 6-anhydro-4-*O*-β-D-galactopyranosyl-L-galactitol (I). Partial methanolysis of the agar with methanolic hydrogen chloride yielded 3 : 6-anhydro-4-*O*-β-D-galactopyranosyl-L-galactose dimethyl acetal (II). This was hydrolysed to the free sugar (III) and reduced to the disaccharide glycitol (I) obtained on hydrolysis. The quantitative separation of the products of methanolysis has allowed certain speculations to be made on the structure of the polysaccharide.

AGAR is a polysaccharide constituent of the cell wall in certain red seaweeds (Rhodophyceae) from which it is extracted by hot water. Although agar has been the subject of many publications [1-4] most authors make no mention of the biological origin of their material, and further, it is not yet known whether the polysaccharides from the various agarophytes have the same structure. In particular the agar from *Gracilaria confervoides*, which is the

[1] Percival and Somerville, *J.*, 1937, 1615.
[2] Jones and Peat, *J.*, 1942, 225.
[3] Araki, *Mem. Coll. Sci. Tech., Kyoto*, 1953, **2**, *B*, 17.
[4] Araki and Hirase, *Bull. Chem. Soc. Japan*, 1954, **27**, 109.

chief agar-bearing weed of the U.S.A., New Zealand, and South Africa, does not appear to have received detailed attention.

Analysis of *Gracilaria* agar, purified by extensive washing, showed that it contained 42% of galactose calculated on an anhydro basis. Paper chromatograms of a partial hydrolysate revealed an almost continuous streak extending from the origin to well past the position of galactose, which was fairly well defined. Despite the streaking there were indications that some disaccharide was present and several attempts were made to fractionate hydrolysates on charcoal [5] by using gradient elution [6] with aqueous ethanol. However, apart from D-galactose no pure materials could be isolated. This streaking was probably due to 3 : 6-anhydro-L-galactose and disaccharide containing it. Chromatograms of synthetic 3 : 6-anhydro-D-galactose streaked badly in the three solvents used.

When a hydrolysate was reduced with sodium borohydride [7] and chromatographed a series of discrete spots was obtained. A hydrolysate reduced in this manner and fractionated on a cellulose column [8] yielded as main products, 1 : 4-anhydro-D-galactitol (= 3 : 6-anhydro-L-galactitol), dulcitol, and a crystalline disaccharide glycitol. Hydrolysis of the last and chromatography of the hydrolysate yielded galactose, and a liquid which was chromatographically identical with 1 : 4-anhydro-D-galactitol and had the correct specific rotation.[9] No esters of this alcohol could be induced to crystallise. The hepta-*O*-methyl derivative of the disaccharide glycitol yielded 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose on hydrolysis, indicating that galactopyranose occupied the non-reducing end in the original molecule. When the disaccharide glycitol was oxidised by periodate it liberated 1 mol. of formic acid, consumed 3 mol. of periodate, and produced formaldehyde. These facts are consistent with its structure being 3 : 6-anhydro-4-*O*-β-D-galactopyranosyl-L-galactitol (I).

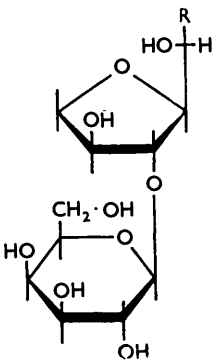
Partial methanolysis of *Gracilaria* agar and fractionation of the products on cellulose yielded 3 : 6-anhydro-L-galactose dimethyl acetal, a mixture of methyl galactosides, and 3 : 6-anhydro-4-*O*-β-D-galactopyranosyl-L-galactose dimethyl acetal (agarobiose dimethyl acetal) (II). On distillation the 3 : 6-anhydro-L-galactose dimethyl acetal was largely converted into methyl 3 : 6-anhydro-α-L-galactoside. The physical constants of the agarobiose dimethyl acetal (II) and the phenylosazone of the free sugar (III) are in agreement with those recorded by Araki and Hirase,[4] who had previously isolated the acetal (II) by the partial methanolysis of agar (species not mentioned). However, the hexa-acetate of the agarobiose dimethyl acetal (II) as prepared by us had m. p. 138° compared with m. p. 88° previously reported.[4] The specific rotations are in both cases identical. The formic acid produced and periodate consumed during oxidation of the dimethyl acetal (II) were also consistent with structure (III). Further evidence for the structure of this disaccharide was provided by reduction of the free sugar (III), isolated by mild hydrolysis of the dimethyl acetal, with sodium borohydride to give the disaccharide glycitol (I), identical with the material previously obtained.

Exhaustive methylation of the agar gave a product which, on hydrolysis and fractionation, yielded as major constituent 2 : 4 : 6-tri-*O*-methyl-D-galactopyranose. Thus, besides the 1 ⟶ 4-links indicated by the isolation of the disaccharide, 1 ⟶ 3-links are also present in the original molecule. This has been demonstrated on several previous occasions; [1,3] unfortunately in no case was the species of plant, from which the agar was extracted, mentioned. No tetra-*O*-methyl-D-galactose was isolated.

Although it was hoped that oligosaccharides higher than disaccharide, might be isolated by partial degradation techniques, only in the case of the fractionation of a reduced hydrolysate was a material isolated (in very low yield) which might have been a trisaccharide glycitol. The separations of products of methanolysis on cellulose columns

[5] Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 677.
[6] Alm, *Acta Chem. Scand.*, 1952, **6**, 1186.
[7] Abdel-Akher, Hamilton, and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 4691.
[8] Hough, Jones, and Wadman, *J.*, 1949, 2511.
[9] Ness, Fletcher, and Hudson, *J. Amer. Chem. Soc.*, 1951, **73**, 3742.

were very nearly quantitative and the results thus afford an accurate analysis of the degradation products. If due allowance was made for the unchanged agar, and the higher oligosaccharides which were eluted from the columns with aqueous ethanol, and which had very low methoxyl values, the yields of sugars from agar which had been refluxed with 1% methanolic hydrogen chloride for 1 hr. were calculated to be 3 : 6-anhydro-L-galactose, 12·7%; D-galactose, 22·6%; and agarobiose, 62%. These calculations are based on the



(I); R = $CH_2 \cdot OH$

(II); R = $CH(OMe)_2$

(III); R = CHO

The β-configuration is assumed because of the low specific rotation.

parent free anhydro-sugar. When the methanolysis was carried out for only 10 min. with 1% methanolic hydrogen chloride the respective yields were 5, 19, and 76%. The oligosaccharides mentioned above gave the same compounds on further treatment with methanolic hydrogen chloride as demonstrated by paper chromatography. Hence it would appear that at least 76% (by weight) of the agar molecule from *Gracilaria confervoides* is composed of agarobiose units, probably united by 1 ⟶ 3-links in an unbranched chain. This work has not demonstrated the form of the 3 : 6-anhydro-L-galactose residues in the macromolecule but since this agar appears very similar in all respects to that examined by Araki [3,4] which is probably mainly from *Gelidium amansii*, it is likely that this sugar residue is present in the pyranose form.[4] If the disaccharide were the sole repeating unit the acetyl and methoxyl values of the acetate and *O*-methyl ether of agar should be 36·3 and 34·2%, respectively. In this work the highest values which it was possible to achieve were 35·4 and 33·8, respectively, which closely support the hypothesis that the macromolecule is composed almost entirely of disaccharide units.

The sulphate content indicated that there was about one sulphate ester for every 40 disaccharide residues.

Experimental

Unless otherwise stated, solutions were concentrated at 40°/20 mm., and specific rotations were measured in aqueous solution. Paper chromatograms (Whatman No. 1 paper) were run in (*a*) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4),[10] (*b*) butanol–pyridine–water (9 : 2 : 2), or (*c*) butanol–ethanol–water (40 : 11 : 19). *p*-Anisidine hydrochloride [11] was used to detect reducing sugars and periodate–benzidine [12a] or periodate-starch [12b] spray to detect glycosides. M. p.s are corrected.

Agar.—For the extraction of the agar [kindly done by Messrs. Vitamin Oils (Pty) Ltd.] the dried *Gracilaria confervoides* was covered with water, and steam passed into the mixture for 1—1·5 hr. After this it was centrifuged, and then filtered hot, a filter aid being used to give a clear solution. It was allowed to set for 24 hr. and then cut up into small pieces, which were frozen at *ca.* −10° for 48 hr. The resulting mass of ice and agar was crushed and thawed, and

[10] Jones, *J.*, 1953, 1672.
[11] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[12a] Cifonelli and Smith, *Analyt. Chem.*, 1954, **26**, 1132.
[12b] Metzenberg and Mitchell, *J. Amer. Chem. Soc.*, 1954, **76**, 4187.

the agar collected on a wire sieve and thoroughly washed with water. The wet agar was collected in a basket centrifuge and dried at 45°. For analysis it was soaked in repeated changes of water and for the final washing it was left in water at 37° for a week. It was finally filtered, washed with ethanol and acetone, and dried at 60°/1 mm. [Found: sulphated ash, 0·67; SO_4'' (after hydrolysis), 0·8; N, 0·5%].

Agar Acetate.—Agar (25 g.) was added in small portions to formamide [13] (250 c.c.), with shaking. Pyridine (250 c.c.) was added and the mixture left at room temperature for 2 days to swell the agar. Acetic anhydride (300 c.c.) was added with shaking and after 16 hr. the mixture was diluted with acetone (1500 c.c.). The clear liquid obtained after centrifugation was poured into ice-water giving a fibrous precipitate, which was collected on a cotton cloth filter and well washed first with water and then with alcohol. This material was redissolved in acetone (1 l.), centrifuged, and precipitated into ether (5 l.). The process was repeated a second time and the product (28 g.) collected, washed with ether, and dried in a vacuum; it had $[\alpha]_D^{20}$ −34° (*c*, 1·0 in $CHCl_3$) (Found: Ac, 35·4%). The acetyl value (determined by Rullen and Pacsu's method [14]) was not increased on reacetylation with acetic anhydride and pyridine.

Quantitative Hydrolysis.—Agar was hydrolysed with N-sulphuric acid at 100° for 17 hr. in a sealed tube. A weighed amount of maltose was added to the hydrolysate,[8] which was then neutralised (IR 4B resin) and chromatographed (Whatman No. 20 paper) in solvent (*a*) for 48 hr. The strips containing the galactose and maltose were extracted with water in a mechanical macerater, the mixture filtered, and the sugars estimated by the Somogyi micro-method.[15] Blank determinations were carried out [Found: galactose (calc. as $C_6H_{10}O_5$), 41·9, 42·0%. Moisture was determined by heating at 90°/0·1 mm.].

Partial Hydrolysis.—Agar was soaked overnight in water (50 parts), and dilute sulphuric acid was then added to make a 0·1N-solution. This mixture was heated by immersion in a boiling-water bath. Samples were removed at regular intervals, neutralised ($BaCO_3$), concentrated and examined on paper chromatograms run in solvents (*a*) and (*b*). The chromatograms were characterised by heavy streaking but it appeared that 3—3·5 hr. under the above conditions gave the maximum concentration of disaccharide.

A larger quantity of agar (15 g.) was then hydrolysed similarly, and the hydrolysate, after neutralisation ($BaCO_3$) and centrifugation, was concentrated to a syrup (12·5 g.). This syrup in water (100 c.c.) was treated with sodium borohydride (1 g.) in water (20 c.c.) with stirring. Next morning the solution was non-reducing (Fehling). It was neutralised by shaking with IR 120 resin, filtered, and then concentrated. Chromatograms run in solvent (*a*) and sprayed with periodate–benzidine [12a] showed a series of discrete spots, R_G 3·1, 1·2, 0·8, 0·3, 0·16 (relative to galactose). This mixture was fractionated on a cellulose column (36 × 5 cm.), butanol–formic acid–water (45 : 1 : 4) being used as irrigating solvent. The first fraction, a syrup (0·4 g.), was chromatographically identical with 1 : 4 anhydro-D-galactitol, R_G 3·1. This was followed by dulcitol (1 g.), R_G 1·2, m. p. and mixed m. p. 185—187°, and disaccharide glycitol (3 g.), R_G 0·67. The next fraction (0·9 g.) was predominantly disaccharide glycitol, but it contained a trace of material of R_G 0·52. The latter (0·5 g.) was obtained chromatographically pure from the subsequent fraction, but has not yet been investigated. No further material could be eluted with this solvent, but elution with water yielded an amorphous mixture (2·6 g.) of oligosaccharide glycitols.

The Disaccharide Glycitol (I).—This substance crystallised from methanol in large needles, m. p. 174°, $[\alpha]_D^{22}$ −15° (*c*, 1·2) (Found: C, 44·4; H, 7·05. $C_{12}H_{22}O_{10}$ requires C, 44·2; H, 6·8%). It (0·69 g.) was hydrolysed in N-sulphuric acid (15 c.c.) at 100° for 10 hr. The hydrolysate was neutralised ($BaCO_3$), filtered, and concentrated, and the residue dissolved in methanol. Next morning the deposited galactose (0·272 g.), m. p. and mixed m. p. 165—166°, was filtered off and the syrup, obtained by evaporation of the filtrate, was streaked on large sheets of paper. The chromatograms were run in solvent (*a*) and the fractions isolated in the usual way. These afforded galactose (0·057 g.; total yield 87%), and a syrup (0·276 g.; 79%), $[\alpha]_D^{18}$ −18° (c, 0·86), chromatographically identical with 1 : 4-anhydro-D-galactitol. Ness, Fletcher, and Hudson [9] reported $[\alpha]_D^{20}$ −18°. Acetylation and benzoylation of the disaccharide glycitol yielded syrups, $[\alpha]_D^{23}$ 0° (*c*, 1·1) and $[\alpha]_D^{16}$ +64° (*c*, 1·4 in $CHCl_3$), respectively.

Methylated Disaccharide Glycitol.—The disaccharide glycitol (0·8 g.) in 40% (w/v) sodium

[13] Carson and Maclay, *J. Amer. Chem. Soc.*, 1946, **68**, 1015.
[14] Rullen and Pacsu, *Ind., Eng. Chem., Anal.*, 1942, **34**, 1209.
[15] Somogyi, *J. Biol. Chem.*, 1952, **195**, 19.

hydroxide solution (23 c.c.) was treated with methyl sulphate (10 c.c.) with stirring at 0° over 3 hr. The mixture was then heated (water-bath) for ½ hr. and again similarly methylated, after which it was neutralised and exhaustively extracted with chloroform. The washed extract was dried (K_2CO_3) and evaporated, leaving a syrup (0·4 g.) which was further twice methylated with Purdie's reagent to give a syrup (0·15 g.), $[\alpha]_D^{22}$ −36° (*c*, 0·55 in MeOH) (Found: C, 54·0; H, 8·5; OMe, 48·0. $C_{19}H_{36}O_{10}$ requires C, 53·8; H, 8·6; OMe, 51·2%).

The methylated *product* (75 mg.) was hydrolysed in N-sulphuric acid at 100° for 5 hr., $[\alpha]_D^{25}$ −15° ⟶ +27°. After neutralisation ($BaCO_3$) and filtration the solution was evaporated, yielding a syrup. Chromatograms of this syrup sprayed with *p*-anisidine hydrochloride [11] only revealed one substance, corresponding to tetra-*O*-methyl-D-galactose. The whole of the syrup was separated on large papers in solvent (*c*) and the strips corresponding to tetra-*O*-methyl-D-galactose were cut out and eluted in the usual way. The material (21 mg.) obtained in this way was refluxed with aniline (7·1 mg.) in ethanol (3 c.c.) for ½ hr. After some time at room temperature the crystals which had formed were filtered off and recrystallised from ethanol; they had m. p. and mixed m. p. 189—190° with authentic 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine.

Periodate Oxidation.—To the disaccharide glycitol (0·1101 g.) in water (25 c.c.) was added 0·4M-sodium metaperiodate (5·0 c.c.), and the mixture was diluted to 50 c.c. The progress of the reaction, which was complete in 24 hr., was followed by titration (0·02N-sodium hydroxide) of aliquot parts at regular intervals, after destruction of excess of periodate with ethylene glycol. The periodate consumed was then determined in the usual way, and the formaldehyde was estimated on an aliquot portion as its dimedone derivative,[16] m. p. and mixed m. p. 188—189° (Found: 0·95, 1·04 mol. of formic acid and 1·25 mol. of formaldehyde produced, and 3·10, 2·94 mol. of periodate consumed).

Methanolysis of Agar.—Agar (25 g.), dried at 60°/1 mm. for 24 hr., was refluxed with 1% methanolic hydrogen chloride for 1 hr. After cooling, undissolved material (4 g.) was filtered off and the filtrate neutralised (Ag_2CO_3). The syrup (25 g.), obtained on concentration, was transferred to a cellulose column (43 × 8 cm.) and eluted with *n*-butanol–water (9 : 1). The fractions (100 c.c.) were sorted by paper chromatography and identical ones combined. This afforded four fractions, after which no more material could be eluted with this solvent. When the column was stripped with ethanol and water oligosaccharides (6·6 g.) of very low methoxyl value were recovered. This weight together with that of the unchanged agar (4 g.) was subtracted from the original weight of agar in calculating the percentages quoted for the fractions below. Furthermore, these percentages represent the proportion of each parent free sugar in the original agar molecule on an anhydro-basis.

Fraction I yielded a syrup (2·8 g.; 12·7% of agar consumed, calc. as $C_6H_8O_4$), $[\alpha]_D^{20}$ −26° (*c*, 1·0), R_R 1·8 (relative to rhamnose) in solvent (*c*) (Found: C, 46·7; H, 7·5; OMe, 24·2. Calc. for $C_8H_{16}O_6$: C, 46·3; H, 7·8; OMe, 29·8%). Araki and Hirase [4] report $[\alpha]_D^{10}$ −28·7° for 3 : 6-anhydro-L-galactose dimethyl acetal (II). When this syrup (0·4 g.) was in contact with N-sulphuric acid for 36 hr. $[\alpha]_D^{20}$ −26° ⟶ −20° (constant). After neutralisation ($BaCO_3$) the solution was concentrated to a syrup which gave positive Schiff and Seliwanoff reactions and reduced cold Fehling's solution. Preparation of the phenylosazone in the usual way yielded yellow crystals, m. p. 203—204·5° (Found: N, 16·5. Calc. for $C_{18}H_{20}O_3N_4$: N, 16·5%). The filtrate from this preparation deposited more crystals, m. p. 212—213° (Found: N, 16·45%). Haworth, Jackson, and Smith [17] found m. p. 216° for 3 : 6-anhydro-D-galactosazone.

Distillation of the remainder of fraction I (2·4 g.) yielded a pale yellow syrup (1·4 g.), b. p. 120—160°/0·03 mm. (bath temp.), which crystallised on trituration with ethyl acetate. Recrystallisation from ethyl acetate gave large needles, m. p. and mixed m. p. 139° with methyl 3 : 6-anhydro-α-L-galactoside, $[\alpha]_D^{20}$ −85° (*c*, 1·0) (Found: C, 47·7; H, 6·9; OMe, 16·7. Calc. for $C_7H_{12}O_5$: C, 47·7; H, 6·8; OMe, 17·6%). Haworth, Jackson, and Smith [17] report $[\alpha]_D^{18}$ +80° for the D-isomer. Attempts to prepare a tri-*p*-nitrobenzoate were unsuccessful.

Fraction II was a syrup (1·77 g.) consisting of two substances R_R 1·3 and 1·1 (solvent *c*), respectively, which were the methyl α- and β-D-galactofuranosides (chromatographic evidence). Fraction III (2·34 g.) crystallised from ethyl acetate; it then had m. p. 108—109°, $[\alpha]_D^{20}$ +180° (*c*, 0·5) and was impure methyl α-D-galactopyranoside (lit., m. p. 112°, $[\alpha]_D^{20}$ +179°). The β-isomer was isolated by crystallisation from ethanol; it had m. p. and mixed m. p. 175—176°,

[16] Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1477.
[17] Haworth, Jackson, and Smith, *J.*, 1940, 620.

$[\alpha]_D^{20}$ 0° (*c*, 1·0). The total weight of methyl galactosides (4·11 g.) represented 22·6% of the agar consumed (calc. as $C_6H_{10}O_5$).

Fraction IV on concentration gave a solid [11·4 g.; 62% (calc. as $C_{12}H_{18}O_9$) of the agar consumed], R_R 0·65 (solvent *c*), which crystallised from ethanol in hexagonal plates, m. p. 163—166°, $[\alpha]_D^{18}$ −36° (*c*, 1·0 in MeOH) (Found: C, 45·5; H, 7·2; OMe, 16·9. Calc. for $C_{14}H_{26}O_{11}$: C, 45·5; H, 7·1; OMe, 16·8%). Araki and Hirase [4] report m. p. 162—164°, $[\alpha]_D^{20}$ −37·4° (*c*, 1·1 in MeOH) for agarobiose dimethyl acetal (II). When this material (0·8 g.) in 0·01N-oxalic acid was heated on a water bath for 2 hr., $[\alpha]_D^{20}$ −25° ⟶ −17° (constant). After neutralisation with IR 4B resin and filtration, the filtrate was evaporated to a solid foam (0·55 g.), which gave positive Schiff and Seliwanoff reactions, and reduced cold Fehling's solution.

Chromatograms of this sugar showed a long streaky spot of R_G approximately 0·27 (in solvent *c*). The phenylosazone had m. p. 222—223·5°, $[\alpha]_D^{20}$ −115° [*c*, 0·8 in pyridine–ethanol (2 : 3)] (Found: C, 54·6; H, 5·9; N, 10·9. Calc. for $C_{24}H_{30}O_8N_4$: C, 57·35; H, 6·0; N, 11·15%). Araki and Hirase [4] report m. p. 220—221°, $[\alpha]_D$ −108°.

The free sugar was reduced with sodium borohydride in the usual way, the solution was treated with Amberlite resin IR 120 to remove sodium ions, and the eluate evaporated to dryness. The residue was fractionated on a cellulose column, butanol–formic acid–water (45 : 4 : 1) being used. The resulting material crystallised from methanol in plates, m. p. and mixed m. p. 173—174° with the disaccharide glycitol previously described.

Agarobiose dimethyl acetal (0·55 g.) in dry pyridine was cooled to 5° and acetic anhydride (5 c.c.) in pyridine (5 c.c.), which had also been cooled to 5°, was added. After 3 days at 5° the mixture was poured into water, from which the hexa-acetate crystallised after 2 days. Recrystallisation from methanol afforded crystals, m. p. 137·5—138·5°, $[\alpha]_D^{18}$ −13·5° (*c*, 1·2 in benzene) (Found: C, 50·2; H, 6·2; OMe, 9·8; Ac, 39·0. Calc. for $C_{26}H_{38}O_{17}$: C, 50·15; H, 6·15; OMe, 10·0; Ac, 41·5%). Araki and Hirase [4] report m. p. 87—88°, $[\alpha]_D^{13}$ −12·5° (in benzene).

Periodate Oxidation of Agarobiose Dimethyl Acetal.—0·4M-Sodium metaperiodate (5·0 c.c.) was added to agarobiose dimethyl acetal (0·0977 g.) in water and the volume adjusted to 50 c.c. Aliquot parts (5 c.c.) were titrated at intervals with 0·01N-sodium hydroxide, bromocresol purple being used as indicator.[18] The reaction was complete in 24 hr. (Found: 0·97 mol. of formic acid produced; 2·02 mol. of periodate consumed). Formaldehyde was absent.[16]

Short Methanolysis of Agar.—Dry agar (10 g.) was refluxed with 1% methanolic hydrogen chloride (200 c.c.) for 10 min., the residue (2·3 g.) filtered off, and the filtrate neutralised (Ag_2CO_3). After silver chloride had been removed, the filtrate was concentrated and treated with hydrogen sulphide. Excess of hydrogen sulphide was removed by bubbling in nitrogen. The precipitate was then filtered off and the filtrate concentrated to syrup (9 g.). This was fractionated as described above to yield the following fractions: Fraction I, 3 : 6-anhydro-L-galactose dimethyl acetal [0·37 g.; 5·0% (calc. as $C_6H_8O_4$) of agar consumed]; fraction II, a mixture of methyl galactosides [1·16 g.; 19% (calc. as $C_6H_{10}O_5$) of agar consumed]; and fraction III, agarobiose dimethyl acetal [4·76 g.; 76% (calc. as $C_{12}H_{18}O_9$) of agar consumed], m. p. and mixed m. p. 164·5—166·5° after recrystallisation from ethanol. Washing of the column with ethanol and water yielded oligosaccharides (2·6 g.). The weight of this material and of the above residue was subtracted from the weight of original agar in calculating the percentages quoted.

Methylated Agar.—Agar acetate (21 g.) was methylated with methyl sulphate and sodium hydroxide in essentially the way described by Percival and Somerville.[1] Five treatments gave a material which was almost completely soluble in chloroform. After centrifugation, the solution was precipitated in light petroleum (b. p. 40—60°), affording a white, fibrous product, $[\alpha]_D^{15}$ −64° (*c*, 0·9 in $CHCl_3$) (Found: OMe, 31·8%). This product (5 g.), dissolved in chloroform (200 c.c.), was precipitated by successive addition of light petroleum (b. p. 40—60°) to yield three fractions: (*A*) 3·2 g., $[\alpha]_D^{15}$ −84° (*c*, 0·5 in $CHCl_3$) (Found: OMe, 32·3%); (*B*) 1·5 g., $[\alpha]_D^{15}$ −76° (*c*, 1·0 in $CHCl_3$) (Found: OMe, 33·8%); and (*C*) 0·2 g., $[\alpha]_D^{15}$ −68° (*c*, 0·7 in $CHCl_3$) (Found: OMe, 33·4%).

Fraction *B* (1·4 g.) was heated with N-hydrochloric acid at 100° for 16 hr. and, after neutralisation (IR 4B resin) and evaporation, afforded a syrup (1·2 g.). Separation of this on a cellulose column, by using butanol–light petroleum (b. p. 100—120°) (2 : 3),[8] yielded mainly

[18] Hartman, *J. Appl., Chem.*, 1953, **3**, 308.

a syrup (0·8 g.), R_{TMG} 0·88 (relative to tetra-*O*-methyl-D-galactose). This syrup crystallised when triturated with ether–light petroleum (b. p. 40—60°), and after recrystallisation from the same solvent had m. p. 104—106°, $[\alpha]_D^{20}$ +124° ⟶ +96° (*c*, 1·4). The aniline derivative had m. p. 169—170° (from ethanol). Percival and Somerville [1] report m. p. 104—105°, $[\alpha]_D$ +93°, for 2 : 4 : 6-tri-*O*-methyl-α-D-galactose, and Hirst and Jones [19] m. p. 179° (sometimes 169°) for the aniline derivative.

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[19] Hirst and Jones, *J.*, 1939, 1482.