166. *Studies on Agar-agar. Part IV.*

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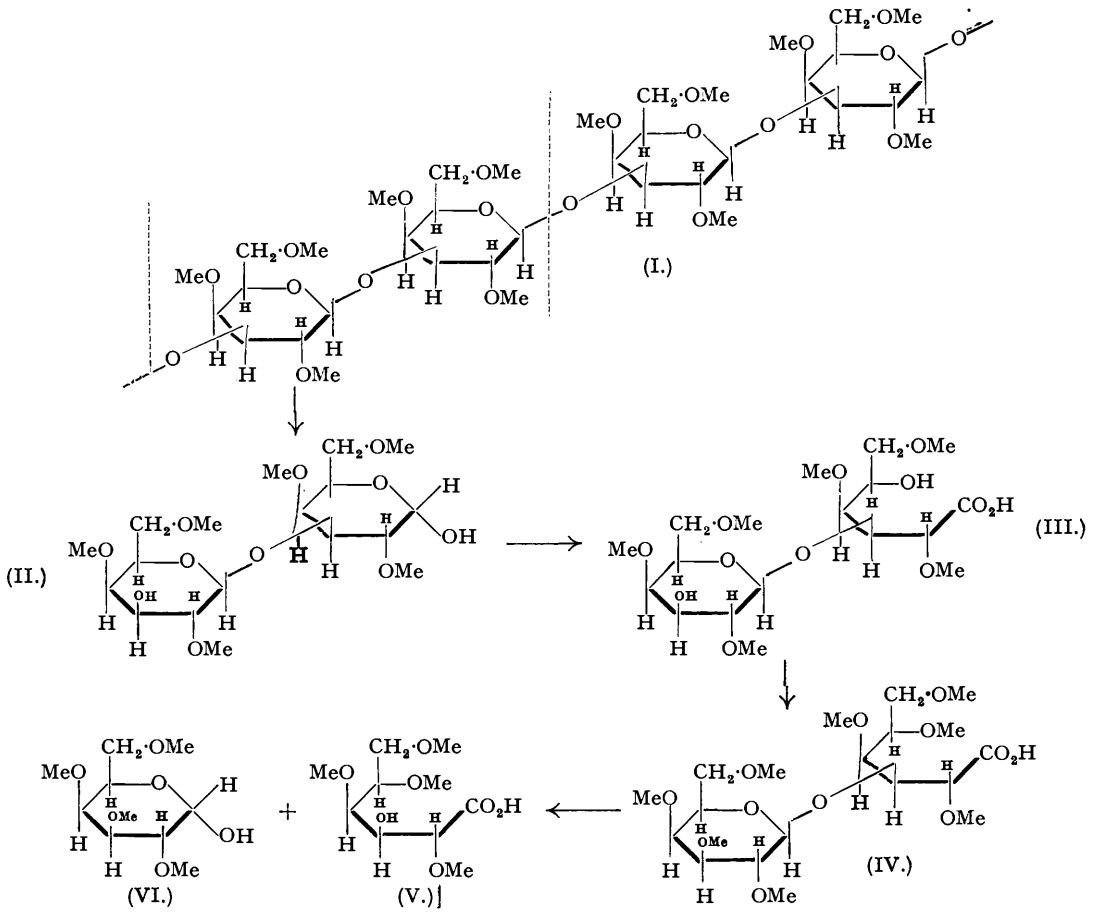
By the acetolysis of methylated agar, followed by oxidation, a mixture of disaccharide esters was obtained from which on hydrolysis and suitable treatment tetramethyl *d*-galactopyranose anilide was isolated together with a mixture of methylated acids. It was concluded that the main acid product was 2 : 4 : 5 : 6-*tetramethyl* d-*galactonic acid* and an accompanying crystalline acid was shown to be 2 : 5-*dimethyl* 3 : 6-*anhydro*-l-*galactonic acid*, thus proving the linkage of the 3 : 6-anhydro-*l*-galactose residues to the *d*-galactopyranose units to be at C_4 as suggested by Jones and Peat (this vol., p. 225).

The absence of a non-reducing end group of tetramethyl galactose is confirmed and evidence for the presence of dimethyl methylgalactosides in the products of hydrolysis is presented. The products obtained on heating agar at 130° with water have been studied and their relation to the agar molecule is discussed.

Haworth and Percival (J., 1931, 1342) used the method of degrading methylated starch and glycogen with acetyl bromide, followed by oxidation, to show that contiguous glucose units in these polysaccharides were pyranose. The application of this method to agar was instituted, not to decide the configuration of the *d*-galactose residues, since the isolation of 2 : 4 : 6-trimethyl galactose from methylated agar by Percival and Somerville (Part I; J., 1937, 1615) had already shown them to be pyranose and linked through C_1 and C_3, but to decide, if possible, the mode of attachment and configuration of the *l*-galactose residues which had been isolated as 2 : 4-dimethyl 3 : 6-anhydro-β-methyl-*l*-galactoside by methylation of the hydrolysis products of methylated agar (Hands and Peat, *Chem. and Ind.*, 1938, **57**, 937; Forbes and Percival, *Nature*, 1938, **142**, 797; Part II, J., 1939, 1844), although the proportion of the disaccharide containing the *l*-galactose fragment, if such could be isolated, would obviously be small because of the preponderating proportion of *d*-galactose units.

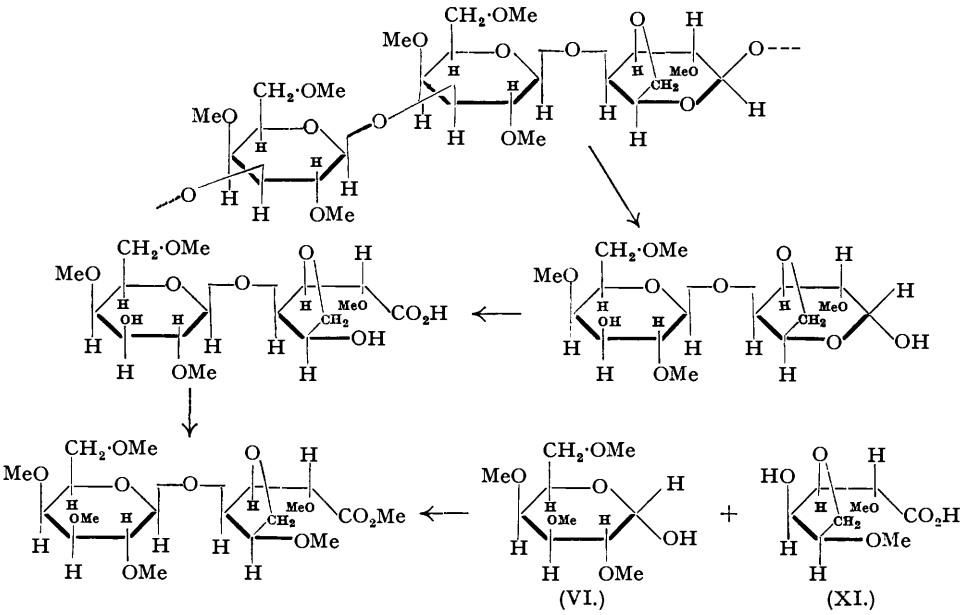
Methylated agar was degraded with acetyl bromide and the products after suitable treatment were oxidised and methylated; distillation in a high vacuum gave mainly crystalline *pentamethyl methyl*-d-*galactonate*, but a fraction was also isolated which appeared to be a mixture of methylated disaccharide esters. Hydrolysis of this disaccharide mixture yielded tetramethyl *d*-galactopyranose (VI), characterised as the anilide. On some occasions the accompanying methylated hexonic acid crystallised, but the major portion was a syrup which would not lactonise, and which was concluded to be mainly 2 : 4 : 5 : 6-tetramethyl *d*-galactonic acid (V), since the corresponding amide gave a negative Weerman test and the ester on methylation yielded crystalline pentamethyl methyl-*d*-galactonate. This evidence does not exclude the possibility that the acid was 2 : 3 : 4 : 5-tetramethyl galactonic acid, but the above interpretation is in harmony with the view that most of the *d*-galactopyranose residues are joined together by 1 : 3-linkages (I); the various stages in the analysis are represented below.



The crystalline acid (m. p. 160°, $[\alpha]_D^{18°}$ −65°) proved to be 2 : 5-*dimethyl* 3 : 6-*anhydro*-l-*galactonic acid* (XI) for the following reasons: The physical constants, apart from the sign of the rotation, closely resemble those for 2 : 4-dimethyl 3 : 6-anhydro-*d*-galactonic acid (Haworth and Smith, J., 1940, 620), but a mixed m. p. determination with the *l*-acid (m. p. 151°) showed a depression of 30°. Jones and Peat (this vol., p. 225) have isolated from methylated agar an amide which they describe as 2 : 5-dimethyl 3 : 6-anhydro-*l*-galactonamide (m. p. 173°). The above acid was also converted into an amide, m. p. 171°, which gave a negative Weerman reaction and appears to be identical with that of Jones and Peat, so the conclusions of these workers as to the mode of union of the 3 : 6-anhydro-*l*-galactose residues to the *d*-galactose units by 1 : 4-linkages are supported by our acetolysis experiments, as shown on p. 752.

In Part I (*loc. cit.*) it was pointed out that no tetramethyl *d*-galactopyranose was detectable in the hydrolysis products of methylated agar; the quantity used, however, was not sufficient to decide definitely that a non-reducing end group of *d*-galactose did not exist. Larger quantities of methylated agar have now been hydrolysed, and the original provisional conclusion confirmed. Jones and Peat (*loc. cit.*) have indeed accepted this view and made use of it to formulate a structure for agar embodying a repeating unit of nine *d*-galactopyranose residues joined by β-linkages through the 1 : 3-positions, terminated by one *l*-galactopyranose-6-sulphate residue attached by position 4, the reducing group of this residue being attached to the terminal *d*-galactopyranose unit of another similar chain. It does not appear to us, however, that this conception of the agar molecule

accords with all the facts, although, as indicated above, we agree as to the mode of union of the *l*-galactopyranose residues.



As pointed out previously, in the specimens of agar used by us (Duff and Percival, J., 1941, 830) there is no question of the hydrolysis of a sulphate group in the *l*-galactose residue to yield a 3 : 6-anhydro-ring during methylation as suggested by Peat (*Ann. Reports*, 1941, 154) because the washed agar employed contained but 0·1% S. A very small quantity of a methylated *l*-galactose (isolated as *tetramethyl* l-*galactopyranose anilide*) was detected in one of the fractions of hydrolysed methylated agar. It is probable that this originated by the acid hydrolysis of a sulphate group at some stage, since it has now been shown (E. E. Percival, Thesis, Edinburgh, 1942) that the acid hydrolysis of β-methylgalactoside sulphate proceeds normally without anhydride formation.

From the yield (11·5%) of 2 : 4-dimethyl 3 : 6-anhydro-β-methyl-*d*-galactoside recorded in Part II (*loc. cit.*) the proportion of *d*- to *l*-galactopyranose units is closer to 15 : 2 than to that of 9 : 1 as suggested by Jones and Peat (*loc. cit.*), although the precise evaluation of this ratio is admittedly difficult and must be stated with reserve.

A point which renders difficult the acceptance of the relatively simple structure of Jones and Peat (*loc. cit.*) concerns the yield of 2 : 4 : 6-trimethyl methylgalactosides. The maximum yield (65%) of crystalline material recorded in Part II admittedly may exclude some of this fraction owing to the difficulties of crystallising a substance of low melting point, but if a methylated agar of the composition proposed by Jones and Peat were hydrolysed, the yield of trimethyl methylgalactosides would be 104·8% and of dimethyl anhydromethylgalactosides 10·1% of the methylated agar used, the figures for a 15 : 2 ratio being 103% and 11·9% respectively.

This discrepancy is serious, as is also the fact that the methoxyl content for a model of methylated agar on the lines suggested by Jones and Peat should be 42%, whereas the highest recorded value is not greater than 35% for a representative sample (Jones and Peat, *loc. cit.*).

The view was expressed in Part II (*loc. cit.*) that dimethyl methylgalactosides were probably present in the fraction containing the monomethyl 3 : 6-anhydromethyl-*l*-galactosides. This is now confirmed and, although precise evaluation of the proportion is difficult owing to the failure to isolate crystalline products, the amount may be as high as the yield of dimethyl anhydromethylgalactosides. Whether one is dealing with a single dimethyl galactose, or a mixture, is uncertain, since the product was contaminated with decomposition products of the monomethyl anhydromethylgalactosides, but tetramethyl *d*-galactopyranose anilide was isolated in good yield on methylation and anilide formation, and the amorphous osazone obtained in the usual way appeared to be a monomethyl hexosazone, which would indicate substitution of methoxyl at C_2. In the absence of non-reducing end group fractions the only explanation, that appears possible in the present state of our knowledge, of the presence of dimethyl galactoses is that complete methylation of agar has not yet been achieved, although it may be remarked that the use of sodium in liquid ammonia (Part II, *loc. cit.*) failed to raise the methoxyl content of methylated agar appreciably.

The treatment of agar with water in an autoclave at 130° was shown by Takahashi and Shirahama (*J. Fac. Agr. Hokkaido Imp. Univer., Japan*, 1934, **35**, 101) to yield two distinct degradation products, hydrato-kanten δ (hereafter called " δ "), which formed a gel with water, and hydrato-kanten λ (" λ "), soluble in water. This work has now been repeated and the products " δ " and " λ " acetylated, methylated, and hydrolysed to

determine the relationship of these and more highly degraded products " δ_1 " and " λ_1 " to agar and to one another. Comparative determinations of molecular weight (which must not be regarded as absolute values) were made by the viscosity method and by the determination of reducing power by alkaline hypoiodite; the results are in Table I.

TABLE I.

	$[\alpha]_D$.	% OMe.	% OAc.	M_v.	M_0.	M_1.
Methylated agar	−86°	32	—	15,500	12,300	16,000
Acetylated agar	−32	—	37	29,500	16,600	—
Methylated " δ "	−82	32·4	—	7,800	6,250	8,100
Acetylated " δ "	−32·5	—	36·5	10,000	5,630	—
Acetylated " δ_1 "	−32	—	38	6,000	3,400	3,300
Methylated " λ "	−86	34	—	4,500	3,600	3,400
Acetylated " λ "	−40	—	40	4,500	2,500	—
Acetylated " λ_1 "	−37	—	44	2,500	1,400	1,200

M_v denotes the apparent molecular weights by the viscosity method, taking $K_m = 10^{-3}$, and are mean values (except for " λ_1 "). M_0 is the apparent molecular weight calculated from M_v, the repeating unit being reckoned as $C_6H_{10}O_5$. M_1 is the apparent molecular weight calculated from the iodine number determined for the unsubstituted polysaccharide.

The chemical composition of " δ " and " λ " was investigated by methanolysis of the methylated derivatives. The trimethyl methylgalactosides were estimated in the usual way, and the proportion of 3 : 6-anhydromethyl-*l*-galactosides found by methylation and isolation of the crystalline β-methylgalactoside. The results are in Table II, and show that the products of hydrolysis of the three substances concerned are closely similar.

TABLE II.

		Agar.	" δ."	" λ."
2 : 4 : 6-Trimethyl methyl-*d*-galactosides, %		65	52	59
2 : 4-Dimethyl 3 : 6-anhydro-β-methyl-*l*-galactoside	(*a*)	8	11	9
(*a*) crystalline (*b*) estimated	(*b*)	11·5	14·9	13·3

The difference in properties of " δ " and " λ " is not explained by this analysis, so the physical differences between them and the failure to convert " δ " into " λ " must arise from some difference in arrangement at present obscure.

EXPERIMENTAL.

The Washing of Agar.—Powdered agar (B.D.H.) (10 g.; ash 3·2%, S 0·3%) was washed ten times with water (2 l. each time) during 10 days. The p_H of the first washing liquid as determined by the quinhydrone electrode was 7·0 as for the subsequent washings. The product after treatment with alcohol and ether and drying had ash 1·2%, S 0·1%. This material was acetylated to yield a product which after precipitation from chloroform solution with light petroleum had $[\alpha]_D^{18°}$ −32°. The specific rotations are determined in chloroform (*c*, 1·0) unless otherwise stated (Found: Ash, 0·4; S, 0·03; $CH_3{\cdot}CO$, 37·1%).

The Acetolysis of Methylated Agar.—Methylated agar (44 g.), prepared from agar (B.D.H.) as described in Part I (*loc. cit.*) ($[\alpha]_D^{18°}$ −86°; OMe, 32·5%), was treated in 4 g. portions in chloroform (40 c.c.) with acetyl bromide (12 c.c. in chloroform, 15 c.c.). After 7½ hours the mixtures were poured into ice-water; more chloroform was added and the chloroform solution was washed with sodium bicarbonate solution and ice-water until free from acid, and dried over sodium sulphate. Evaporation gave a yellow syrup which contained bromine and reduced Fehling's solution. The subsequent operations were essentially as described by Haworth and Percival (*loc. cit.*) and yielded finally a mobile syrup (6 g.), which was fractionally distilled at 0·03 mm.; (1) 2·2 g., b. p. 105—125° (all the b. p.'s in this paper are bath temperatures), $n_D^{14°}$ 1·4506; (2) 1·6 g., b. p. 125—140°, $n_D^{14°}$ 1·4498, OMe 58%; (3) 0·2 g., b. p. 145—170°, $n_D^{14°}$ 1·4556; (4) 1·1 g., b. p. 180—220°, $n_D^{14°}$ 1·4734, $[\alpha]_D^{14°}$ −4° (Found: C, 52·5; H, 8·0; OMe, 47·5; CO_2Me, 12·5%).

Pentamethyl methyl d-*galactonate.* After standing in the refrigerator, fraction (2) partially crystallised; the crystals were separated on a porous tile and recrystallised from light petroleum (b. p. 60—80°), yielding 0·9 g., m. p. 46°, $[\alpha]_D^{14°}$ +20° in water (*c*, 0·4) (Found: C, 51·6; H, 8·7; OMe, 64·3; CO_2Me, 19·6. $C_{12}H_{24}O_7$ requires C, 51·4; H, 8·6; OMe, 66·4; CO_2Me, 21·0%).

2 : 5-*Dimethyl* 3 : 6-*anhydro*-l-*galactonic acid.* Fraction (4) (0·63 g.) was heated at 95—100° with 5% sulphuric acid (20 c.c.); $[\alpha]_D^{14°}$ −8° (initial); +16° (160 mins., constant value). Neutralisation with barium carbonate and extraction with ether gave a mobile syrup (0·3 g.), which yielded on treatment with aniline tetramethyl *d*-galactopyranose anilide (0·2 g.), m. p. and mixed m. p. 196°. The residue of barium salts was treated with sulphuric acid, and a syrup was obtained which crystallised on the addition of chloroform to yield an acid (0·08 g.), m. p. 160°, $[\alpha]_D^{16°}$ −65° in water (*c*, 0·5)(Found: C, 46·2; H, 7·0; OMe, 28·0; equiv. by titration, 206. $C_8H_{14}O_6$ requires C, 46·6; H, 6·8; OMe, 30·0%; equiv., 212).

2 : 4-Dimethyl 3 : 6-anhydro-*l*-galactonic acid, prepared from the corresponding β-methylgalactoside by Haworth and Smith's method (*loc. cit.*), had m. p. 151°, mixed m. p. with the above 125—130°. A small portion of the acid on treatment with diazomethane and then with methyl-alcoholic ammonia yielded a crystalline amide, which gave a negative Weerman reaction and after recrystallisation from acetone had m. p. 171°.

Further Experiments on the Disaccharide Acids.—The syrupy product accompanying the above crystalline acid was not investigated further on this occasion, but, in other experiments, probably owing to the difficulty of controlling the conditions of acetolysis, the main product was a syrup.

A fraction (1·9 g.), b. p. 180—210°/0·08 mm., $n_D^{14°}$ 1·4755, $[\alpha]_D^{14°}$ −7° (OMe, 54·2%), corresponding to fraction (4) above, obtained from 32 g. of methylated agar, after a further methylation with methyl iodide and silver oxide (1·4 g.), was hydrolysed for 3 hours with 5% sulphuric acid. Neutralisation with barium carbonate and treatment as before gave tetramethyl *d*-galactopyranose anilide (0·5 g.), m. p. and mixed m. p. 197°, $[\alpha]_D^{16°}$ −78° in acetone (*c*, 1·0) (Found: OMe, 38·5. Calc. for $C_{16}H_{25}O_5N$: OMe, 39·9%). Treatment of the insoluble residue with sulphuric acid gave a syrup 0·65 g.), $[\alpha]_D^{14°}$ −3° in water (*c*, 1·5) (Found: OMe, 46·1; equiv. by titration, 245. $C_{10}H_{20}O_7$ requires OMe, 49·2%; equiv., 251). This acid was heated at 100°/15 mm. for 10 hours, but no change in properties was observed.

Esterification of the acid (0·5 g.) with diazomethane, followed by distillation at 110—135°/0·07 mm., gave an ester

(0·4 g.), $n_D^{18°}$ 1·4503, $[\alpha]_D^{15°}$ +11° in water (*c*, 1·0) (Found: OMe, 56·2; CO_2Me, 20·1. $C_{11}H_{22}O_7$ requires OMe, 58·3; CO_2Me, 22·2%). A portion (0·1 g.), treated with methyl-alcoholic ammonia, gave a syrupy amide which gave a negative Weerman test. The remaining ester on methylation with methyl iodide and silver oxide, followed by distillation at 110°/0·50 mm., gave crystalline pentamethyl methyl galactonate, m. p. 46°, not depressed by an authentic specimen, $[\alpha]_D^{16°}$ +19° in water (*c*, 1·1).

The Hydrolysis of Methylated Agar and End Group Investigation.—Methylated agar (32 g.) (OMe, 32·9%) was hydrolysed with methyl-alcoholic hydrogen chloride (1 l.) at 75° for 40 hours. Neutralisation with silver carbonate, filtration, and removal of solvent gave a syrup (33·4 g.), which was distilled at 0·03 mm. to yield the following fractions: (1) 15·6 g., b. p. 125—145°, crystalline; (2) 1·4 g., b. p. 145—150°; (3) 2·4 g., b. p. 165—175°; (4) 14·0 g., residue. Fraction (1) was recrystallised from light petroleum (b. p. 60—80°) (500 c.c.) so that about 80% of the trimethyl methyl-galactoside content was deposited rapidly, and the filtrate was evaporated to yield a semi-solid mass (3·1 g.), which was then distilled from a flask with a vacuum-jacketed spiral column to yield three crystalline fractions: (*a*) 0·67 g., b. p. 120—130°, $[\alpha]_D^{14°}$ +100° in water (c, 1·0), OMe 51·1%; (*b*) 1·3 g., b. p. 130—140°, OMe 50·9%; (*c*) 0·24 g., b. p. 140—160°.

Fraction (*a*), kept for 24 hours at room temperature with 0·8N-sulphuric acid, suffered no change in rotation, proving the absence of 2 : 4-dimethyl 3 : 6-anhydro-β-methyl-*l*-galactoside. Subsequent hydrolysis of the glycosides at 95—100°, followed by anilide formation in the usual way, gave none of the easily crystallisable tetramethyl galactopyranose anilide, but 2 : 4 : 6-trimethyl *d*-galactose anilide, m. p. 178°, was obtained.

Fraction (3) was extracted with light petroleum (b. p. 60—80°), and gave a residual syrup (2·0 g.) which after two methylations with methyl iodide and silver oxide gave 2 : 4-dimethyl 3 : 6-anhydro-β-methyl-*l*-galactoside (0·4 g.). The light petroleum extracts were evaporated and the syrup (0·3 g.) obtained was methylated three times with methyl iodide and silver oxide. Hydrolysis and anilide formation gave an anilide (0·03 g.), m. p. 197° $[\alpha]_D^{20°}$ +70° in acetone (*c*, 0·8), mixed m. p. with tetramethyl *d*-galactopyranose anilide, 185°. This must therefore be characterised as *tetramethyl* l-*galactose anilide* (Found: C, 61·1; H, 7·9; OMe, 37·6; N, 4·6. $C_{16}H_{25}O_5N$ requires C, 61·7; H, 8·1; OMe, 39·9; N, 4·5%).

Fraction (4) was hydrolysed as above for a further 24 hours and the glycosides on distillation at 0·05 mm. gave the following fractions: (5) 2·5 g., b. p. 115—125°, crystalline; (6) 4·0 g., b. p. 125—145°, crystalline; (7) 3·9 g., b. p. 150—175°; (8) 2·9 g., b. p. 175—200°; (9) 1·5 g., residue.

Fractions (5) and (6) were combined and recrystallised from light petroleum to give 2 : 4 : 6-trimethyl methylgalactosides (5·5 g.), m. p. 60°. The residual syrup on hydrolysis and anilide formation gave no tetramethyl galactopyranose anilide. Fractions (7) and (8) were combined and distilled from a smaller flask to give (10) 1·7 g., b. p. 135—150°, $n_D^{16°}$ 1·4712, partly crystalline; (11) 2·8 g., b. p. 155—175°, $n_D^{16°}$ 1·4706, $[\alpha]_D^{16°}$ +4° in water, OMe 40·2%; (12) 1·0 g., b. p. 175—200°, $n_D^{16°}$ 1·4688, $[\alpha]_D^{16°}$ +3° in water, OMe 38·7%; (13) 1·4 g., residue.

Fraction (11) was extracted repeatedly with boiling light petroleum (b. p. 60—80°) and the residue was combined with fraction (12) and heated at 95—100° with 5% sulphuric acid for 24 hours to decompose anhydro-sugars. After neutralisation with barium carbonate, filtration, and evaporation the residual syrup (3·4 g.; OMe 26%) was submitted to glycoside formation with 5% methyl-alcoholic hydrogen chloride at 70° for 8 hours; the product, obtained in the usual way, distilled at 0·03 mm. as a syrup (2·9 g.), b. p. 170—185°, $n_D^{16°}$ 1·4700 (Found: OMe, 41·0. Calc. for $C_9H_{18}O_6$:OMe, 41·9%).

Complete methylation and isolation of tetramethyl galactopyranose anilide. The syrup (0·5 g.) was methylated four times with silver oxide and methyl iodide, and the product converted into the anilide. Tetramethyl *d*-galactopyranose anilide (0·3 g.), m. p. 197°, not depressed by an authentic specimen, $[\alpha]_D^{17°}$ −72° in acetone (*c*, 1·0), was obtained.

Osazone formation. The syrup (1·0 g.; OMe, 41·0%) was hydrolysed with 8% hydrochloric acid at 95—100° for 2½ hours. The sugar isolated had $[\alpha]_D^{16°}$ +30° in water (*c*, 1·5) (Found: OMe, 26·4. Calc. for $C_8H_{16}O_6$:OMe, 29·8%).

Treatment with phenylhydrazine acetate gave a tarry osazone, which could be precipitated from chloroform solution by light petroleum as a brown powder (Found: OMe, 7·5. Calc. for $C_{19}H_{24}O_4N_4$: OMe, 8·3%).

The Hydrolysis of Agar at 130°.—Washed agar (100 g.) was heated in an autoclave at 130° with water (2 l.) for 5 hours. The gel was separated in a centrifuge and purified by solution thrice in hot water, cooling, and centrifuging; dehydration with alcohol followed and the powdered product "δ" (40 g.) was dried in a vacuum over calcium chloride. The clear brown solution accompanying this material had p_H 4·7, determined by the quinhydrone electrode. It was concentrated at 48°/15 mm. to 500 c.c. and poured into alcohol (2 l.). The precipitated product "λ" was purified thrice by solution in water and precipitation with alcohol, dehydrated, and dried as before (17 g.).

Experiments on Hydrato-kanten "δ."—This substance was a greyish-white powder (ash, 0·5%), slightly reducing to Fehling's solution, and 0·03 g. in 5 c.c. of water formed a gel. The iodine number determined according to Bergmann and Machemer (*Ber.*, 1930, **63**, 316) was 2·47, corresponding to a unit of 8100 per reducing group. The iodine number for washed agar was 1·25, corresponding to a value of 16,000.

Acetylation and fractionation of the "δ" acetate. Powdered "δ" (45 g.) was acetylated with pyridine and acetic anhydride. Fractional precipitation (48 g.) from chloroform by light petroleum yielded three fractions (Table III). When deacetylated, each of these fractions formed a gel with water (0·05 g. in 3 c.c.). The apparent molecular weight (M_v) was calculated from $\eta_{sp.} = c.M_v.10^{-3}$, the repeating unit being reckoned as $[C_6H_7O_2(OAc)_3]$.

TABLE III.

	% CH_3·CO.	$[\alpha]_D^{14°}$.	$\eta_{sp.}^{20°}$.	*c*.	M_v.
Agar acetate	36·4	−32·1°	0·343	0·336	29,400
(1) (6 g.)	34·1	−33·3	0·125	0·427	8,400
2) (24 g.)	36·0	−32·5	0·158	0·428	10,600
(3) (9 g.)	36·5	−31·8	0·131	0·415	9,100

Deacetylation and methylation. Fraction (2), dissolved in acetone, was deacetylated and methylated, the product being remethylated twice, purified, and fractionated (17 g.) (Table IV).

TABLE IV.

	% OMe.	$[\alpha]_D^{14°}$.	$\eta_{sp.}^{20°}$.	*c*.	M_v.
Methylated agar	32·1	−86°	0·266	0·349	15,500
(1) (3·1 g.)	32·0	−79	0·172	0·418	8,400
(2) (7·0 g.)	32·5	−80	0·168	0·441	7,800
(3) (6·9 g.)	32·1	−86	0·152	0·418	7,400

Hydrolysis of methylated " δ." Methylated " δ " (9·5 g.) was hydrolysed with methyl-alcoholic hydrogen chloride, and the products, divided into 10 fractions by distillation in a high vacuum, treated as described in Part II (*loc. cit.*) to yield trimethyl methylgalactosides (4·96 g., 52%) and 2 : 4-dimethyl 3 : 6-anhydro-β-methyl-*l*-galactoside (1·05 g., 11%). On the assumption that the completely methylated syrups (2·3 g.) from which the 2 : 4-dimethyl β-methyl-*l*-galactoside crystallised contained 16% of this material (see Part II, *loc. cit.*) the proportion of the latter substance becomes 14·9%.

Hydrato-kanten " λ."—This substance was a white powder (ash, 2·5%), soluble in cold water and reducing to Fehling's solution. The iodine number was 5·89, corresponding to a unit of 3,400 per reducing group.

Acetylation was carried out as above and the acetate (14 g.), obtained in good yield, was divided into three fractions by precipitation by light petroleum from chloroform solution (Table V). Methylation, carried out as described above, gave a product (4·5 g.), which was fractionally precipitated (Table VI).

TABLE V.

	% CH_3·CO.	$[\alpha]_D^{14°}$.	$\eta_{sp.}^{20°}$.	*c*.	M_v.
1	38·7	−45°	0·077	0·410	5,400
2	40·7	−37	0·053	0·368	4,100
3	41	−36	0·062	0·437	4,100

TABLE VI.

	% OMe.	$[\alpha]_D^{14°}$.	$\eta_{sp.}^{20°}$.	*c*.	M_v.
1	32·5	−100°	0·115	0·452	5,200
2	34·5	− 80	0·092	0·446	4,200
3	35·0	− 78	0·081	0·401	4,100

Hydrolysis of methylated " λ." Methylated " λ " (11·2 g.) was hydrolysed, and the products (8 fractions) investigated as for " δ " to yield trimethyl methylgalactosides (6·21 g., 59·1%) and 2 : 4-dimethyl 3 : 6-anhydro-β-methyl-*l*-galactoside (0·95 g., 9·1%). On the assumption that the syrups from which the latter substance crystallised contained 16% of this material, the proportion becomes 13·3%.

Autohydrolysis of " δ."—The finely powdered substance (20 g.) was heated with water (200 c.c.) for 2½ hours at 130°. The resultant thin gel was centrifuged, and the product purified as described previously. The greyish-white powder (8 g.) was reducing to Fehling's solution and the iodine number of 6·1 corresponds to a unit of 3,300 per reducing group. This substance " δ_1" formed a gel with water (0·08 g. in 2 c.c.).

Acetylation as described above, followed by fractionation (3·8 g.), gave: fraction (1) 1·5 g., $[\alpha]_D^{14°}$ −35°, $\eta_{sp.}^{20°}$ 0·090 in *m*-cresol, (*c*, 0·441), M_v 5,900, CH_3·CO 37·8%; fraction (2) 2·1 g., $[\alpha]_D^{14°}$ −30°, $\eta_{sp.}^{20°}$ 0·087 in *m*-cresol (*c*, 0·412), M_v 6,100, CH_3·CO 38·0%.

Powdered " δ " (20 g.) was heated for 4½ hours at 130° with water (400 c.c.). No solid remained and no substance was obtained on precipitation with alcohol. " δ " is thus not transformed into " λ " in this way.

Autohydrolysis of " λ."—The powdered " λ " (10 g.) was heated with water (200 c.c.) for 1½ hours at 130°. The product on precipitation with alcohol appeared unchanged and gave the same iodine number (5·87) as the starting material. Further autohydrolysis (3½ hours) gave a product " λ_1" (7·1 g.) with an iodine number of 17·0, corresponding to a unit of 1,200 per reducing group.

Acetylation was followed by fractionation (3·4 g.): fraction (1) 0·8 g., $[\alpha]_D^{14°}$ −43°, $\eta_{sp.}^{20°}$ 0·073 in *m*-cresol (*c*, 0·417), M_v 5,000, CH_3·CO 40·0%; fraction (2) 2·5 g., $[\alpha]_D^{14°}$ −37°, $\eta_{sp.}^{20°}$ 0·037 in *m*-cresol (*c*, 0·433), M_v 2,500, CH_3·CO 44·1%.

Methylated agar was recovered unchanged on heating with water for 3½ hours at 130°

Thanks are expressed to the Earl of Moray Endowment and Imperial Chemical Industries Ltd. for grants and to the Carnegie Trust for the award of a Teaching Fellowship to one of us (E. G. V. P.).

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