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The Degradation of Agar-Agar and Similar Substances.

THE DEGRADATION OF AGAR-AGAR AND SIMILAR SUBSTANCES.

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I N T R O D U C T I O N

The elucidation of the problems of carbohydrate chemistry depends on our knowledge of the simplest units. From these units, the monosaccharides generally and the hexoses in particular, we can build up a continuous framework to include many of the familiar polysaccharides.

Following the researches of Fischer (1) on the stereochemical structure of the sugars, it is now generally accepted, largely as a result of the work of Haworth (2), that hexoses exist as six-membered rings, involving the linkage of one oxygen atom and five carbon atoms. These investigations required the study of many sugars and their numerous derivatives, with problems both chemical and stereochemical. It is in the light of such discoveries that investigations of the more complex carbohydrates are undertaken.

The disaccharides, which are the next simplest form, may be theoretically considered as two hexose units, condensed by the elimination of a water molecule. By the Haworth method of complete methylation, with subsequent hydrolysis and careful examination of the scission products, the common disaccharides have been allotted formulae which account for the majority of their properties. Thus maltose is considered to consist of two α-glucopyranose residues, linked by the reducing group of one unit to position four of the other; that is, 4-α-glucopyranosido-glucopyranose. Lactose, on the other hand, is understood to be a condensation product of glucose and galactose, or simply a glucoside of galactose. These compounds are in every way comparable to the methylglucosides, which exist similarly in α- and β-forms.

In several cases, these disaccharides are themselves scission products of the much more complex polysaccharides. Starch, for example, can be hydrolysed by enzyme action to give an 80% yield of maltose. Such degradation products throw a great deal of light on the constituents of these complexes, and also on the type of linkage uniting the hexose units.

Rapid progress has been made in the past decade on the structure of the best known polysaccharides. The results of these researches, chiefly by Haworth (3) and

co-workers, have been to offer indisputable proof that these complexes are built up of long chains of monosaccharides residues, united by glycosidic linkages. The molecules may, by this theory, exist as true chains with two terminal groups, or the possibility exists that the units may be linked together to form a loop, either with or without side-chains.

Furthermore, these macro-molecules may be built up of only one type of monosaccharide, or there may be two ormore distinct types. It has been found that starch (4) contains only α-glucopyranose units, while xylan (5) is understood to consist of xylopyranose (93%) with an arabofuranose residue (6%) as the terminating unit.

The earlier work on the molecular weights of polysaccharides was done by physical means, of which the most notable was the application of Staudinger's viscosity method on solution in m-cresol (6). From X-ray methods (7), also, the dimensions of the crystallographic cell may be calculated, and from a knowledge of the density, the molecular weight may be estimated. The ultracentrifugal method of Svedberg (8), which he applied to proteins, has also been used to determine the molecular weight of cellulose.

More recently, however, a chemical method of assay has been evolved by Haworth (3). This involves the complete methylation and hydrolysis of the polysaccharide,

examination of the scission products, and isolation of the terminal units of the chains. Since in a straight chain this terminal residue will have only one linkage belonging to the macro-molecule, it will obviously be different from the other residues, and the proportion of it in the hydrolysed methylated polysaccharide will give a clue to the chain length. By this method the molecular size of many polysaccharides and polysaccharide degradation products, e.g. starches, glycogen, inulin, levan, xylan, cellulose and cellulose dextrins have been elucidated, and although there is a certain amount of controversy regarding results, and their direct applicability to the unsubstituted polysaccharide, definite macro-molecules of fixed length appear to have been established. The mean length of the two starch dextrins, according to Haworth and Miss Plant (9), is eight and twelve glucose units respectively, while inulin (10) is calculated to consist of thirty fructofuranose residues.

The evidence, however, by no means points conclusively to the fact that all the polysaccharides exist as straight chains; there is still the possibility that certain of the more complex may be in loop form. Alternatively, there may be side-chains or branched chains. Recent work on glycogen (11) illustrates this new complexity: each macro-molecule is represented as a chain of eighteen (and alternatively of twelve) 4-α-glucopyranosido-

glucopyranose residues, joined to another chain by a link between a reducing terminal unit and a hydroxyl other than that in position four. This does not exclude the possibility of a continuous main chain of α-glucopyranose units, linked between positions one and four in a loop, and having other similar units linked as side-chains at intervals, since about the same proportion of dimethyl glucoses as of tetramethyl glucopyranose can be isolated, although this may simply be due to incomplete methylation.

It will be realised, therefore, that some of these polysaccharides are exceedingly complex, both in the methods of linkage and the diversity of their products. One of the most complex is agar-agar.

This is the name given to the polysaccharide which constitutes the stems of certain marine algae, of which the group Florideae or red algae are the most common. They grow almost exclusively in tropical waters, and have a characteristic leaf-like growth and upright thallus. When purified, agar-agar is widely used in pharmaceutical and bacteriological work.

It has been studied, generally in a physical way, since the middle of last century, and a considerable amount of work has been done on its gel-forming properties. Hardy (12) showed that the liquid phase of the system agar-water is a solution of agar in water, while the solid phase is a solution of water in agar. The

phenomenon of gel-formation is due to the attempts of the macro-molecules to go into solution, and the resistance offered by the inherent linkages. This characteristic of agar must be accounted for by any formula which attempts to portray the residue-linkage system.

The extreme complexity of agar is revealed by the wide variety of sugars which the early workers claimed to have isolated from it. In 1859, it was shown by Payen (13) to contain "gelose", or d-galactan, as its basic principle. He claimed that this galactan, or composition of anhydro-galactose units, gave agar its gel-forming property, and assigned to it the empirical formula $C_6H_{10}O_5$. Morin (14) obtained from gelose both mucic and oxalic acids by hydrolysis with dilute nitric acid. In 1882, most of the work already done on agar was repeated by Greenish (15), who claimed the separation of no less than seven carbohydrates, including arabinose and xylose. Tollens (16) stated that he had crystallised lactose and a mixture of glucoses from agar by treatment with dilute acids. The presence of pentoses was reported by Czapek (17) and Reichardt (18), fructose by Seber (19) and Matsui (20), while Furuchi (21) has reported uronic acid.

The position was made much clearer in 1929 by Lüdtke (22), who, by the study of hydrolysis products, concluded that agar contained 34 - 40% of d-galactose. Further progress resulted when Pirie (23) isolated hepta-acetyl-

dl-galactose by acetolysis; this indicated that a derivative of l-galactose was present.

An advance was made in 1933 by Percival and Sim (24), who prepared a chloroform-soluble acetate by suitable treatment. This brought agar into line with the other complex polysaccharides, and when Percival, Munro and Somerville (25) succeeded in forming methylated agar from the acetate the way was open for the application of the Haworth method of methylation and hydrolysis. This resulted in the isolation by Percival and Somerville (26) of a new trimethyl d-galactose. This was identified as 2:4:6-trimethyl-α- d-galactose, and they calculated that d-galactose comprised no less than 55 - 60% of agar. A feature, unique at the time, was that this showed that in one portion of the agar molecule the d-galactopyranose units were linked between positions one and three. This linkage has since been found in damson gum (27) and in the galactogen of the edible snail (28). More recent work still has resulted in the identification of 3:6-anhydro-l-galactose. (29, 30)

There have also been various conflicting views published regarding the sulphur content of agar. In 1929, Neuberg and Ohle (31) detected the presence of sulphuric acid when agar was hydrolysed, and claimed that the sulphate group played a part in the linkage of the molecule. Samec and Isajevic (32) considered that the

sulphuric ester in agar would bear a relation to the gel-forming property although this view was later denied by Takahashi and Shirahama (33) who suggested the following formula for gelose. $R\text{-}R_1\text{-}(O\text{-}SO_2\text{-}O)M$; M denotes metals chiefly calcium. They performed a series of experiments on the hydrolysis of agar by superheating with water in an autoclave, and they held that the two carbohydrate residues ($R\text{-}R_1$) were the cause of gel-formation. Their results showed that after various times of heating agar lost its gel-forming ability, and they considered that to be due to the fission of those fragments ($R\text{-}R_1$), not to the disruption of the sulphate linkage.

Lately, Neuberg and Schwietzer (34) have obtained a 90% yield of almost sulphur-free material by shaking agar with water: this substance can form a gel exactly as crude agar. Since neither acetylated nor methylated agar contain sulphur, its importance in the main portion of the polysaccharide appears to have been overstressed.

The agar employed in the following experiments was washed with water for several days, and is therefore the water-insoluble portion.

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PART I

THE PARTIAL HYDROLYSIS OF AGAR.

INTRODUCTION

Several workers have studied the solubility of agar in water at various temperatures and concentrations. This followed naturally from the interest shown in its gel-forming properties, which have been examined fairly thoroughly.

A number of experiments were carried out by Fellers (1), with a view to sterilising and purifying agar for bacteriological purposes. He found that small concentrations of sodium hydroxide (5%) and hydrochloric acid (2%) inhibited gel-formation, after heating for fifteen minutes at one atmosphere pressure in an autoclave. Most of the later work, however, was done on the sulphur content, which was generally held to have some relation to the gel-formation, exactly as phosphorus was formerly regarded as the cause of the insolubility of the amylodextrin portion of starch (2). This view was supported by Samec and Isajevic (3), who regarded agar as gelose sulphate, and assigned to it the formula $(C_6H_{10}O_5)_{57}$ SO_4H_2, claiming that rupture of the sulphate linkage resulted in the loss of gel-forming ability. Various attempts were made to isolate the free "agar acid" by dialysis methods, notably by Fairbrother and Mastin (4) and Hoffman and Gortner (5) who agreed in allotting to it

the formula $R \cdot O \cdot SO_2 \cdot OH$, where R was a large polysaccharide residue. Hoffman and Gortner calculated its molecular weight to be about three thousand, and stated that the gelation of agar was the gelation of a salt, not of a complex polysaccharide.

In 1931, the position was reëxamined by Takahashi and Shirahama (6), who conducted a series of experiments on the effects of high temperature and pressure on an agar-water mixture. They found that by autoclaving the mixture at 130° for varying times, they could destroy the gel-forming power, and divide the agar into two portions. Both fragments, when isolated, resembled agar in appearance, but one of them was soluble in cold water. They called the insoluble portion "hydrato-kanten-δ" and decided that it was a simple polysaccharide. It dissolved in hot water, forming a sticky precipitate when cooled; it did not reduce Fehling's solution, and on oxidation with concentrated nitric acid it gave mucic and oxalic acids. They claimed that it was one of the principal constituents of agar, and that its fission from the molecule was the cause of the loss of the gel-forming ability of the agar.

The water soluble portion (hydrato-kanten-λ) appeared to be much more complex. It contained both sulphur and calcium, and they suggested for it the formula $R_1(O \cdot SO_2 \cdot O)_2 Ca$: by electrodialysis they isolated from it a free acid,

which they called "kanten acid". They therefore allotted to gelose the formula $R \cdot R_1(O \cdot SO_2 \cdot O)M$, where M denotes metals, that is, $\delta \cdot R_1(O \cdot SO_2 \cdot O)M$, thus opposing the view of Samec and Isajevic, that the sulphate group was the essential link in the molecule.

It appeared to be desirable to attempt the isolation of these "δ" and "λ" portions, determine if they are fundamentally different in construction, and get some indication of their respective molecular weights. The possibility exists that the solubility of λ is due to the comparatively small size of its molecule, evidence of which would be found by examination of its acetylated and methylated derivatives by viscosity methods. Furthermore, by hydrolysis of the methylated derivatives of δ and λ, isolation of the scission products, and comparison of the δ and λ fractions with each other and with those from methylated agar, evidence should be obtained as to whether these polysaccharides have any inherent structural differences.

EXPERIMENTAL

Powdered agar (100g.) was washed ten times by decantation with distilled water (100l.) and filtered. The agar was placed in a covered beaker with distilled water (2l.), and the mixture heated in an autoclave for 5 hours at 130° and a pressure of 30lbs. per square inch. The autoclave had previously been calibrated by the use of substances of known m.p. in sealed tubes. (Benzoic acid, m.p. 121°, succinimide, m.p. 125°, o-tolidine, m.p. 129° all melted. Urea, m.p. 132° and malonic acid, m.p. 133° did not melt under these conditions.) On cooling, the mixture consisted of a jelly-like precipitate, a brown liquid, and a small amount (2·0g.) of residue. The gel was separated by centrifuging, and purified by redissolving three times in hot water, cooling and centrifuging. It was then dehydrated by alcohol and ether, and the powdered product (40g.) kept in a vacuum desiccator over calcium chloride.

Hydrato-kanten-δ.

This substance was a light, greyish-white powder (ash, 0·5%). It was slightly reducing to boiling Fehling's solution, and gave a positive Selwanoff test (7). (For this test, a few crystals of resorcinol are added to water (2c.c.) and concentrated hydrochloric acid (2c.c.). A

small amount of the substance (1mg.) is then added, and after heating the mixture for 30 seconds a precipitate appears. This precipitate is readily soluble in cold alcohol. Control experiments must be done concurrently.)

Hydrato-kanten-δ was soluble in hot water and formed a gel on cooling (0·03g. in 5c.c.). The iodine number by Bergmann and Machemer's method (Ber. 1930, 63, 316) was 2·47, corresponding to a molecular weight of 8100, assuming that each macro-molecule had only one reducing group. The iodine number of washed agar, by the same method, was 1·25, corresponding to an apparent molecular weight of 16,000.

Hydrato-kanten-λ.

After removal of the hydrato-kanten-δ, the brown solution was evaporated at 40°/15m.m. to 500c.c., and poured into alcohol (2l.). The white precipitate was filtered, and purified three times by dissolving in water (50c.c.), and precipitating. The substance was then dehydrated by alcohol and ether, and dried in a vacuum desiccator over calcium chloride.

The product (17g.) was a light white powder (ash 2·5%). It was reducing to Fehling's solution, and gave a positive Schuanoff test. It was soluble in cold water and did not form a gel. The iodine number was 5·89, corresponding to an apparent molecular weight of 3400.

Evaporation of the solution, after separation of Hydrato-kanten-λ.

The alcohol-water mixture was evaporated to dryness, and a crisp, brown residue (25g.) was obtained. It was reducing to cold Fehling's solution, gave a positive Schwanoff test, and the iodine number was 56·8, corresponding to an apparent molecular weight of 352. Found: $[\alpha]_D^{14^\circ}$ - 14·5° in water (c, 1·6). This iodine number, and those relating to similar substances which will be quoted later, must be accepted with reserve, owing to the formation of iodoform. This may be due to the adsorbed alcohol, (despite rigorous drying at 100°/15m.m. over phosphorous pentoxide) or to the presence of some decomposition product. It is clear that some highly reducing substances are present on account of the fact that the material reduces cold Fehling's solution, and the apparent molecular weight calculated from the iodine number may have no significance.

Acetylation of hydrato-kanten-δ

The powdered substance (45g.) and pyridine (250c.c.) were heated at 70° for 3 hours. Acetic anhydride (250c.c.) and pyridine (100c.c.) were added, and the mixture heated at 70° for 15 hours. After standing for 40 hours, the brown solution was poured down a thin stream of water into cold water (9l.). The product, when dried, was a brown powder (48g.). It was purified three times by dissolving in

chloroform (25c.c.), filtering and precipitating in light petroleum (500c.c.). The brownish powder (40g.) was then precipitated in three fractions: fraction (1) was a brown, friable glass (6g.), fraction (2) a white powder (24g.) and fraction (3) a white powder (9g.). When deacetylated by alkali, each of these fractions formed a gel with water (0·05g. in 3c.c.).

	$\%CH_3 \cdot CO$	$[\alpha]_D^{14}$ in $CHCl_3$	$\eta_{sp.}$	c.	Apparent M.W.
Acetylated Agar	36·4	-32·1° (c,1·0)	0·343	0·336	29,400
Frac.1.	34·1	-33·3°	0·125	0·427	8,400
2.	36·0	-32·5°	0·158	0·428	10,600
3.	36·5	-31·8°	0·131	0·415	9,100

The acetyl content was determined by dissolving 0·2g. in acetone (50c.c.) adding 0·1N NaOH and back titrating with 0·1N H_2SO_4 after 4 hours.

The apparent molecular weight was calculated by Staudinger's formula ($\eta_{sp.} = C \cdot M \cdot K_m$) with $K_m = 10^{-3}$ and reckoning the repeating unit as $[C_6H_7O_2(O \cdot CO \cdot CH_3)_3]$. Agar acetate (0·0336g.) was dissolved in m-cresol (10·00c.c.). The mean time of flow, T_c, at 20·0°, of m-cresol (7·00c.c.) in the viscometer was 404·6 seconds; the mean time of flow, T_s, of the solution (7·00c.c.) was 543·2 seconds.

$\therefore \quad T_s/T_c = \frac{543 \cdot 2}{404 \cdot 6} = 1 \cdot 343$

$\therefore$ specific viscosity, $\eta_{sp.} = 0 \cdot 343$

$\therefore \quad M = \frac{0 \cdot 343 \times 1000 \times 288}{3 \cdot 36} = 29,400.$

Methylation of Acetylated Hydrato-kanten-δ.

The powdered substance (25g. $CH_3 \cdot CO$, 36·0%, $[\alpha]_D^{14^\circ}$, - 32·6) was dissolved in acetone (300c.c.) at 40°, and treated with dimethyl sulphate (125c.c.) and 30% sodium hydroxide (325c.c.) in one-tenth portions every ten minutes at 55°. The temperature was finally raised to 70° for 15 minutes, and the yellow powder filtered off. The methylation was repeated three times with the same quantities of dimethyl sulphate and alkali. The product was then extracted with chloroform (2l.) and precipitated in light petroleum. It was purified twice by dissolving in chloroform, filtering, and re-precipitating. The white substance (17g.) was then precipitated in three fractions; fraction (1) was a brownish powder (3·1g.), fraction (2) a white powder (7·0g.), and fraction (3) a white powder (6·9g.).

Methylated	$\%OCH_3$	$[\alpha]_D^{14^\circ}$ in $CHCl_3$	$\eta_{sp.}$	c.	Apparent M.W.
Agar	32·1	-92° (c, 1 0)	0·266	0·349	15,500
Frac.1	32·0	-79°	0·172	0·418	8,400
2.	32·5	-80°	0·168	0·441	7,800
3.	32·1	-86°	0·152	0·418	7,400

The apparent molecular weight was calculated using Staudinger's formula, and reckoning the repeating unit as $[C_6H_7O_2(OCH_3)_3]$.

Hydrolysis of Methylated Hydrato-kanten-δ.

The powdered substance (9·5g. OCH_3, 32·1%, $[\alpha]_D^{14°}$, - 80°) and 2% methyl-alcoholic hydrogen chloride (250 c.c.) were refluxed at 80°. The substance dissolved after 4 hours. and polarimetric readings were taken at intervals; $[\alpha]_D^{14°}$, + 3·2° (4 hours); + 13·7° (10 hours); + 16·6° (14 hours); + 21·2° (18 hours); + 23·2° (20 hours); + 24·5° (22 hours, Constant value.) After neutralisation with silver carbonate and evaporation of the solvent, the yellow syrup (8·96g.) was distilled at 0·04m.m.

Fraction.	Bath Temp.	$n_D^{14°}$	%OCH_3	$[\alpha]_D^{14°}$ in $CHCl_3$	Weight.
1.	110 - 120°	solidified	51·1	+ 67° (c, 1·0)	2·87g.
2.	145 - 150°	solidified	47·3	+ 37°	2·26g.
3.	175 - 180°	1·4671	40·1	- 16°	0·81g.
4.	180 - 210°	1·4674	40·2	- 17°	0·25g.
5.	210 - 240°	1·4725	44·4	- 25°	1·43g.

Residue 1·26g.

Fraction (1) b.p. 110 - 120°. When recrystallised from light petroleum this fraction (2·81g.) had $[\alpha]_D^{14°}$ + 104° in water, (c, 1·0) and m.p. 63°. It was therefore identified as a mixture of the α- and β- forms of 2:4:6-trimethyl methylgalactoside, as isolated from agar (8) Mixed m.p. with authentic specimen, 62°.

Fraction (2) b.p. 145 - 150°. This fraction was extracted eight times with boiling petroleum (b.p. 60 - 80°), and

gave a soluble portion (A) and an insoluble portion(B). (A) was recrystallised three times from petroleum and the trimethyl methylgalactoside (m.p. 63°) removed (0·66g.) The residual syrup (0·48g.) had $[\alpha]_D^{14°}$, + 20° in chloroform (c, 0·4) and OCH_3, 28%. It was methylated twice with silver oxide and methyl iodide and distilled at 120°/ 0·03m.m. to give a clear, mobile syrup. (0·45g.) Found: $n_D^{14°}$, 1·4498; $[\alpha]_D^{14°}$, + 41 ; OCH_3, 52·5%. From this portion, a crystalline solid settled out on standing, which by rotation ($[\alpha]_D^{14°}$, + 85° in chloroform) m.p. (82°) and mixed m.p.(82°) was identified as 2:4-dimethyl-(3:6-anhydro-) β -. methyl-l- galactoside (0·10g.), the presence of which in agar was already known. (9.10). The residual syrup had $[\alpha]_D^{14°}$, + 31° in chloroform and OCH_3, 40·2%.

(B) had $[\alpha]_D^{14°}$ - 15·5° and OCH_3, 40·2%. It was methylated twice with silver oxide and methyl iodide and distilled at 120°/ 0·03m.m. to give a clear, mobile syrup (1·15g.), which had $n_D^{14°}$, 1·4488, $[\alpha]_D^{14°}$, + 20°, and OCH_3, 53·0%. A small amount (0·02g.) of 2:4-dimethyl-(3:6 anhydro-)-β-methyl-l-galactoside crystallised out on standing. The residual syrup (1·11g.) had $[\alpha]_D^{14°}$, + 5° and OCH_3, 56%

Fraction (3) b.p. 175 - 180°. This fraction was extracted in the same way as the previous fraction. From the portion soluble in petroleum, trimethyl methylgalactoside m.p. 63°

(0·11g.), 2:4 dimethyl-3:6-anhydro-β -methyl-l-galactoside m.p. 83° (0·0 6g.) and a clear, mobile syrup (0·19g.) with $[\alpha]_D^{14°}$, + 37° and OCH_3, 56·1%, were obtained. The insoluble portion (0·45g.) had $[\alpha]_D^{14°}$, - 29°, OCH_3, 40·3%, and on methylation gave a clear syrup with $n_D^{14°}$, 1·4462, $[\alpha]_D^{14°}$, + 21°, and OCH_3, 52·1%. This syrup in turn yielded 2:4-dimethyl-3:6-anhydro-β -methyl-l-galactoside (0·08g.) and a clear, mobile syrup with $[\alpha]_D^{14°}$, + 2°, and OCH_3, 56·7%. Fraction (4) b.p. 180 - 210°. This fraction was insoluble in light petroleum. On methylation it gave a clear, mobile syrup (0·23g.) with $n_D^{14°}$, 1·4500, $[\alpha]_D^{14°}$, + 27°, and OCH_3, 54%. Fraction (5) b.p. 210 - 240°. Using Staudinger's formula, and reckoning the repeating unit as $[C_6H_7O_2(OCH_3)_3]$, this fraction had η_{sp} 0·015 (c, 0·449), corresponding to an apparent molecular weight of 700.

Hydrolysis of Fraction 5.

The brown, viscous syrup (1·03g.) was refluxed at 80° with 2% methyl-alcoholic hydrogen chloride (30c.c.). Polarimetric readings were taken at intervals; $[\alpha]_D^{14°}$, - 28° (0 hours); -5° (1 hour); + 8° (3 hours); + 16° (4 hours); + 26° (5 hours); + 28° (6 hours. Constant value.) The resultant yellow syrup (0·86g.) was distilled at 0·03m.m.

Fraction.	Bath Temp.	$n_D^{14°}$	Weight.
1.	110 - 130°	1·4690	0·16g.
2.	130 - 150°	1·4690	0·15g.

Fraction.	Bath Temp.	$n_D^{14^\circ}$	Weight.
3.	150 - 180°	1·4654	0·06g.
4.	180 - 240°	1·4742	0·04g.

Residue 0·42g.

Both fraction 4 and the residue resembled the original substance (fraction5) very closely in appearance.

Fractions 1 and 2, which partly solidified on standing, were extracted with petroleum together and the trimethyl methylgalactoside m.p. 63 (0·25g.) separated. Fraction 3 was insoluble in petroleum and was methylated together with the insoluble portion from fractions 1 and 2. From the clear, mobile syrup (0·12g. $n_D^{14^\circ}$, 1·4550) 2:4-dimethyl-3:6-anhydro-β-methyl-l-galactoside m.p. 83° (0·10g.) separated out. This indicated that fraction 5 consisted of approximately two parts trimethyl methyl -d-galactoside m.p. 63° and one part anhydro methyl-galactoside m.p. 83°.

Composition of Methylated Hydrato-kanten-6.

Fraction.	Trimethyl methylgalactoside m.p.63°.	Anhydro methylgalactoside m.p. 83°.	Clear Syrup.
1.	2·81g.	-	-
2.	0·66g.	0·12g.	1·48g.
3.	0·11g.	0·14g.	0·56g.
4.	-	-	0·23g.
5. calc.	0·35g.	0·14g.	0.03g.
	3·93g.	o·40g.	2·30g.
Percentage	43·8	4·5	25·7

Calc. from 8·96g.

Reautoclaving of Hydrato-kanten-δ.

Reautoclaving for 2½ hours.

The finely powdered substance (20g.) was autoclaved for 2½ hours at 130° with water (400c.c.) The resultant thin gel was centrifuged and the solid product purified three times by dissolving in hot water and precipitating in alcohol. After dehydration by alcohol and ether, the product was a greyish-white powder (8g.). It was slightly reducing to boiling Fehling's solution and gave a positive Seliwanoff test. The iodine number was 6·10, corresponding to an apparent molecular weight of 3,300. This substance, "hydrato-kanten-δ'" formed a gel with water (0·08g. in 2c.c.)

Acetylation of Hydrato-kanten-δ'.

The powdered substance (5g.) was acetylated with pyridine (45c.c.) and acetic anhydride (35c.c.), by the same process as for the acetylation of hydrato-kanten-δ. The purified product was a white powder (3·8g.), which, on deacetylation with alkali formed a gel with water. The substance was dissolved in chloroform and precipitated in light petroleum into two fractions, both of which were white powders.

Fraction	$\%CH_3 \cdot CO$.	$[\alpha]_D^{14°}$ in $CHCl_3$.	$\eta_{s.p.}$	c.	Apparent M.W.+
1.(1·5g.)	37·8	- 35° (c, 1·0)	0·090	0·441	5·900
2.(2·1g.)	38·0	- 30°	0·087	0·412	6·100

+Calculated according to Staudinger's equation, and reckoning the repeating unit as $[C_6H_7O_2 \cdot (O \cdot CO \cdot CH_3)_3]$.

Evaporation of solution, after separation of Hydrato-kanten-δ'.

A small portion (15c.c.) of the brownish solution was poured into alcohol (100c.c.) There was no precipitate. The entire solution was then evaporated down to dryness, and a crisp, brown residue obtained. [9·2g. $[\alpha]_D^{14°}$,+ 1·0° in water (c, 1·0)] . The product was reducing to cold Fehling's solution and gave a positive Seliwanoff test. The iodine number was 35·4, corresponding to an apparent molecular weight of 565. (Iodoform was present.)

Reautoclaving for 4½ hours.

Powdered hydrato-kanten-δ (20g.) was autoclaved for 4½ hours at 130° in water (400c.c.) No solid remained after this length of time, and the brown solution gave no precipitate in alcohol. The solution was evaporated to dryness at 35°/15m.m. and a crisp, brown residue (14g.) obtained. Found: $[\alpha]_D^{14°}$, + 11·9° in water. (c, 1·0) The iodine number was 50·2, corresponding to an apparent molecular weight of 398. (Iodoform was present.)

Estimation of free galactose in the above degradation products.

A small amount of the substance (0·35g.) was dissolved in water (10c.c.) and methylphenylhydrazine (0·5g.) added in alcohol (10c.c.) After addition of 50% acetic acid (0·1c.c.), the mixture was shaken and set aside for twelve hours. The galactose methylphenylhydrazone was recrystallised from boiling ethyl alcohol, in which it was soluble

with difficulty.

Substance.		Crude product.	Recrystallised product.	
Galactose	0·03g.	0·038g.	0·036g.	m.p. 188°
	0·35g.	0·47g.	0·45g.	m.p. 188°
Residue/2½ hours.	0·35g.	0·005g.	0·005g.	m.p. 188°
Residue/4½ hours.	0·35g.	0·01g.	0·01g.	m.p. 188°
Residue/ Agar.	0·70g.	0·005g.	0·005g.	m.p. 188°

Free galactose in residue from 2½ hours period = 1·0%, and from the 4½ hours period, 2·0%.

Free galactose in Agar residue = 0·5%

Methylation of Residue from 4½ hours period.

The dry, crisp, brown residue (11·0g.) was dissolved in acetone (250c.c.), and dimethyl sulphate (55c.c.) and 30% sodium hydroxide (143c.c.) added. Since a reducing sugar was present, half the usual one-tenth portions of alkali were run in during the first three additions. The neutralised solution was extracted with chloroform (1500c.c.) and the methylation process repeated three times. After two methylations with silver oxide and methyl iodide, the product was a crisp, brown substance (7·1g.)

The methylated product (7·1g.) was dissolved in chloroform (20c.c.) and precipitated in light petroleum

(500c.c.) The precipitate, (6·5g.) when dried, was a light, crisp, yellow substance. (Fraction A.) The petroleum-chloroform mixture was evaporated to dryness, and a small amount (061g.) of a yellow syrup was obtained. (Fraction B.) Found for (B): OCH_3, 52%; $[\alpha]_D^{14^\circ}$, + 31° in chloroform; $n_D^{14^\circ}$, 1·4630.

Hydrolysis of Fraction B.

A portion of this substance (0·16g.) was hydrolysed for 3 hours with 7% sulphuric acid (10c.c.) After refluxing the final product ($[\alpha]_D^{14^\circ}$, + 24°) with alcohol (3c.c.) and aniline (0·5c.c.), a small amount (0·005g.) of a crystalline substance was obtained. By the m.p. (195°) and mixed m.p. with an authentic specimen (195°), it was identified as 2:3:4:6- tetramethylgalactose anilide. If an approximately quantitative yield of the anilide is obtained, the percentage free galactose in the original product by this method is 2%.

Hydrolysis of Fraction A.

The light, crisp, yellow substance (5·2g.) was methylated again with silver oxide and methyl iodide. Found: $[\alpha]_D^{14^\circ}$, - 8·2° in chloroform (c. 1·0), OCH_3, 35·3% $\eta_{sp.}$ 0·021, (c, 0·397) corresponding to an apparent molecular weight of 1,100 by Staudinger's equation, reckoning

the repeating unit as $[C_6H_7O_2(OCH_3)_3]$ This substance (5·0g.) was refluxed with methyl-alcoholic hydrogen chloride (200c.c.) at 80°. Polarimetric readings were taken at intervals; $[\alpha]_D^{14°}$, + 3° (1 hour); + 5° (2 hours); + 11° (4 hours); + 12° (6 hours); + 14° (7 hours. Constant value.) The resulting yellow syrup (4·63g.) was distilled at 0·03m.m.

Fraction.	Bath Temp.	$n_D^{14°}$	$\%OCH_3$	$[\alpha]_D^{14°}$ in $CHCl_3$	Yield.
1.	120 - 125°	1·4577	50·1	+ 76° (c, 0·4)	0·70g.
2.	130 - 140°	solidified	51·0	+ 74°	0·91g.
3.	140 - 180°	1·4640	41·1	- 10°	0·64g.
4.	150 - 180°	1·4700	43·2	- 17°	0·15g.
5.	200 - 240°	1·4720	43·9	- 19°	0·67g.
				Residue	1·50g.

Fraction (1) partly solidified on standing. The mobile portion (0·15g.) was decanted. Found: OCH_3, 49·1%; $[\alpha]_D^{14°}$, + 75° in chloroform (c, 0·4); $n_D^{14°}$, 1·4570. The entire fraction was therefore trimethyl methylgalactoside m.p.63°.

Fractions (2), (3) and (4) were extracted with petroleum together. The soluble portion gave, trimethyl methylgalactoside m.p.63° (0·10g.) and a colourless syrup (0·15g.), with $[\alpha]_D^{14°}$, + 35° in chloroform and OCH_3, 56·4%.

The insoluble portion gave 2:4-dimethyl-3:6-anhydro-β-methyl-l-galactoside m.p. 83° (0·22g.) and a colourless syrup (0·10g.) with $[\alpha]_D^{14°}$, + 2° in chloroform, and OCH_3, 56·1%.

Fraction (5) resembled fraction (5) from the hydrolysis of methylated hydrato-kanten-δ in appearance, boiling point, methoxyl content, rotation and refractive index.

Composition of Fraction A.

Fraction.	Trimethyl methylgalactoside. m.p.63°	Anhydro methylgalactoside. m.p. 83°	Clear syrup.
1.	0·70g.	-	-
2,3,4,	1·09g.	0·32g.	0·25g.
5. calc.	0·18g.	0·07g.	0·01g.
	1·97g.	0·39g.	0·26g.
Percentage.	42·6%	8·4%	5·6%

calc. from 4·63g.

Acetylation of Hydrato-kanten-λ.

The powdered substance (14g.) was heated with pyridine (70c.c.) at 70° for 3 hours. Acetic anhydride (70c.c.) and pyridine (30c.c.) were added, and the mixture heated at 70° for 15 hours. After standing for 40 hours,

the brown solution was poured down a thin stream of water into cold water (2 ℓ.). The product, a light, brown powder (16g.), was purified three times by dissolving in chloroform, filtering and precipitating in petroleum. It was then precipitated into three fractions; fraction (1) was a brown powder (4·6g.), fraction (2) a white powder (4·7g.), and fraction (3) also a white powder (4·4g.)

	$\%CH_3CO$	$[\alpha]_D^{14^\circ}$	in $CHCl_3$.	$\eta_{sp.}$	c.	Apparent M.W.†
Acetylated Agar.	36·4	- 32·1°	(c, 1·0)	0·343	0·336	29,400
Fraction 1.	38·7	- 45·1°		0·077	0·410	5,400
2.	40·7	- 37·1°		0·053	0·368	4,100
3.	41·0	- 36·9°		0·062	0·437	4,100

† Calculated using Staudinger's formula and reckoning the repeating unit as $[C_6H_7O_2(O\cdot CO\cdot CH_3)_3]$.

Methylation of Acetylated hydrato-kanten-λ.

The finely ground product (10g. CH_3CO, 40%, $[\alpha]_D^{14^\circ}$ - 40° in chloroform) was dissolved in acetone (200c.c.) and methylated with dimethyl sulphate (50c.c.) and 30% sodium hydroxide (130c.c.) This process was repeated three times. After extraction with chloroform and precipitation in petroleum, the purified product (4·5g.) was precipitated into three fractions. Fraction (1) was a brown, friable glass, which, when dissolved in chloroform and precipitated,

formed a brown powder (1·1g.); fraction 2 was a white powder (1·5g.) and fraction 3 also a white powder (1·5g.)

	$\%OCH_3$.	$[\alpha]_D^{14^\circ}$ in $CHCl_3$.	$\eta_{sp.}$	c.	Apparent M.W.†
Methylated Agar.	32·1	-92° (c, 1·0)	0·266	0·349	15,500
Fraction 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

† Calculated by Staudinger's formula, and reckoning the repeating unit as $[C_6H_7O_2\cdot(OCH_3)_3]$.

Hydrolysis of Methylated hydrato-kanten-λ.

The powered substance (9·0g. OCH_3, 34·1%; $[\alpha]_D^{14^\circ}$ -84° in chloroform) was refluxed at 80° with 2% methyl-alcoholic hydrogen chloride (250c.c.). Polarimetric readings were taken at intervals; $[\alpha]_D^{14^\circ}$, -7° (1½ hours); +6° (3 hours); +15° (7hours); +18° (9 hours); +20° (10 hours); +21° (11hours. Constant value.) After neutralisation and evaporation, the yellow syrup (8·15g.) was distilled at 0·03m.m.

Fraction.	Bath. Temp.	$n_D^{14^\circ}$	%OCH_3	in $CHCl_3$.	Yield.
1.	110-120°	solidified.	50·2	+70° (c,1·0)	3·31g.
2.	145-150°	1·4674	47·1	+38°	1·60g.
3.	175-180°	1·4680	40·3	-17°	0·40g.
4.	180-210°	1·4700	40·4	-17°	0·38g.
5.	210-240°	1·4770	43·2	-25°	0·55g.
				Residue	0·83g.

Fraction 1. b.p. 110-120°.

This fraction gave on recrystallisation 2:4:6-trimethyl methylgalactoside m.p.63° (3·28g.)

Fraction 2. b.p. 145-150°.

This fraction was extracted eight times with light petroleum, and gave a soluble portion (A) and an insoluble portion (B).

(A) gave finally trimethyl methylgalactoside m.p.63° (0·30g.), 2:4-dimethyl-3:6-anhydro-β-methyl-l-galactoside m.p. 83° (0·06g.), and a colourless syrup (0·30g.) with $[\alpha]_D^{14^\circ}$, +30° in chloroform, and OCH_3, 56·0%.

(B) gave finally 2:4-dimethyl-3:6-anhydro-β-methyl-l-galactoside m.p. 83° (0·08g.) and a colourless syrup (0·85g.) with $[\alpha]_D^{14^\circ}$, +3° in chloroform, and OCH_3. 57·1%.

Fraction (3) b.p. 175 - 180°. The soluble portion of this fraction consisted entirely of trimethyl methyl-galactoside m.p. 63° (0·16g.) The insoluble portion

gave finally 2:4-dimethyl-3:6-anhydro-β-methyl-l-galactoside (0·02g.) and a colourless syrup (0·13) with $[\alpha]_D^{14^\circ}$, + 4° and OCH_3, 54·7%.

Fraction (5) b.p. 210 - 240°. This fraction resembled fraction (5) from the hydrolysis of methylated hydrato-kanten-δ in appearance, boiling point, methoxyl content, rotation and refractive index.

Composition of Methylated Hydrato-kanten-λ .

Fraction.	Trimethyl methylgalactoside m.p. 63°	Anhydro methylgalactoside m.p. 83°	Clear syrup.
1.	3·28g.	-	-
2.	0·30g.	0·14g.	1·15g.
3.	0·16g.	0·02g.	0·21g.
4.	-	0·25g.	0·13g.
5.calc.	0·38g.	0·15g.	0·03g.
	4·12g.	0·56g.	1·52g.
Percentage.	50·6%	6·9%	18·6%

calc. from 8·15g.

Reautoclaving of Hydrato-kanten- λ .

The powdered product (10g.) was dissolved in water (200c.c.) and heated at 130° for 1½ hours. A portion was then precipitated in alcohol; when dried, this precipitate gave an iodine number of 5·87, corresponding to an apparent molecular weight of 3400, which was identical with the value found for the original substance. The solution was then autoclaved for a further 3½ hours, making 5 hours in all. Precipitation in alcohol (1 ℓ.) gave a white powder (7·1g.), "hydrato-kanten- λ'." The iodine number was 17·0, corresponding to an apparent molecular weight of 1,200.

Acetylation of Hydrato-kanten- λ' .

The finely ground substance, (5·2g,) was acetylated with pyridine (46c.c.) and acetic anhydride (32c.c.). The purified product (3·4g.) was precipitated in petroleum into two fractions; fraction (1) was a grey powder (0·8g.) and fraction (2) a white powder (2·5g.)

Fraction,	%CH_3CO.	$[\alpha]_D^{14^\circ}$ in $CHCl_3$	$\eta_{sp.}$	c.	Apparent M.W.†
1.	40·0	- 43°	0·073	0·417	5,000
2.	44·1	- 37°	0·037	0·433	2,500

The constants of fraction (1) are similar to those of acetylated hydrato-kanten-λ .

†Calculated by Staudinger's formula, and reckoning the repeating unit as $[C_6H_7O_2 \cdot (O \cdot CO \cdot CH_3)_3]$.

Evaporation of the solution, after precipitation of Hydrato-kanten-λ' .

The alcohol-water mixture was evaporated down to dryness, and a crisp, brown residue (2·0g.) obtained, with $[\alpha]_D^{14^\circ}$, - 12·2° in water (c, 1·0). The iodine number was 62·9, corresponding to an apparent molecular weight of 318. (Iodoform was present.)

No galactose methylphenylhydrazone could be prepared from this residue.

Determination of Molecular Weights by the Rast Method.

A small amount of the substance (0·01g.) was weighed into a small tube with freshly sublimed camphor (0·10g.) The tube was sealed off, immersed completely in an oil bath at 180°, and the temperature at which the first crystal appeared was noted.

The m.p. of camphor, under these conditions, was 172·2°.

	m.p.	Depression.	Apparent M.W.
Acetylated Hydrato-kanten-δ	169·1°	3·1°	1,352
Acetylated Hydrato-kanten-λ	169·6°	2·6°	1,202
Acetylated Agar.	170·7°	1·5°	2.011
Methylated Hydrato-kanten-δ	169·5°	2·7°	1,309
Methylated Hydrato-kanten-λ	170·7°	1·5°	1,352
Methylated Agar.	Insoluble in camphor.		

The substances are apparently degraded at the high temperature.

Table of Apparent Molecular Weights.

M_v denotes the apparent molecular weight of the substituted derivatives from viscosity measurements. The values of M_v quoted for acetylated and methylated hydrato-kanten-δ, acetylated and methylated hydrato-kanten-λ, and acetylated hydrato-kanten-δ', are the mean values of their various fractions; for acetylated hydrato-kanten-λ' the value of fraction (2) is quoted.

M_o denotes the apparent molecular weight of the unsubstituted polysaccharide calculated from the M_v values, the repeating unit being reckoned as $(C_6H_{10}O_5)$.

M_i denotes the apparent molecular weight of the polysaccharide, calculated from the iodine number.

	M_v.	M_o.	M_i.
Acetylated Agar.	29,500	16,600	16,000
Acetylated hydrato-kanten-δ.	9,400	7,800	8,100
Acetylated hydrato-kanten-λ.	4,500	2,500	3,400
Acetylated hydrato-kanten-δ'.	6,000	3,500	3,300
Acetylated hydrato-kanten-λ'.	2,500	1,400	1,200
Methylated Agar.	15,500	12,300	16,000
Methylated hydrato-kanten-δ.	7,900	6,300	8,100
Methylated hydrato-kanten-λ.	4,500	3,600	3,400

DISCUSSION.

The work already done on the partial hydrolysis of agar, by Takahashi and Shirahama, (6) indicates that the agar molecule can be broken down into two distinct portions. The portion insoluble in cold water, hydrato-kanten-δ resembles agar in its physical properties and the Japanese workers concluded, as a result of their investigations, that the former is a simple polysaccharide. As proof of this, they produced evidence that it had a small ash content (0·5%), it could be hydrolysed to yield reducing sugars, and on oxidation with nitric acid gave mucic and oxalic acids. On the other hand, they found that hydrato-kanten-λ, the water soluble portion, contained sulphur (SO_4, 3·6%) and calcium (2%), so they claimed that this fragment contained a sulphate linkage.

Since the agar employed here in the preparation of hydrato-kantan-δ and λ had been thoroughly washed, it follows from the work of Neuberg and Schwietzer (11) that it is almost entirely free from sulphur. The two degradation products, therefore, can contain exceedingly little sulphur, a fact which appears to oppose the view of Takahashi and Shirahama, namely, that hydrato-kanten-λ is

not a simple polysaccharide like hydrato-kanten-δ, but contains a sulphuric ester linkage. The ash content of the hydrato-kanten-δ isolated here is very small (0·5%), while that of the hydrato-kanten-λ (2·7%) is much lower than the value found by Takahashi and Shirahama (7·8%)

The hydrato-kanten-λ fragment must be some essential part of the agar molecule, and not just a degradation product of the insoluble hydrato-kanten-δ, since further hydrolysis of this insoluble product does not produce more hydrato-kanten-λ. Assuming that each molecule had only one reducing group, the iodine number of the latter indicates an apparent molecular weight of approximately 3,400, or about 21 anhydro-galactose units, if the entire molecule is regarded as being composed of these residues. This is known not to be the case, but it is a convenient basis for calculation. This value (3,400) is less than half that found for hydrato-kanten-δ (8,100, or about 50 anhydro-galactose units) Agar, itself, by this method, gives an apparent molecular weight of 16,000, or about 100 anhydro-galactose units. While these molecular weights may not have any absolute value, comparison of the results shows that the hydrato-kanten-δ molecule is at least twice the size of the hydrato-kanten-λ molecule, and about half the size of the agar molecule.

The phenomenon of gel formation, which may be observed in solutions of agar and hydrato-kanten-δ, may be due to some system of cross linkages in the macromolecules. If such a complicated system exists, some of the linkages in agar must be ruptured by hydrolysis at 130°, and it may be expected that the component parts of the two fractions isolated will not be strictly identical.

Evidence of small differences in these fragments is obtained by the study of the products obtained by acetylation and methylation. When acetylated or methylated hydrato-kanten-δ is precipitated in three fractions, it is found that these fractions are a little different from each other in specific rotation and acetyl or methoxyl content, although they bear a close resemblance to the values found for the similar agar derivatives. Viscosity measurements, however, carried out by Staudinger's method, using the formula $\eta_{sp} = c.K_m M$, with $K_m = 10^{-3}$, indicate that the apparent molecular weight of agar acetate (29,400) is about three times that of acetylated hydrato-kanten-δ (mean value of fractions 9,400). Reckoning the repeating unit in the acetates as $[C_6H_7O_2 \cdot (O \cdot CO \cdot CH_3)_3]$, the values for the unsubstituted polysaccharides are 16,600 and 7,800 respectively.

The acetylated hydrato-kanten-λ fractions have higher rotations and greater acetyl contents than agar acetate; the apparent molecular weight from viscosity measurements is 4,500 (mean value), which gives a value of 2,500 for the unsubstituted ploysaccharide. This is somewhat lower than the value from the iodine number estimation (3,400), but the methylated derivative has an apparent molecular weight of 4,500, which corresponds to 3,600 for the unsubstituted polysaccharide, reckoning the repeating methylated unit as $[C_6H_7O_2(OCH_3)_3]$.

An attempt to determine the molecular weights of these acetylated and methylated compounds by Rast's method was unsuccessful. With the exception of methylated agar, the substances dissolved in camphor at 180°, but the results indicate that the macro-molecules are degraded at the high temperature.

If either of these fragments in turn are autoclaved for a time, the molecule is still further degraded. Hydrolysis of hydrato-kanten-δ for 2½ hours at 130° produces a substance, hydrato-kanten-δ', which has an apparent molecular weight of 3,300 by the iodine number method. Acetylation and precipitation into two fractions gives products which have greater acetyl contents than acetylated hydrato-kanten-δ ; this indicates that further linkages are ruptured, and

the molecular size decreased. Viscosity measurements show an apparent molecular weight of 6,000 for the acetate (mean value), from which the unsubstituted compound can be reckoned as 3,500; this is very similar to the value found for hydrato-kanten-λ, but this fragment is essentially different in that it is insoluble in cold water; moreover, it forms a gel when the solution in hot water is cooled. It therefore appears that although the molecule may be approximately of the same size as the hydrato-kanten-λ molecule, the linkage phenomenon which prevents solution still persists, as it does in normal hydrato-kanten-δ. Autoclaving hydrato-kanten-δ for 4½ hours at 130°, on the other hand, degrades it still more extensively, as no water insoluble portion remains; no hydrato-kanten-λ, however, is formed.

The hydrato-kanten-λ fragment appears to be more resistant to further autoclaving. A period of 2½ hours at 130° has no effect, but after 5 hours a part of the substance is degraded. This new product, hydrato-kanten-λ', has an iodine number of 17·0, which corresponds to an apparent molecular weight of 1,200. Fraction (1) of the acetylated product is very similar to the normal acetylated hydrato-kanten-λ fractions in rotation, acetyl content and apparent molecular weight,

but fraction (2) has a high acetyl content (44%) and an apparent molecular weight of 2,500, which corresponds to 1,400 for the unsubstituted polysaccharide. It therefore appears that the hydrato-kanten-λ molecule is more resistant to rupture than the hydrato-kanten-δ molecule, since it is not completely degraded by hydrolysis for five hours at 130°. It would seem that they are differently constructed, and the latter product apparently contains the type of linkage concerned in gel formation, which appears to be fairly easily ruptured.

No mention is made by Takahashi and Shirahama regarding the substance which may be obtained by evaporation of the alcohol-water mixture, after filtration of the hydrato-kanten-λ, although the yield is about 25% of the original material. This crisp, brown substance reduces cold Fehling's solution, and has an iodine number of 56·8, corresponding to an apparent molecular weight of 352. Iodoform is produced during the estimation, so that the apparent molecular weight had probably no significance; moreover, since the substance is reducing to cold Fehling's solution, there may be more than one reducing group per molecule. Very few substances, apart from ascorbic acid, are known which reduce cold Fehling's solution; gluco-reductone(14)

has this property, so that similar highly reducing substances may be present in this residue. A small amount of galactose methylphenylhydrazone can be prepared from this residue(galactose 0·5%), so that galactose residues must be in a position to be split off from the macro-molecule.

Similar substances are obtained by evaporation of the solutions employed in the autoclaving of hydrato-kanten-δ ; the residue after the $2\frac{1}{2}$ hour period had an apparent molecular weight of 565 and contained free galactose (1%), whereas the residue after the $4\frac{1}{2}$ hour period had an apparent molecular weight of 398 and free galactose(2%). In both cases iodoform was produced during the estimation of reducing power. In the same way, the residue obtained after the autoclaving of hydrato-kanten-λ had an apparent molecular weight of 318 (iodoform being present), but no galactose methyl-phenylhydrazone could be prepared in this case. All these residues reduce cold Fehling's solution.

It is already known that agar contains both d- and l-galactose, and it was desirable, therefore, to attempt the isolation of each, in the form of their derivatives, from hydrato-kanten-δ and λ, and thus determine if there is any essential difference in their

chemical compositions. This was attempted by the hydrolysis of the methylated derivatives.

The only definitely identified products isolated by the hydrolysis of methylated agar are a mixture of the α and β- forms of 2:4:6-trimethyl methyl-d-galactoside (8), and on further methylation, 2:4-dimethyl-3:6-anhydro β-methyl-l-galactoside(9)(10). In the case of hydrato-kanten-δ and λ, the methylated derivatives were heated at 80° under reflux with 2% methyl-alcoholic hydrogen chloride. The former product was more resistant to hydrolysis, since it took 22 hours for the specific rotation to reach a constant value ($[\alpha]_D^{14°}$, +24·5°), while the latter took 11 hours ($[\alpha]_D^{14°}$, +21°); this may, however be accounted for by the difference in solubility, since methylated hydrato-kanten-δ took 4½ hours to dissolve. After neutralisation, extraction and evaporation, the resulting syrups were fractionally distilled, and the scission products examined.

It is found that 2:4-dimethyl-3:6-anhydro-β-methyl-l-galactoside and the mixture of α- and β-forms of 2:4:6-trimethyl methyl-d-galactoside already known to be present in methylated agar, can be obtained from both the hydrato-kanten-δ and -λ derivatives. It is difficult to estimate the amounts exactly, and the results must be accepted with

reserve, since a laborious extraction process is required, and the third product, a clear, mobile syrup (S) of varying composition, has been shown to contain some of the anhydro-galactose derivative(12). Another factor is the presence of a large amount of high boiling point material; an equilibrium appears to be attained during the hydrolysis, after which no further hydrolysis occurs. Four fractions below 210° (total 6·31g. 68%) can be distilled after the hydrolysis of hydrato-kanten-δ, then a fifth fraction b.p.210-240° is taken (1·43g. 16%) The residue, however, (1·26g. 14%) is very similar in appearance to fraction 5, and it is doubtful if its chemical composition is very different.

This fraction 5 may be hydrolysed further by treatment 2% methyl-alcoholic hydrogen chloride, and small yields of 2:4:6-trimethyl methylgalactoside (29%), 2:4-dimethyl-3:6-anhydro-β-methyl-l-galactoside(11·5%) and the clear, mobile syrup (S) (2·3%) are finally obtained. The residue (49%), again, is very similar in appearance to fraction 5, and it is probable that its chemical composition is very similar.

From the amounts of the various substances actually isolated, the product from methylated hydrato-kanten-δ is found to contain 2:4:6-trimethyl methyl-d-galactoside

43·8% and on further methylation 2:4-dimethyl-3:6-anhydro-β-methyl-l-galactoside 4·5% and S 25·7%. The products obtained after a similar treatment of methylated hydrato-kanten-λ give somewhat similar values, namely, 2:4:6-trimethyl methylgalactoside 50·6%, 2:4-dimethyl-3:6-anhydro-β-methyl-l-galactoside 6·9% and S 18·6%. Moreover, since S has been estimated to contain about 15% of the anhydro-l-galactose derivative (12), the quantities of this compound from the methylated derivatives may be calculated to be 8·3% and 9·7% respectively.

Furthermore, if the whole of fraction 5 and the residue from the hydrolysis of hydrato-kanten-δ is calculated as trimethyl methylgalactoside, dimethyl-3:6-anhydro-methyl-l-galactoside and S in the proportions found by the hydrolysis of fraction5, values are obtained which are probably nearer to the true composition. Methylated hydrato-kanten-δ thus yields on hydrolysis trimethyl methylgalactoside 60·3%, and on further methylation 2:4-dimethyl-3:6-anhydro-β-methyl-l-galactoside 11·1% and S 27·0%; the proportions of the products obtained after a similar treatment of hydrato-kanten-λ are fairly similar, namely, 65·5%, 12·9% and 19·8% respectively. If the estimated amount (15%) of the anhydro-l-galactose derivative in S is added, the values for this substance

become 15·2% and 15·9% respectively.

It will be observed that the amount of the anhydro-l-galactose derivative in the two fragments is very similar; on the other hand, hydrato-kanten-λ has a larger amount (5%) of the d-galactose derivative, and a smaller amount of S, - the composition of which is so far unknown.

No tetramethyl galactose could be isolated from either of these hydrolyses. It appears, therefore, that these hydrato-kanten-δ and -λ products either have no end-group, or the end-group is not a galactopyranose residue. The examination of a more degraded product was therefore undertaken; the product actually selected was the residue obtained after autoclaving hydrato-kanten-δ for 4½ hours.

This crisp, brown residue was methylated and the monosaccharide portion (2%) was removed by precipitation in petroleum. The methylated product (fraction A), which by viscosity measurement had an apparent molecular weight of 1,100 (cf. iodine number method, 398 for the unmethylated product), was hydrolysed with 2% methyl-alcoholic hydrogen chloride, and the fractions examined as before. The composition of the product was found to be trimethyl methylgalactoside 42·6%, and on further methylation, 2:4-dimethyl-3:6-anhydro-β-methyl-l-

galactoside 8·4% and S 5·6%. Calculating for complete hydrolysis of fraction 5 and the residue as before, the values become trimethyl methylgalactoside 70·2%, S 8·0% and the anhydro-l-galactose derivative 19·6%, or 20·8% by reckoning the amount (15%) in solution in S. The maximum amounts of these substances derived from the methylated products can be seen in the table.

	Methylated Agar. (12)	Methylated Hydrato-kanten-β	Methylated Hydrato-kanten-λ	A.
Trimethyl methylgalactoside.	62·1	60·3	65·5	70·2%
Dimethyl anhydro-l-galactoside.	11·6	15·1	15·9	20·8%
S.	26·0	23·0	16·8	7·0%

No tetramethyl galactose was found in this fraction A, although if the chains are very long, the amount of the end product formed would necessarily be small, and since only a fairly small amount of fraction A was available, the end product may have escaped detection. The apparent molecular weight, however, (1,100, or 5 - 6 methylated anhydro-galactose units) appears to indicate that the molecular size is comparatively small, so that some form of ring combination may be present even in

this more highly degraded product.

During the investigations on the hydrolytic products of methylated agar by Percival and Somerville (8), and later Hands and Peat (9), no trace of tetramethyl galactose was detected, and it would appear that if agar exists in straight chains, the end-groups are not normal galactopyranose residues. It may be that the hydratokanten-δ and -λ fragments exist in the molecule as chains which are cross-linked in such a way as to close the ends of the chains entirely, and to cause the phenomenon of gel formation. On the other hand, no recognisable end product has been isolated from either of these fragments, and it appears that they, also, do not exist in terminated chains.

It is possible from geometrical considerations for six galactopyranose residues, united by β-linkages between positions one and three, to form a closed ring (8), and it may be that such a ring exists in these molecules. Since fraction A, by viscosity measurement, has an apparent molecular weight of 1,100, which is approximately six methylated anhydrogalactose residues, there is a possibility that this loop is present in the structure of the agar molecule. There may be, also, rings containing 3:6-anhydro-l-

galactose residues as well as d-galactose residues, and since from Part II there is evidence that a galactopyranose residue may be linked to a 3:6-anhydro-l-galactose residue, these anhydro-l-galactose residues may be important links between the d-galactose units. They are clearly fundamental building stones in the structure, since 2:4-dimethyl-3:6-anhydro-β-methyl-l-galactoside has been found in all the fragments examined.

Another consideration may be advanced regarding the linkage of the l-galactose residue. It may be linked in positions one, three and six to three d-galactose units, and on hydrolysis, the 3:6-anhydro ring may be formed by union of the third and sixth carbon atoms. From this reasoning, it is also possible to postulate 1:3- and 1:6-anhydro rings; this latter ring, however, has been shown by McCreath and Smith (13) to be opened by treatment with acid. However, it is much more probable that the 3:6-anhydro ring exists as such in the agar molecule, on account of the low methoxyl and acetyl contents of the derivatives.

The possibility exists that the agar molecule may be composed of a large number of fairly small rings, which are interlinked in a complicated manner, and on

heating at 130° a characteristic fragment is split off. This latter fragment, hydrato-kanten-λ (about one fifth of the whole molecule) does not have the type of linkage concerned in gel formation, and on autoclaving for a further period the most degraded product which can be precipitated from water has an apparent molecular weight of 1300 (mean value), which is approaching the basic size of the postulated rings. This system of rings may also contain short side chains at intervals, which could account for the small amounts of free galactose which are split off, or the system may contain some substance which is at present unknown.

Thereis, however, also the possibibity of a combination of the above constructions. The hydrato-kanteh products may consist of long chains terminated by loops, which would account for the lack of an end-group, or loops may be spaced along the length of a terminated, and perhaps branched, chain.

If branched chains are present it should be possible to isolate methylated monosaccharide residues containing less than the normal number of methyl groups, e.g. dimethyl galactoses (15). Although this has not been done, it is quite likely that substances of this type are present in the higher boiling fractions. On

account of the subsequent methylations required to isolate the 2:4-dimethyl-3:6-anhydro-β-methyl-l-galactoside no direct evidence for this view is available, but the mobile syrup S will contain these substances in the fully methylated state.

Many tentative structures can be advanced to account for the physical architecture of the agar molecule by considering the properties of the above compounds, and it is apparent that the polysaccharide is exceedingly complex. Further study will be required before any approach to a more detailed structure of the agar molecule can be attempted.

SUMMARY.

1. Washed agar can be partially hydrolysed by heating with water at 130°, as shown by Takahashi and Shirahama.

2. One of the products, hydrato-kanten-λ, is soluble in cold water, while the other, hydrato-kanten-δ, has properties very similar to those of agar. Further autoclaving of hydrato-kanten-δ does not produce hydrato-kanten-λ, so that these two substances are distinctive fragments of the agar molecule.

3. Other degradation products are also obtained by evaporation of the solutions employed. These products reduce cold Fehling's solution and therefore contain some exceedingly reactive substances.

4. The agar molecule is found to be about twice the size of the hydrato-kanten-δ molecule which in turn is about twice the size of the hydrato-kanten-λ molecule. The apparent molecular weights by the iodine number method agree closely with those calculated from viscosity measurements.

5. Further autoclaving of hydrato-kanten-δ for $2\frac{1}{2}$ hours results in the formation of a more degraded, water insoluble product, with approximately the same apparent molecular weight as hydrato-kanten-λ. Autoclaving for $4\frac{1}{2}$ hours degrades it completely to the crisp, active residue.

6. Hydrato-kanten-λ is more resistant to further hydrolysis by autoclaving, but after heating for 5 hours at 130° a water soluble substance can be isolated, with an apparent molecular weight of 1,300.

7. Although the hydrato-kanten products differ in physical properties, their chemical compositions are fairly similar, and resemble that of agar. Hydrolysis of the methylated derivatives gives in both cases 2:4:6-trimethyl methyl-d-galactoside, and on further methylation, 2:4-dimethyl-3:6-anhydro-β-methyl-l-galactoside and a clear mobile syrup. It is clear that they are both complex polysaccharides.

8. No tetramethyl galactose was isolated by the hydrolysis of the methylated hydrato-kanten products or of a more degraded product. Therefore, either the end-group is not a galactopyranose residue, or these substances do not exist in terminated chains.

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PART II

THE DEGRADATION OF METHYLATED AGAR BY CONTACT WITH ACETYL BROMIDE.

INTRODUCTION

It is well known that degradation of the complex polysaccharides by chemical means does not give rise to products of equal chain length. These scission products vary in length from the familiar monosaccharides and disaccharides, to the much more complex dextrins which may be almost equal in chain length to the original polysaccharide. In 1932, Haworth and Machemer (1) brought forward evidence that cellulose breaks down progressively to cellodextrins which have chain lengths determinable as $(C_6)_{25}$, $(C_6)_{24}$--- $(C_6)_{10}$. Starch also gives rise to a variety of dextrins. Haworth and Miss Plant (2) have examined starch dextrins with chain lengths of eight and twelve units respectively, and also an α- amylodextrin which is two thirds of the length of the starch molecule. The latest work on glycogen (3,4) indicates that it may contain chains of eighteen and twelve units respectively, depending on its origin, while inulin (5) is known definitely to consist of about thirty fructofuranose residues. These chain lengths were determined by Haworth's method of gravimetric assay of the terminal units.

It is also of interest to examine the positions of the linkages between the monosaccharide units, since the

residues may be joined to each other in either α- or β- forms. For stereochemical reasons, the method of linkage determines whether the macro-molecule shall exist in long, straight threads, or whether this is spatially impossible. The simplest linked compounds, the disaccharides, have been studied in this connection. In 1926, maltose (6), a scission product of starch and glycogen, was proved to be 4-α-glucosido-glucose, that is, two glucopyranose units joined by an α-linkage. Similarly, cellobiose (7), the disaccharide from cellulose, was found to be β-glucosido-glucose; thus chemically similar to, and stereoisomeric with, maltose. By the study of models, it is obvious that cellulose molecules may form straight chains, while the starch molecules cannot, and because of the α- linkages take up a zig-zag form.

From these conceptions, it may be said that starch, cellulose and glycogen are analogues of the simplest glucosides. It was necessary to prove, however, that the disaccharides prëexisted in the macro-molecules, and were not formed by secondary reactions during hydrolysis. For this purpose, it was desirable to protect the remaining hydroxyl groups in the polysaccharide by some process such as acetylation or methylation.

The presence of maltose in the starch molecule, apart from its hydrolysis by the use of enzymes, was indicated

in 1921 by Karrer and Nägeli (8), who isolated hepta-acetymaltosidobromide from starch by the action of acetyl bromide. This view was proved in 1931 by Haworth and Percival (9), who succeeded in degrading trimethyl amylose at room temperature with acetyl bromide. One of the products of degradation was a disaccharide, which on oxidation and hydrolysis gave tetramethylglucopyranose and 2:3:5:6-tetramethyl-γ-gluconolactone. The disaccharide was therefore identified as octamethyl methylmaltobionate. A similar series of experiments proved that maltose was also preformed in glycogen.

The object of the experiments carried out here was to attempt the isolation of a disaccharide by the degradation of methylated agar by acetyl bromide. It is known from the work of Percival and Somerville (10) that agar contains a linkage in position three of the galactose residue, so that it ought to be possible to isolate a disaccharide or trisaccharide containing this linkage. Evidence would therefore be forthcoming as to whether the galactose residues are linked as a series of units, or whether a disaccharide, composed of galactose residues, linked in positions one and three, preëxists in the molecule, or whether the galactose residues are linked together by some intervening unit.

EXPERIMENTAL.

Preliminary Experiment I.

The methylated agar (5g.) employed in this trial experiment was prepared by the method of Percival and Somerville (9). The reaction was carried out on two portions of methylated agar (3g. in chloroform 45c.c. and 2g. in chloroform 30c.c.) and acetyl bromide (9c.c. in chloroform 10c.c. and 6c.c. in chloroform 8c.c.), in small flasks fitted with calcium chloride tubes. After standing for 12 hours with occasional shaking, the contents of each flask were poured into ice water (200c.c.) and chloroform (15c.c.); the chloroform layer was washed with dilute sodium bicarbonate solution and ice water until free from acid. The chloroform layer was dried over anhydrous sodium sulphate, and on evaporation to dryness at 30°/15m.m. gave a yellow syrup (4.5g.) which was reducing to Fehling's solution and contained bromide ions. This product was refluxed for 12 hours at 78° with dry methyl alcohol (75c.c.) and silver carbonate (10g.), and yielded a light yellow syrup (3·2g.) which was free from bromide ions.

Methylation.

This substance (3·2g.) was dissolved in acetone (50c,c.) and methylated at 35°, and later at 65°, with dimethyl sulphate (30c.c.) and 30% sodium hydroxide (70c.c.), which were added in one tenth portions at ten minute intervals. The mixture was cooled to 0°, made slightly acid with dilute sulphuric acid and extracted with chloroform (1 l.). This methylation process was repeated; the chloroform extract (2·0g.) was methylated twice with silver oxide (20g.) and methyl iodide (50c.c.), and gave a colourless, mobile syrup (1·8g. OCH_3, 49%) which was fractionally distilled at 0·03m.m.

Fraction.	Bath Temp.	$n_D^{14°}$	$\%OCH_3$	$[\alpha]_D^{14°}$ in $CHCl_3$	Yield.
1.	130 - 140°	1·4509	60	+ 23° (c, 0·4)	0·61g.
2.	160 - 170°	1·4606	58	+ 22°	0·10g.
3.	190 - 200°	1·4654	46	+ 3°	0·07g.
4.	220 - 230°	1·4760	40	± 0°	0·21g.

Residue 0·8g. OCH_3, 38%.

Fraction 1. b.p. 130 - 140°.

A small amount (0·03g.) of a crystalline substance was obtained from this fraction. By the m.p. (46°) and rotation ($[\alpha]_D^{14°}$ + 19° in water,) it appeared to be 2:3:4:6-tetramethyl-β-methylgalactoside (m.p. 45°, $[\alpha]_D^{14°}$ +20° in water.)

Hydrolysis of fraction 1.

The remainder of the fraction (0·45g.) was hydrolysed with 7% hydrochloric acid. Polarimetric readings were taken at intervals; $[\alpha]_D^{14°}$, + 28° (0 mins.), + 36° (30 mins.), + 40° (45 mins.), +44° (60 mins.), + 47° (75 mins.), + 50° (90mins.), + 53° (115 mins.), +54° (120 mins. Constant value.)

Anilide Formation.

The neutralised product, on boiling under reflux with alcohol (5c.c.) and aniline (1c.c.) for 2 hours at 90°, gave a crystalline anilide (0·4g.). By the m.p. (196°) and mixed m.p. with an authentic specimen (196°), it was identified as 2:3:4:6-tetramethyl galactose anilide.

Found: C, 61·7; H, 8·0; OCH_3, 38·8; N, 4·6%.

Calc. for $C_{16}H_{25}O_5N$. C, 61·8; H, 8·0; OCH_3, 39·9; N, 4·5%

The small amount of high boiling fraction was probably due to an excessive time of contact with the acetyl bromide.

Preliminary Experiment 2.

Methylated agar (6g.) and acetyl bromide (18c.c.) were allowed to react at 15° for 5 hours 15 mins. and worked up in the same manner as above. The final product, (3·4g. OCH_3, 46%), was fractionally distilled at 0·03m.m.

Fraction.	Bath Temp.	$n_D^{14°}$	$[\alpha]_D^{14°}$	in $CHCl_3$	%OCH_3	Yield
1.	130 - 140°	1·4541	+ 31°	(c, 0·4)	61	0·40g.
2.	170 - 175°	1·4553	+ 26°		58	0·30g.
3.	190 - 220°	1·4656	+ 2°		54	0·16g.

Residue 2·4g. $[\alpha]_D^{14°}$, ± 0°; OCH_3, 44%.

The large amount of residue indicates that the time of reaction was too short.

Experiment 3.

The reaction was carried out on four portions of methylated agar (4g. in chloroform 40 c.c.) and acetyl bromide (12 c.c. in chloroform 15c.c.), that is, 16g. of methylated agar in all. After standing for 8 hours the product was poured into ice water, and on neutralisation and extraction with chloroform as before, gave a thick, yellow syrup (18g.) which was reducing to Fehling's solution and contained bromide ions.

This product was dissolved in ether (500c.c.) and shaken with silver carbonate (25g.) and water (10c.c.) for 50 hours, until the solution was free from bromide ions. The silver residues were extracted with boiling ether, and the combined ethereal extracts gave a brownish syrup (13g.).

Oxidation with bromine.

The syrup was dissolved in ice water (500c.c.) (insoluble portion 3g.) and barium benzoate (16g.) and bromine (2c.c.) added, and the mixture kept in the dark. After standing for 3 days, and adding more bromine (2c.c.) the solution was non-reducing. Sodium sulphate (9g.) was added to precipitate the barium, and the bromine removed by aeration. The solution was made slightly

alkaline with sodium carbonate and concentrated to 150c.c. at 45°/15m.m. Acidification of the cold solution precipitated more benzoic acid, the last traces of which were removed by extraction with ether. The filtrate was again made alkaline and concentrated to 70c.c.

Methylation.

Acetone (200c.c.) was added to the mixture, which was methylated at 35°, and later at 65°, with dimethyl sulphate (30c.c.) and 30% sodium hydroxide (70c.c.) in one tenth portions at ten minute intervals. The methylated mixture was cooled to 0°, acidified with ice cold dilute sulphuric acid, and extracted with chloroform (1500c.c.). The aqueous layer was made alkaline with sodium carbonate and evaporated to dryness at 45°/15m.m., and the residue extracted with boiling alcohol(1500c.c.). The mixture of sodium salts obtained from this extract, together with the extract from the chloroform layer, were methylated with dimethyl sulphate (40c.c.) and 30% sodium hydroxide (107c.c.) as before. The chloroform extract gave a yellow, viscous syrup (5g.), which was methylated twice with methyl iodide (45c.c.) and silver oxide (30g.). The resultant brownish, mobile syrup (4·6g.) was fractionally distilled at 0·03m.m.

Fraction.	Bath Temp.	$n_D^{14^\circ}$	%OCH_3	$[\alpha]_D^{14^\circ}$ in $CHCl_3$	Yield.
1.	100-150°	1·4490	55	-12° (c, 0·4)	0·9g.
2.	150-180°	1·4512	54	-12°	0·2g.
3.	180-220°	1·4697	46	-21°	2·5g.
				Residue	0·8g.

Fraction 3 gave a positive Seliwanoff test, indicating the presence of a ketose or an anhydro aldose residue. It was remethylated and distilled at 210-220°/0·03m.m. $n_D^{14^\circ}$ 1·4701.

Found: C, 52·4; H, 7·9; OCH_3, 48·9; $COOCH_3$, 13·9%.

Calc. for $C_{19}H_{34}O_{11}$; C, 52·1; H, 7·8; OCH_3, 49·8; $COOCH_3$, 13·5%.

Hydrolysis of fraction 3.

A portion of this fraction (0·9g.) was heated at 100° with 5% sulphuric acid (20c.c.), polarimetric readings being taken at intervals: $[\alpha]_D^{14^\circ}$ -20° (0 mins.), -11° (60 mins.) -5° (120 mins.), +1° (180 mins.), +6° (120 mins.), +11° (300 mins.), +14° (360 mins.), +15° (420 mins.), +18° (460 mins. Constant value.)

Isolation of tetramethyl galactose.

The solution was neutralised at 50° with barium carbonate, evaporated to dryness at 40°/15m.m., and dried with alcohol and benzene. The white, solid residue was extracted with ether (500c.c.), which on evaporation gave a yellow, mobile

syrup (0·4g.). Yield, 82% of theoretical.

Anilide formation.

The syrup was heated under reflux at 90° for 2 hours with alcohol (5c.c.) and aniline (1c.c.); on cooling a crystalline substance (0·2g.) was deposited. By the m.p. (196°), and mixed m.p. (196°) with an authentic specimen, it was identified as 2:3:4:6-tetramethyl galactose anilide. The mother liquor was evaporated to dryness and a further amount of the substance obtained, (Total yield 0·25g.) No trimethyl galactose was obtained, as the anilide.

Attempted Isolation of Acid portion.

The dried barium salts remaining after the ether extraction were dissolved in water (50c.c.) and acidified with dilute sulphuric acid. The solution was then adjusted with barium hydroxide to contain a very slight excess of barium and evaporated to dryness. On extraction with boiling ether (800c.c.) no syrup was obtained. The residue was optically inactive.

Experiment 4.

The above experiment was repeated, methylated agar (44g.) (in eleven portions of 4g.) and acetyl bromide (132c.c.) (in eleven portions of 12c.c.) being allowed to react for 7½ hours. The final product (6g.) after the oxidation and methylation processes as described above was fractionally distilled at 0·03m.m.

Fraction.	Bath Temp.	$n_D^{14^\circ}$	$\%OCH_3$	$[\alpha]_D^{14^\circ}$ in $CHCl_3$	Yield.
1.	105-125°	1·4506	53·2	-	2·19g.
2.	125-140°	1·4498	58·0	-8°(c, 0·4)	1·64g.
3.	145-170°	1·4556	-	-	0·21g.
4.	185-220°	1·4734	47·5	-4°	1·10g.
				Residue	0·60g.

Pentamethyl methyl-d-galactonate.

Fraction 2. b.p. 125-140°.

After standing for several weeks in a refrigerator, crystals were observed in this fraction. They were separated on a porous tile, recrystallised from light petroleum b.p. 60 - 80° (0·9g). The m.p. was 46°, $[\alpha]_D^{14^\circ}$ in water (c, 0·4), +20°

Found: C, 51·64; H, 8·66; OCH_3, 64·3; $COOCH_3$, 19·6%.

Calc. for $C_{12}H_{24}O_7$: C, 51·42; H, 8·58; OCH_3, 66·4; $COOCH_3$, 21%.

Hydrolysis of fraction 4.

Found for fraction 4: C, 52·5; H, 8·0; OCH_3, 47·5; $COOCH_3$, 12·5%. These figures show it to be very similar to fraction 3 of experiment 3.

A portion of fraction 4 (0·63g.) was heated at 100° with 5% sulphuric acid (20c.c.), polarimetric readings being taken at intervals; $[\alpha]_D^{14°}$ -8° (0 mins.), +5° (30 mins.), +10° (70 mins.), +13° (130 mins.), +16° (160 mins. Constant value). The neutralised product was evaporated and extracted with ether as before, and a yellow, mobile syrup (0·3g.) was obtained, from which 2:3:4:6-tetramethyl galactose anilide (0·2g.), m.p. and mixed m.p. 196°, was prepared. Yield of 2:3:4:6-tetramethyl galactose was 88% of theoretical.

Isolation of Acid portion.

The residue after ethereal extraction was dissolved in water and adjusted to a slight excess of barium with dilute sulphuric acid and barium hydroxide. The solution was evaporated to dryness, and the ethereal extract gave a small amount of a colourless syrup (0·09g.), which was acid to litmus. On addition of chloroform, a white crystalline substance formed in the syrup. This was filtered off, and the remainder of the syrup left for one week in a refrigerator; after this length of time the

entire portion had crystallised. The white crystalline solid (total yield 0·08g.) was acid to litmus when dissolved in water, and contained no barium. The m.p. was 158° and $[\alpha]_D^{14°}$ -63·8° →-65·6° after 24 hours, in water (c, 0·2).

Found: C, 46·16; H, 7·01; OCH_3, 28%.

By titration, 1 litre N NaOH was equivalent to 206g.

Calc. for $C_8H_{14}O_6$: C, 46·6; H, 6·8; OCH_3, 30%.

1 litre N NaOH is equivalent to 212g.

DISCUSSION.

The experiments carried out in this section, in the attempt to isolate a di- or tri- saccharide from methylated agar, are similar to those of Haworth and Percival (8), who succeeded in isolating a maltose derivative from trimethyl amylose and also from methylated glycogen.

The procedure consists of degrading the methylated product by contact with acetyl bromide, oxidising the products with bromine, and methylating, so that a mixture of methyl esters is produced. In the case of trimethyl amylose, one of the products is methyl octamethyl maltobionate (which can be hydrolysed to 2:3:4:6-tetramethyl glucose and 2:3:5:6-tetramethyl-γ-gluconolactone) and it was hoped, in these experiments, to isolate a similar typical di- or tri- saccharide composed of galactose residues. Furthermore, since it was known that galactose residues in agar are linked in position 3 (9) it was thought possible that such a disaccharide might contain a linkage between positions 1 and 3.

It is possible to degrade methylated agar, mixed with chloroform to form a thin paste, at room temperature (15°) by contact with acetyl bromide in chloroform solution. Trial experiments were necessary to determine the time of reaction to give an adequate yield of a fraction boiling about 180°/ 0·03m.m. which is the b.p. of the corresponding maltose derivative. Preliminary Experiment 1 indicated that after 12 hours the yield of what appeared to be monosaccharide (fractions (1) and (2) was about 70% of the distillable product, while fraction (3), which appeared to have approximately the constants of the desired di- or tri- saccharide, only accounted for 7% of the product distillable below 230°/ 0·03m.m. On the other hand, a time of reaction of 5¼ hours, as in Preliminary Experiment 2, gave a very small amount of distillable product and a large amount of residue (71%), so that the reaction time was obviously inadequate. The monosaccharide fraction of these experiments was proved by hydrolysis and anilide formation to contain a large proportion of 2:3:4:6-tetramethyl galactose.

Experiment 3 gave a satisfactory yield of a fraction b.p. 180 - 230°/ 0·03m.m. (69% of distillable product, 16% of starting material) which resembled the methyl octamethyl maltobionate. This fraction, however, had

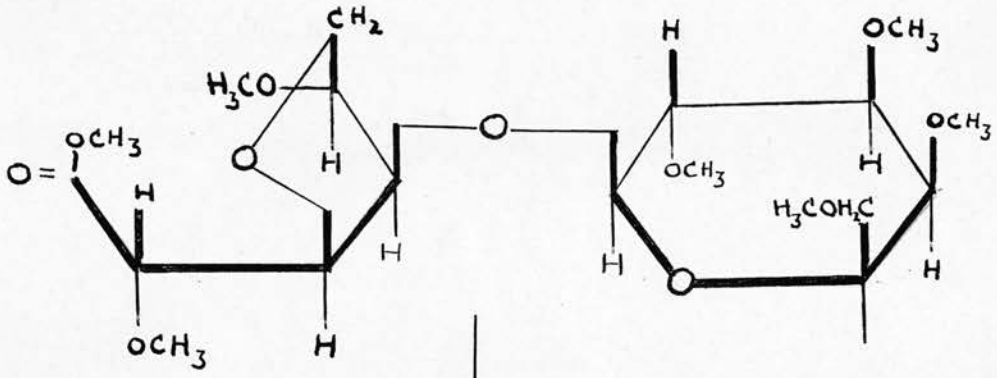
a methoxyl content of 49%, whereas for a methylated disaccharide composed of the methylated derivatives of a normal galactopyranose residue and a galactonic acid residue, the value should be 58%, and for a trisaccharide, 54%. A clue to the composition of this substance, however, was provided by the fact that it gave a positive Seliwanoff test, indicating that it contained a ketose or perhaps an anhydro aldose residue. Since it was known that 3:6- anhydro-*l*-galactose derivatives (11) (12) are obtained from methylated agar, it was thought that a di- or tri- saccharide containing such a residue might be present, and accordingly the required constants were worked out and compared with the analytical figures for fraction 3. It was found that a formula for a disaccharide based on a structure such as figure A page 75 gave values which correspond very closely to the results of the analysis.

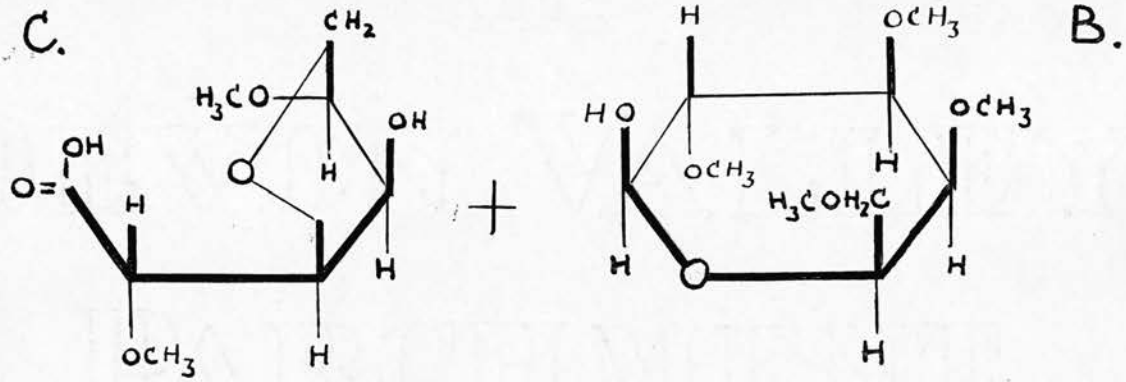
Found for fraction 3: C, 52·4; H, 7·9; OCH_3, 48·9; $COOCH_3$, 13·9%.

Calc. for $C_{19}H_{24}O_{11}$: C, 52·1; H, 7·9; OCH_3, 49·8; $COOCH_3$, 13·5%.

To obtain evidence for this structure it was necessary to hydrolyse the product, and isolate the respective components.

A.





C.

B.

Accordingly, a portion of fraction 3 was hydrolysed with 5% sulphuric acid. After neutralisation with barium carbonate and evaporation to dryness, the ethereal extract gave a yellow, mobile syrup, which was identified by means of its crystalline anilide as 2:3:4:6- tetramethyl galactose (figure B page 75). This proves that the compound contains a normal galactopyranose residue, which is linked in position 1; this tetramethyl galactose could not have been due to an incomplete separation during distillation, since it is clear that the free reducing group had been removed by oxidation and that all the methylated sugars were present as esters. This tetramethyl galactopyranose must therefore have been liberated by the hydrolysis. The possibility that the substance was a trisaccharide with a central galactopyranose residue appears to be ruled out by the analytical data, and by the failure to isolate any of the known 2:4:6- trimethyl galactose anilide. The quantity of tetramethyl galactose obtained also indicates that the product was mainly a disaccharide.

The acid portion of the compound should have been present as the barium salt in the residue after extraction of the tetramethyl galactose with ether. On neutralisation, however, no acid product whatever could be extracted and it appears that either the

postulated acid anhydro- galactose residue (figure C page 75) is broken up by the prolonged treatment with acid (460 mins. at 100°) or some other residue unstable to acid is present. In this connection it is significant to note that hydrolysis of 2:4-dimethyl- 3:6- anhydro-α-methyl- d - galactoside, and 3:6- anhydro-α-methyl- d - galactoside is accompanied by a drastic loss of material, so that it appears that these derivatives are disrupted by acid. Furthermore, on addition of iodine and alkali to the cold solution, iodoform is produced; it is highly probable that this is due to the presence of laevulinic acid, although a sufficient quantity to do confirmatory tests has not yet been obtained.

This experiment was repeated (Experiment 4.) under approximately the same conditions, with a view to obtaining a further quantity of the disaccharide, and repeating the hydrolysis. A larger quantity of methylated agar (44g.) was employed, and the reaction was allowed to proceed for 7½ hours. After oxidation and methylation, four fractions were separated by fractional distillation.

After standing for several weeks at -5°, crystals were observed in fraction 2. The crystals were separated, recrystallised from petroleum, and by the analytical figures, the substance was shown to be the fully methylated straight chain ester, pentamethyl methyl- d - galactonate ($[\alpha]_D^{14^\circ}$, + 20° in water).

Found: C, 51·64; H, 8·66; OCH_3, 64·3; $COOCH_3$, 19·6%

Calc. for $C_{12}H_{24}O_7$: C, 51·42; H, 8·58; OCH_3, 66·4; $COOCH_3$, 21%.

This compound does not appear to have been described previously.

Fraction 4. of Experiment 4. had approximately the same constants as fraction 3. of the previous experiment, although the specific rotation was considerably different ($[\alpha]_D^{14°}$, - 4° compared with $[\alpha]_D^{14°}$, - 22°) Furthermore, although the time of reaction was only 30 minutes less than in Experiment 3, the yield of the fraction b.p. 180 - 230° was much smaller (21% of distillable product, 2·5% of starting material.) Analytical figures, however, were almost identical with those of fraction 3. of Experiment 3.

Another point of difference is that fraction 4. was completely hydrolysed after 160 minutes at 100° (c.f. 460 minutes at 100° for fraction 3.) to give almost the same final specific rotation ($[\alpha]_D^{14°}$ + 16° compared with $[\alpha]_D^{14°}$ + 18°). 2:3:4:6-Tetramethyl galactose was proved to be present and isolated as before as the crystalline anilide; no 2:4:6- trimethyl galactose anilide was obtained, indicating again that the substance was not a trisaccharide.

In this case, however, the mixture of barium salts

gave, on neutralisation, a small yield (0·09g.) of a yellow syrup, which was acid to litmus, and which was crystallised later to a white powder. (0·08g.) This substance ($[\alpha]_D^{14}$ - 63·8° → -65·6° in water) contained no barium, and was readily soluble in cold water to give an acid solution. The analytical figures indicate that it may possess the structure shown in figure C page 75

Found: C, 46·2; H, 7·0; OCH_3, 28%.

Calc. for $C_8H_{14}O_6$: C, 46·6; H, 6·8; OCH_3, 30%.

This substance, on solution in water, appeared to titrate as an acid rather than as a lactone; one litre of normal sodium hydroxide was found to be equivalent to 206g., the theoretical value being 212g. The methoxyl content (28%) appears to rule out the possibility of the original fraction 4. being a trisaccharide composed of one anhydro- galactose residue linked to two normal galactopyranose residues, since the monomethyl anhydro galactose residue isolated from such a compound would have a methoxyl content of 16%. Although up to the present only a small amount of this acid has been isolated, it appears that a disaccharide composed of a normal galactose residue and an anhydro- galactose residue (figure A) may be present in the acetolysis products of methylated agar. Although in figure A the linkage is inserted between positions 1 and 4., no evidence is available so far to

determine if this linkage, or a linkage between positions 1. and 5., or positions 1. and 2., is correct. Since derivatives of 3:6- anhydro- l -galactose have been isolated from agar (11) (12), it is assumed that this anhydro ring is present.

It is difficult to obtain reproducible results by this method of acetolysis, but there is no evidence at the moment of the presence of a di- or tri- saccharide composed exclusively of galactopyranose residues, since if this were the case it would have been expected that a tetramethyl galactonic acid would have been isolated on hydrolysis of the disaccharide fraction. Moreover, the poor yields of the high boiling fraction obtained prevent large amounts being available for hydrolysis.

It is possible that the linkage between positions 1. and 3., which is considered to be present in agar (9), is relatively unstable on hydrolysis, so that only chiefly monosaccharide units are obtained. The isolation of the disaccharide (fraction A.) indicates that in the agar molecule a normal galactopyranose residue is linked to an anhydro-galactose residue. The poor yields (2·5%) of this substance which are obtained, indicate that it only accounts for a small proportion of the agar molecule.

SUMMARY.

1. Methylated agar was degraded by contact with acetyl bromide at room temperature.
2. Pentamethyl methyl - d - galactonate, which does not appear to have been described previously, was obtained, after oxidation, from the monosaccharide fraction.
3. A small yield of a disaccharide has been isolated. This appears to be composed of an anhydro-galactose residue and a normal galactopyranose residue. On hydrolysis, 2:3:4:6-tetramethyl galactose is obtained, and a small yield of what appears to be the dimethyl anhydro-galactonic acid. The results are difficult to reproduce.
4. There is so far no evidence of the existence of a di- or tri- saccharide composed of contiguous galactopyranose residues.

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PART III

THE ACETOLYSIS OF AGAR BY CONTACT WITH ACETIC ANHYDRIDE AND CONCENTRATED SULPHURIC ACID.

INTRODUCTION

The presence of d-galactose in agar has been proved by several workers, notably Lüdtke (1), who isolated galactose methylphenylhydrazone from hydrolysed agar, and Percival and Somerville (2), who prepared a trimethyl d-galactoside by hydrolysing methylated agar. In 1936, however, Pirie (3) claimed to have isolated heptaacetyl-dl-galactose from agar by catalytic acetolysis, thus indicating the presence of l-galactose. This heptaacetyl-dl-galactose had been synthesised by Micheel (4) in 1935.

In addition, Pirie prepared galactose methylphenyl-hydrazone from agar by Lüdtke's method, decomposed it with acetaldehyde (5), and by the use of galactose-trained yeasts he isolated small amounts of l-galactose, corresponding to 1·6% of the dl-galactose. The yield of dl-galactose from the acetolysis of agar was about 7·2%, so he claimed that at least some of the dl-galactose preexisted in the molecule, and that it was not all formed by secondary reactions. Recently, the presence of l-galactose in agar has been proved by the isolation of derivatives of 3:6-anhydro-l-galactose (6,7). It is known that dl-galactose occurs in Chagual gum (8) and quince gum (9), while Anderson (10) has isolated l-galactose from flax-seed mucilage.

Since the heptaacetyl-dl-galactose must exist in the

open chain form, Pirie advanced the view that at least some part of the galactose in agar occurred in the aldehydic form. He found it impossible to prepare heptaacetyl-dl-galactose from derivatives of galactofuranose and galactopyranose by ordinary acetylation methods, but by the method of Wolfrom (11) he succeeded in preparing the heptaacetyl derivatives of d-galactose, d-glucose and d-mannose, and also the hexaacetyl derivatives of l-arabinose, l-rhamnose and d-xylose. He concluded, therefore, that his acetolysis method did not open the oxygen ring of normal galactopyranose. Although Wolfrom and Christman (12) have reported that galactose methylphenylhydrazone is a derivative of aldehydogalactose, Pirie could not prepare heptaacetyl galactose from it by acetolysis.

By hydrolysing agar with normal sulphuric acid for two hours, and acetylating the product, Pirie obtained α-pentaacetyl-d-galactose and a pentaacetyl-dl-galactose, which he stated to be in the α-form, but no heptaacetyl derivative. On the other hand, agar which had been mildly hydrolysed (one-tenth normal sulphuric acid for forty minutes) yielded heptaacetyl-dl-galactose on acetolysis in the usual way. He therefore concluded that in agar dl-galactose exists in a form other than the furanose or pyranose forms, and that heptaacetyl-dl-galactose could be isolated only if acetylation preceded

complete hydrolysis.

Pirie's method of acetolysis was carried out here with a view to preparing the heptaacetyl-di-galactose, and examining the effects of methylation on the other products formed at the same time, and if possible, isolating from them a typical disaccharide or trisaccharide.

EXPERIMENTAL

The agar employed in this experiment was dried at 100° and 15m.m. pressure with frequent shaking. The reaction was carried out in four portions (15g. agar with acetic anhydride 60 c.c. and concentrated sulphuric acid 9 c.c.) that is, 60g. in all of agar. The ice-cold mixture of acetic anhydride and sulphuric acid was added to the agar in small flasks, fitted with air condensers. The mixtures were shaken, left at room temperature for one hour, then heated at 38° for twenty-six hours with occasional shaking. After this length of time, the solutions were black and viscous. The four solutions were filtered through a sintered glass funnel, and the residue washed with normal acetic acid (120 c.c.). [The residue (16·4g.) was chocolate-brown in colour, and formed a rigid gel with water. (0·05g. in 3c.c.) The acetylated product gave $CH_3 \cdot CO$, 35·6% and $[\alpha]_D^{15°}$ -32° in chloroform, so that the residue had all the properties of untreated

agar.]

The filtrate and washings were poured into water (1800c.c.), containing sodium acetate (140g.) and ice (100g.). On standing for two hours, with occasional stirring, a putty-like solid separated out from the brown solution. The mixture was distilled to dryness, neutralised with sodium bicarbonate solution, and distilled to dryness again. The mixture of sodium sulphate and syrup was dissolved in water (200c.c.) and chloroform (400c.c.). The chloroform layer was separated, washed with water, and distilled to dryness. Hot water (1200c.c.) was added, and the syrup shaken to an emulsion. When cold, the water was decanted from the thick, yellow syrup, which was dissolved in hot alcohol (160c.c.). This solution was almost completely decolourised by heating with charcoal at 70° for three hours. The solution was then filtered, and distilled to dryness. The product was an immobile, slightly yellow syrup (36·1g.). Found: $CH_3 \cdot CO$, 56·9%; $[\alpha]_D^{15^\circ}$ + 21·3° in chloroform (c, 0·4).

The syrup was dissolved in hot alcohol (160c.c.) and placed in a refrigerator. No crystallisation took place. The solution was then evaporated to 80c.c. and kept at -5° for seven days, when crystallisation took place. The white crystals (3·0g.) were filtered off, and recrystallised from ethyl alcohol.

Heptaacetyl-dl-galactose.

After 6 recrystallisations and drying over phosphorus pentoxide at 100° / 15m.m. for 2 hours, the m.p. was 124°. (cf. Pirie). No amount of purification could raise the m.p. to Pirie's value of 132°.

Found: $CH_3 \cdot CO$, 60·8%. $C_{20}H_{28}O_{14}$ requires $CH_3 \cdot CO$, 61·1% $[\alpha]_D^{15°}$, ± 0·00° in chloroform. (c, 1·0).

Methylation of heptaacetyl-dl-galactose.

An unsuccessful attempt was made to methylate the heptaacetate (0·76g) with dimethyl sulphate (4c.c.) and 30% sodium hydroxide (11c.c.) in presence of acetone (15c.c.). After two such methylations, a small amount (0·21g.) of acetone-soluble syrup was isolated. This syrup was methylated twice with silver oxide (1g.) and methyl iodide (5c.c.). The product so formed did not distil at 150° / 0·03m.m. pressure, and charred completely.

Methylation of residual syrup.

After removal of the crystalline heptaacetate, the mother liquor was evaporated at 35° / 15m.m. to a syrup (33g.).

Found: $CH_3 \cdot CO$, 53·4%; $[\alpha]_D^{15°}$, + 22·4° in chloroform. (c, 0·4). Methylation was carried out at an initial

temperature of 40°, dimethyl sulphate (165c.c.) and 30% sodium hydroxide (425c.c.) being used in the presence of acetone (300c.c.). The temperature was raised finally to 65°; the solution was cooled, neutralised and the product (20g.) extracted with chloroform (1500c.c.). Found: OCH_3, 44·1%. The methylation was repeated with the same quantities and the product (7·1g.) extracted. Found: OCH_3, 47·9%. The substance was then methylated three times with silver oxide (15g.) and methyl iodide (40c.c.), and yielded a thick, yellow syrup (6·6g.). Found: OCH_3, 54·0%. This syrup was subjected to fractional distillation at 0·04m.m. pressure.

Fraction	Bath Temp.	$n_D^{15°}$	OCH_3	$[\alpha]_D^{15°}$ in $CHCl_3$	Weight
1.	85 - 90°	1·4431	22·5%	± 0° (c, 0·4)	0·12g.
2.	120 - 130°	1·4536	56·1	+ 16·0°	3·40g.
3.	155 - 165°	1·4574	59·0	+ 15·3°	0·31g.
4.	185 - 220°	1·4869	41·7	- 2·8°	0·91g.
5.	220 - 270°	1·4892	41·4	- 3·2°	0·71g.

Residue 1·10g.

All these fractions gave a positive Seliwanoff test. By the rotation, methoxyl content and boiling point, fraction 1. appeared to be methyl laevulate, but the high refractive index indicates a contaminant, possibly a furfural derivative.

Fraction 4. was remethylated with silver oxide (3g.) and methyl iodide (15c.c.) and distilled at 0·03m.m. pressure.

Fraction	Bath Temp.	$n_D^{15^\circ}$	OCH_3 $[\alpha]_D^{15^\circ}$ in $CHCl_3$	Weight
1.	140 - 155°	1·4751	44·7% + 11·1° (c, 0·4)	0·06g.
2.	160 - 180°	1·4954	33·1 + 10·7°	0·21g.
3.	180 - 220°	1·4932	37·6 - 2·6°	0·20g.
4.	220 - 240°	1·4860	42·1 - 2·5°	0·35g.
			Residue 0·08g.	

All these fractions gave a positive Seliwanoff test.

Acetolysis for 44 hours at 38°.

The method used was similar to that of the previous experiment, except that the reaction was allowed to proceed for 44 hours at 38°.

Dried agar (15g.) gave heptaacetyl-dl-galactose (1·2g.) and a syrupy residue (6·0g.). The residue of unchamged agar was 1·6g. The putty-like solid and brown solution, obtained by pouring the filtered reaction mixture into water, were worked up separately. The heptaacetate was isolated from both portions, and the resultant syrups were similar in composition. [Found: $CH_3 \cdot CO$, 57·0%; $[\alpha]_D^{15^\circ}$ + 21·1° before, and $CH_3 \cdot CO$, 53·8%; $[\alpha]_D^{15^\circ}$, + 22·0° after, the removal of the heptaacetate.]

Methylation gave a yellow syrup (0·66g.) which was distilled at 0·03m.m. pressure.

Fraction	Bath Temp.	$n_D^{15^\circ}$	$\%OCH_3$	$[\alpha]_D^{15^\circ}$ in $CHCl_3$	Weight
1.	122 - 128°	1·4508	58·3	+ 15·1° (c, 0·4)	0·13g.
2.	148 - 165°	1·4518	57·9	+ 14·9°	0·09g.
3.	180 - 235°	1·4699	47·3	+ 10·0°	0·30g.
			Residue 0·11g.		

All these fractions gave a positive Seliwanoff test.

Acetolysis for 2 hours at 45°.

Dried agar (15g.) was allowed to react with the acetylation mixture for 2 hours at 45°. Unchanged agar (4·5g.) was recovered. No heptaacetyl-di-galactose could be isolated from the reaction mixture. The resultant syrup had $CH_3 \cdot CO$, 37·9% and $[\alpha]_D^{15^\circ}$, + 25·1° in chloroform (c, 1·0).

DISCUSSION OF RESULTS.

When agar is acetylated by the pyridine-acetic anhydride method, the acetate so formed may be hydrolysed by alkali to give a substance which forms a rigid gel with water. This substance has all the properties of untreated agar, and it is therefore claimed that no degradation takes place on acetylation in this way. More drastic methods, however, such as the acetyl bromide method, or Pirie's method (3), do result in the degradation of the molecule, the amount of degradation depending,

within limits, on the time of reaction.

If the acetyl content is taken as a guide to the composition of the products from Pirie's method, it is observed that a short time of reaction, e.g. two hours at 45°, yields a product containing 37·9% acetyl, whereas if the reaction is allowed to proceed for twenty-six hours at 38°, the acetyl content is 53·4%, after removal of the heptaacetate. It is apparent, therefore, that the residue from the short term acetolysis contains more complex carbohydrates than the residue from the longer term.

Increasing the time of reaction to forty-four hours at 38° appears to make little difference to the acetyl content, either before or after the removal of the hepta-acetyl-dl-galactose. The acetyl content of the product of the forty-four hour period is 57·0% before, and 53·8% after, the separation of the heptaacetate, whereas the twenty-six hour method gives 57·0% and 53·4% respectively. These results, however, may be affected by the presence of small amounts of the heptaacetyl-dl-galactose.

If these acetylated syrups are methylated and separated into fractions in a high vacuum, the methoxyl contents and refractive indices of the fractions from the different experiments may be compared. The forty-four hour method gives fractions which vary in refractive index from $n_D^{15°}$ 1·4508 to $n_D^{15°}$ 1·4699, and in methoxyl content from 58·3% to 47·3% ; the twenty - six

hour method, on the other hand, gives a fraction (fraction 5) with a refractive index as high as $n_D^{15^\circ}$ 1·4892 and a methoxyl content as low as 41·4%. Redistillation indicates that these fractions may not be homogeneous; fraction 4. of the twenty-six hour method on remethylation and distillation yields four fractions which range in refractive index from $n_D^{15^\circ}$ 1·4751 to $n_D^{15^\circ}$ 1·4932, methoxyl content from 44·7% to 32·1%, and in rotation in chloroform from $[\alpha]_D^{15^\circ}$ + 11° to $[\alpha]_D^{15^\circ}$ - 3°. All these fractions from this method show a positive Seliwanoff reaction, indicating that at least some of them contain the 3:6-anhydro-galactose residue, or a ketose residue. The time of reaction, naturally, has a direct effect on the state of degradation of the products.

There is no evidence that any of these fractions contain an appreciable amount of di- or tri-saccharide: by their high refractive indices and low methoxyl contents they appear to be more complex carbohydrates. By this method, then, no disaccharide can be isolated from agar to compare with cellobiose, which can be isolated from cellulose. The low yields obtained after the Haworth methylation processes appear to indicate that some products are destroyed by the strong alkali; the failure to methylate the heptaacetate, moreover, indicates that the alkali is also destructive to the terminal aldehydic group.

The time of reaction has an effect on the yield of

heptaacetyl-dl-galactose. No heptaacetate could be isolated from the two hours acetolysis at 45°; the yield after twenty-six hours at 38° was 5% and after forty-four hours, 8%. It may be that some part at least of the heptaacetate is formed by secondary reactions, such as the decomposition of the 3:6-anhydro-l-galactose, since shown to be present in the molecule (6,7).

Although Pirie could not prepare heptaacetates by acetylation of the normal hexoses or their derivatives, Freudenberg and Soff (13) in 1937 succeeded in isolating small amounts of heptaacetyl-dl-glucose from α-methyl-glucoside tetraacetate by acetolysis with an acetic anhydride - acetic acid - sulphuric acid mixture. Micheel (4) prepared heptaacetyl-dl-galactose synthetically from tetraacetyl aldehydo-d-galactose-6-iodohydrin, and he postulated that the breaking down of an intermediate cyclohexane derivative gave rise to equal amounts of the d- and l-heptaacetates. Furthermore, although Pirie claims that at least some part of the galactose in agar exists in the open chain form, there is no evidence of the aldehydo galactose derivatives when agar is hydrolysed by other means, such as the hydrolysis of methylated agar by methyl-alcoholic hydrogen chloride.

SUMMARY

1. A heptaacetyl galactose has been prepared from agar by Pirie's method. It agrees in rotation and acetyl content with Pirie's compound, but the melting point is eight degrees lower.

2. After separation of the heptaacetate, the remaining products were methylated, and the fragments examined.

3. No methylated di- nor tri-saccharide could be isolated from the methylated reaction products.

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