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This is to certify that Mr JAMES CARGILL SOMERVILLE has successfully sustained an oral examination on the subject matter of his thesis by a committee of the Department of Chemistry.

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Title

INVESTIGATIONS ON THE STRUCTURE OF AGAR-AGAR.

CONTENTS

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

Investigations on the Structure of Agar-Agar.

In the literature there are to be found many references to chemical and physicochemical investigations on agar-agar, but until recently no successful attempt has been made to elucidate the structure of this polysaccharide, presumably owing to its complex nature, whereas great strides have been made in throwing light upon the structure of such compounds as starch and cellulose.

In 1859 Payen (1) called it gelose believing it to be similar to pectin and even in 1912 De Touze (2) thought that it had a connection with pectin because it is found as an intracellular substance in algae. Agar-agar is considered in fact to be a type of hemicellulose

filling up the cellular tissue in the plant, thus corresponding to pectin in its physiological function. It was classified as an acid hemicellulose by Anderson (3) who stated further that because hemicelluloses are dissolved out of the plant tissue by alkali, they might either contain an acid group and be combined by it to some constituent, possibly the cellulose of the plant material, or be held by a glucosidic union in the plant. In either case alkali might break the union and set free the hemicellulose; he also drew attention to the fact that agar-agar, in contrast to all other acid hemicelluloses studied so far does not contain an acid belonging to the uronic group, which would suggest that uronic acids are not necessarily constituents of these substances.

Agar-agar may not contain an organic acid in the molecule, since there is a possibility that the acid formed on hydrolysis of agar-agar with dilute mineral acid may be due to the breakdown of a sugar fragment which is very susceptible to the action of mineral acids, vide Lüdtke (4). This fact may exclude agar-agar from the category of an acid hemicellulose. Anderson (3), however, has stated that there are a few hemicelluloses

known which give only sugars on hydrolysis and agar-agar may belong to this group.

The presence of sulphur has also been detected in agar-agar to the extent of 1-1·2% by Neuberg and Ohle (5) and Lüdtke (4). How the sulphur is attached or what function it has in the agar-agar molecule has not yet been definitely ascertained, though many theories have been formed by different authors.

In 1921 Samec and Ssajevic (6) put forward the ideas that agar-agar is a sulphuric ester of gelose in much the same way as amylopectin is a phosphoric ester of the amyloses, and that the great viscosity of agar-agar is due to the relatively high content of the sulphate ion. In the same year Neuberg and Ohle (5) showed the existence of sulphur in organic combination in agar-agar. Hydrogen sulphide was evolved by bacterial action and sulphuric acid was set free on hydrolysis. In the following year Samec and Ssajevic (7) published another paper, in which they showed that agar-agar contained electrolytes which were so closely bound up with the organic substance, that neither dialysis nor electrodialysis was sufficient to remove them. They found that the chief constituent of agar-ash was calcium

sulphate and that on boiling the solution of agar-agar under pressure the sulphuric acid passed into the solution and was easily removed by dialysis. Simultaneously the whole of the physico-chemical properties of the solution changed so that a causal connection between the sulphur content and the power of forming jellies was deduced. In 1923 Fairbrother and Mastin (8) stated, that the agar-ash contained calcium sulphate, magnesium sulphate and silica as well as traces of other salts, that they obtained almost all the sulphur as sulphuric acid on hydrolysis and that agar-agar breaks down in the presence of even dilute acids. They also showed that the calcium may be replaced by other metals. On heating the agar-agar with dilute hydrochloric acid and water alternately they stated that they obtained an almost ash free gel, which could not be heated without hydrolysis occurring, and which did not set to a gel again on cooling. They thought that agar-agar appeared to consist principally of the calcium salt of an acid sulphuric ester. Hoffman and Gortner (9) agreed with the opinion that the free agar-acid was an acid sulphuric ester, that all the sulphur was in the form of sulphuric acid, that sols of the free agar-acid did not gelatinize on cooling and that auto hydrolysis took place when sols of

the agar-acid were heated. These authors also found that calcium could be removed quantitatively by electro dialysis and that when the sol of the free agar-acid was neutralised by the addition of a base rigid gels were obtained. They stated finally that the gelation of agar-agar was the gelation of a salt and not of a complex polysaccharide.

In 1934 Takahashi and Shirahama (10) published a paper giving the results obtained on heating agar-agar under pressure at 130°C and measurements of the decrease in viscosity due to hydrolysis of the agar-agar in this way. On suitable treatment two products were separated; one appeared to be a simple polysaccharide while the other contained an ethereal sulphate group. Electro dialysis of this latter substance gave a free acid which was unstable to heat and decomposed on standing ($[\alpha]_D$ - 31° in water). It appeared to be a very complex substance and to distinguish it from the "agar-acid" of Hoffman and Gortner (9), it was called "Kanten-acid". These authors concluded that agar-agar consisted of the above two components linked together and that the loss of gelatinising power was due to the fission of the linkage between these polysaccharides and they thus opposed the view of Samec and Ssajevic (7) that

the gelatinising property of agar-agar was dependent on the ethereal sulphate linkage. Cran (11) working with bacterial cultures, presumed from the experimental results that the gelose must consist of two kinds of carbohydrates, one producing a violet colour with iodine and used as nutrient by bacteria, the other showing no colour reaction with the same reagent.

Most of the chemical work which has been undertaken on agar-agar has been on the sugars obtained on acid hydrolysis and while galactose seems to be the sugar occurring in the largest quantity in the hydrolysate, the presence of a number of different sugars has been reported.

König and Bettels (12), estimated, by the mucic acid method, the amount of galactose present to be 33% and this is supported by Lüdtke (4) who estimated it both by the mucic acid method and by the isolation of galactose methylphenylhydrazone. According to Ludtke there was another reducing substance present, the reduction value of the hydrolysate being 60%, 30% due to the galactose and the removal of this sugar quantitatively by Neuberg's (13) method the excess methyl phenyl hydrazine by benzaldehyde, a reducing liquid, which had equivalent reduction values for both sodium hypoiodite

and Fehling's solution was obtained. This appeared to show that it was not a ketose.

Sugars other than galactose whose presence have been detected are a pentose such as xylose or arabinose (Reichardt (14), Czapek (19), Seber (15), Takao (16), Matsui (17), Furuichi (18)), a ketose such as fructose (Takao (16), Matsui (17), Furuichi (18)) and as already stated a reducing hexose by Lüdtke (4), and as well Furuichi (18) stated that a uronic acid was present. Lüdtke (4) also reported the presence of an acid since in the hydrolysis solution of agar-agar neutralised with Barium Carbonate, he estimated the filtrate to contain 16% Barium which increased to 28·9% by precipitation of the Barium salt by alcohol. On removing the Barium he obtained an acid liquid which on extraction with ether gave a small quantity of laevulic acid. He noticed, that during the hydrolysis the acid content increased while the reduction value fell and the yield of galactose methyl-phenyl-hydrazone reached a constant maximum, he assumed, therefore, that the acid is not a primary product, but was developed from the second reducing portion. Another possibility, however, was that the acid is present in the bound state and was gradually released by

hydrolysis. In his opinion, the presence of uronic acid could not be considered, no furfural could be detected on distillation with hydrochloric acid, nor was the naphtho resorcin test positive. This was also borne out by Takahashi and Shirahama (10).

In 1936 Pirie (20) stated that he was not able to acetylate agar by the milder methods of acetylation i.e. with pyridine and acetic anhydride or with acetic anhydride in the presence of sulphur dioxide, chlorine, hydrochloric acid, hydrothiocyanic acid or zinc chloride. Percival and Sim (21), however, showed that agar-agar subjected to proper preliminary treatment, could be acetylated easily to yield a chloroform soluble agar-acetate, which on deacetylation regenerated a substance identical with the original polysaccharide in its ability to form a gel. This was regarded as evidence that no substantial degradation had taken place during acetylation. The substance obtained on the deacetylation of agar acetate, forms a rigid gel when dissolved in boiling water, it does not contain any sulphur and this fact agrees with the assertion of Takahashi and Shirahama (10) that, contrary to the view of Samec and Ssajevic (7), the gelation of agar-agar is merely the gelation of a complex polysaccharide and is not due to the

presence of an ethereal sulphate residue.

Neuberg and Schwiezter (22) have recently shown that agar-agar can be separated into two portions by simple shaking with water at room temperature. The insoluble portion (90%) thus obtained possesses the power of gelation of untreated agar-agar and is almost free from sulphur, while the soluble portion (10%) contains sulphate residues and does not form a gel. Since all the agar-agar preparations used in this thesis were prepared from powdered agar-agar which had been subjected to thorough washing with water, the results described apply to this insoluble portion only, which is considered to be the essential carbohydrate of agar-agar.

DISCUSSION OF RESULTS

The line of attack in order to ascertain the structure of the polysaccharide of agar-agar was the classical method of complete methylation, followed by hydrolysis and the attempted separation of the fragments, such method having proved of value in the hands of Haworth and his co-workers for cellulose, starch, glycogen, xylan etc. Agar acetate was prepared by the treatment of prepared agar-agar with pyridine and acetic anhydride. It was obtained either as a tough colourless glass from chloroform solution or as a white powder by precipitation from this solution by light petroleum. Further acetylation had no effect on its properties and almost ash free agar-agar was obtained on deacetylation. Simultaneous deacetylation and methylation with 30% sodium hydroxide solution and dimethyl sulphate, followed by three further methylations with the same reagents, yielded

a product which was soluble in chloroform and acetone and which remained unchanged on further methylation. Fractional precipitation from chloroform and acetone gave four identical fractions, indicating the homogeneous character of the product.

The methylated agar was hydrolysed completely in 4 hours on heating with 6% sulphuric acid. Methyl alcohol 3·4% was evolved and a brown resin (3%) was deposited. No furfural derivatives could be detected. The filtrate from the solution after neutralisation with Barium Carbonate contained 6·7% Barium and the Barium salt obtained by precipitation with alcohol, contained no methoxyl residue. It was found impossible owing to the solubility of the Barium salt to separate it quantitatively from the sugars (ca 75%) formed on hydrolysis, so that simultaneous glycoside formation and esterification were carried out on the entire hydrolytic product. Distillation in a high vacuum yielded three fractions.

A. An optically inactive mobile ester 16%

B. A crystalline trimethyl methylgalactoside 65%

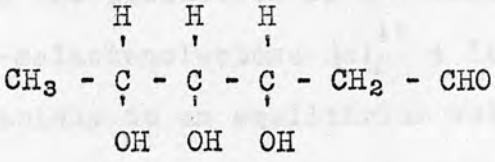
C. A syrup 14%.

The yield of (B) thus isolated from methylated agar was ca. 50% of the starting material and by the

hydrolysis of methylated agar with methyl-alcoholic hydrogen chloride a yield of ca. 60% was obtained. Assuming that methylated agar, as appears likely, is a true derivative of agar-agar, it follows from this that the polysaccharide is composed of galactose residues to the extent of at least 55% whereas previous authors e.g. Lüdtke (4) were able to detect only 30-40%.

Fraction (A) from its physical properties, analytical composition and comparison of the melting points and mixed melting points of the 2:4-dinitrophenylhydrazone and the p-nitrophenylhydrazone prepared from an authentic specimen, proved to be methyl laevulate. There would seem no doubt, therefore, that the acid formerly regarded as a constituent of agar-agar is laevulic acid arising from the decomposition by mineral acid of a fragment of the molecule. Lüdtke (4), indeed, isolated a small amount of laevulic acid from the hydrolysis product of agar-agar itself. It is well known that this acid is more readily produced from ketohexoses than from aldohexoses and under the experimental conditions employed it was found that galactose gave rise to less than 2% and the trimethyl galactose from (B) to none at all. The hydrolysis of methylated agar with methyl-

alcoholic hydrogen chloride yielded only a small quantity of the laevulic ester, fractions (B) and (C) being obtained as before. The application of Bredereck's test and the Seliwanoff reaction on hydrolysed agar-agar, methylated agar and fraction (C) gave positive indication of the presence of a ketose and it is considered, therefore, that the laevulic acid is derived from this source. At the same time it must be remembered that desoxy sugars such as l-desoxy glucose give rise to large amounts of laevulic acid. Levene and Mori (26), Takahashi and Shirahama (10) also found a substance giving a ketose reaction. The above ketose tests were negative with fractions (A) and (B). The same tests performed on d-digitoxose (a didesoxy hexose)



and on l-fucose (6-desoxy galactose) also gave negative results.

The crystalline non-reducing substance (B), $C_{10}H_{20}O_6$ m.p. 70°C $[\alpha]_D^{17}$ + 107° in water. The rotation in conjunction with the rotation of the corresponding trimethyl galactose (+ 124°), indicating it in all probability to be a mixture of the α- and β- forms with a preponderance of the former proved to be a 2:4:6-tri-

methyl methyl-galactoside, this structure being assigned for the following reasons.

1. Complete methylation yielded almost quantitatively 2:3:4:6-tetramethyl galactose which was identified by its crystalline anilide. This observation at once proved it to be a derivative of galactose and excluded the possibility of substitution at position 5.

2. Hydrolysis yielded a crystalline trimethyl galactose $C_9H_{18}O_6$ m.p. 104°-105°C $[\alpha]_D^{17}$ + 124° in water, which readily formed a crystalline dimethyl galactose phenylosazone indicating the presence of a methoxyl group in position 2 of the trimethyl galactose. A crystalline trimethyl galactose anilide was also obtained. Oxidation with bromine water under conditions favourable to the production of a γ-lactone yielded a trimethyl δ-galactonolactone $[\alpha]_D^{18}$ + 150° in water, which fell rapidly to an equilibrium value of +50° in 16 hours. The probability that position 4 was occupied by a methoxyl residue was confirmed by the observation that at room temperature in contact with 1% methyl-alcoholic hydrogen chloride, the crystalline trimethyl methyl-galacto-pyranoside was regenerated, no galacto-furanoside being detected. Both positions 2 and 4 were occupied, therefore, by methoxyl residues. The

trimethyl galactose could not therefore be the 2:3:6-trimethyl galactose of Haworth, Raistrick and Stacey (23) and indeed the physical constants confirmed this fact. It remained, therefore, to decide between 2:3:4- and 2:4:6-trimethyl galactose. The former derivative had been isolated by Challinor, Haworth and Hirst (24) from the aldobionic acid of gum arabic and by Onuki (25) from stachyose. The properties of the sugar in question, the rotation of the lactone formed by oxidation and the properties of its derivatives appear to exclude the possibility of identity. Unfortunately we could not obtain a crystalline phenyl hydrazide for comparison, but a crystalline amide m.p. 167°C $[\alpha]_D^{20}$ + 74° in water was isolated. On applying Weerman's reaction to this amide further evidence that position 2 was occupied by a methoxyl residue was obtained. Its physical constants only served to confirm the absence of identity with the 2:3:6-trimethyl galactose which yields a 2:3:6-trimethyl galactonamide m.p. 135°C, $[\alpha]_D^{20}$ + 20·5° and a crystalline phenyl hydrazide m.p. 175°C, while an amide of 2:3:4-trimethyl galactose has not been described. Repeated attempts to obtain the trimethyl mucic acid which characterised the 2:3:4-

trimethyl galactose of Challiner, Haworth and Hirst (24) failed, small yields of compounds of indefinite composition being obtained, of which the reducing properties seemed to indicate them to be derivatives of tartronic or mesoxalic acid. This result is in accordance with the assignment of the structure 2:4:6-trimethyl α-galactose to the methylated galactose fragment.

Since the completion of this work, 2:4:6-trimethyl α-galactose has been synthesised by Dr. D. J. Bell (27) of the Biochemical Laboratories, University of Cambridge. His derivative was obtained by the following route,

4:6-Benzylidene 2-methyl β-methyl galactoside ⟶ 4:6-Benzylidene 3-p-toluenesulphonyl 2-methyl β-methyl galactoside ⟶ 3-p-toluenesulphonyl 2-methyl β-methyl galactoside ⟶ 3-p-toluenesulphonyl 2:4:6-trimethyl β-methyl galactoside ⟶ 2:4:6-trimethyl β-methyl galactoside ⟶ 2:4:6-trimethyl α-galactose

which leaves no doubt as to the structure assigned. The specific rotation and mutarotation of the synthetic product as well as its melting point and mixed melting point with the trimethyl galactose derived from methylated agar confirmed the identity of the two products.

The galactose residues in agar-agar are thus present in the pyranose form and if one assumes for the moment that they are joined directly together they must be linked by positions 1 and 3. So far this linkage has not yet been demonstrated in any other polysaccharide.

The most common linkage which is found in polysaccharides is that between positions 1 and 4, and this has been shown to be present in such polysaccharides as starch and cellulose. Inulin, another substance whose structure is definitely proved, gives fructose on hydrolysis and consists of fructofuranose units with linkage at positions 1 and 2. A few disaccharides and trisaccharides are known with linkage at positions 1 and 6. This 1:3 link in agar-agar thus appears to be unique. It is possible however that it will be discovered in other polysaccharides such as plant gums or hemicelluloses.

From the strongly negative rotations of acetylated and methylated agar and the fact that d-galactose derivatives are isolated after hydrolysis, it is made clear that there is a preponderance of β-linkages in the molecule. If it is assumed, therefore, that the agar-agar molecule consists entirely of β-galactopyranose units

linked together at positions 1 and 3, the most probable structure is either a zig-zag chain Fig.I, or a closed loop of six units Fig.II. Diagrams of these structures are given. However there is the fact that a significant quantity of a methylated ketose can also be isolated and if a chain of β-galactopyranose units is present, these ketose residues may be contained at points along its length, projecting from the main chain. On the other hand the possibility remains that these residues may be incorporated in the chain itself and this would clearly affect the above conclusions based on the geometry of the β-galactopyranose units linked in positions 1 and 3.

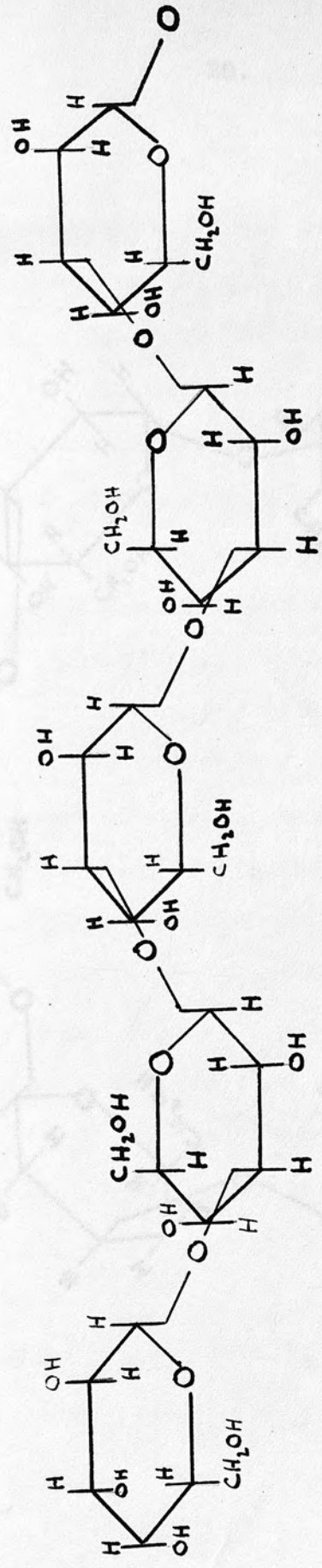


Fig. I

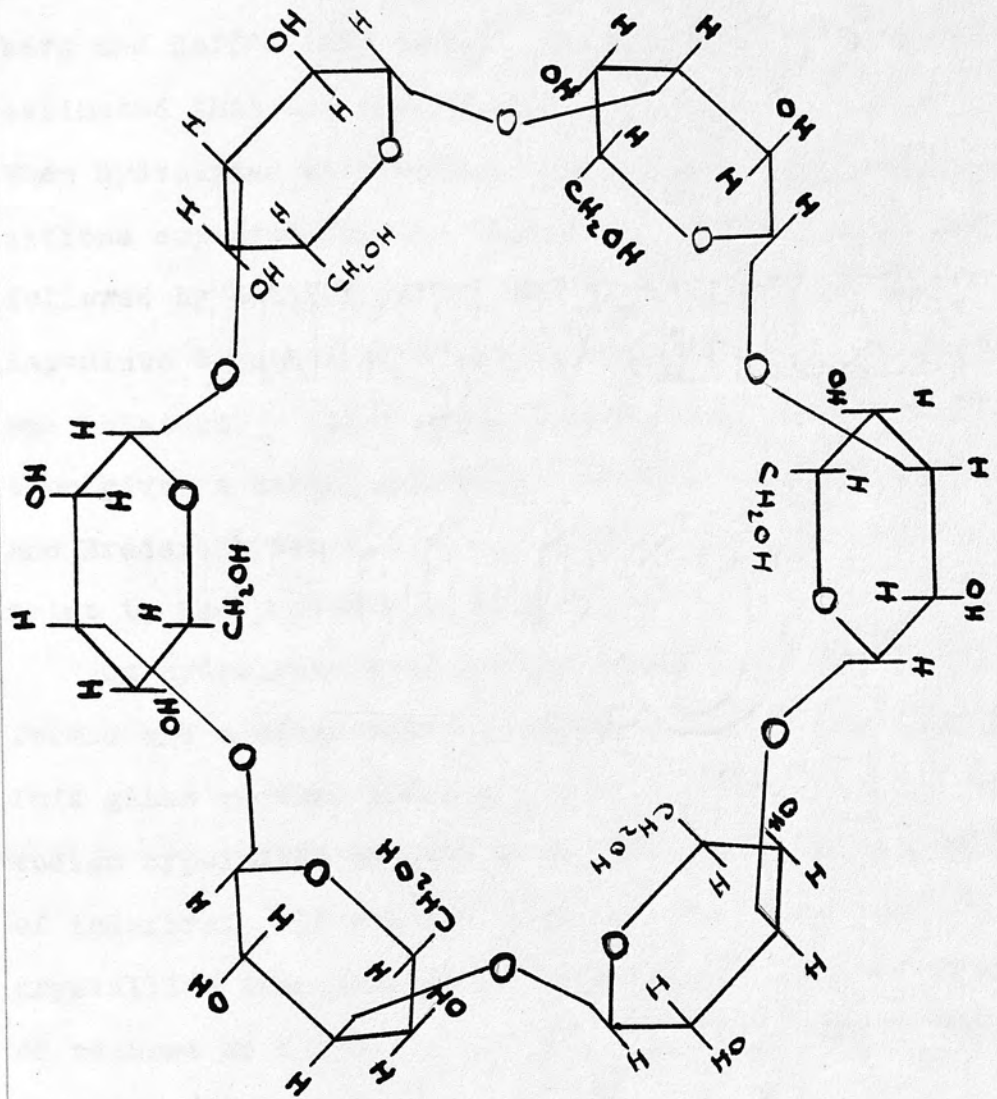


Fig. II

It has not yet been found possible to identify the non-reducing fraction (C) which appears to be a dimethyl methyl-ketoside (OMe, 39%). By the modification of Freudenberg and Soff's (28) method already described, it was estimated that one methoxyl residue was glycosidic. When hydrolysed with dilute mineral acids under the conditions employed for the hydrolysis of methylated agar, followed by esterification and distillation, methyl laevulate together with the unchanged starting material was obtained. This, along with the fact that the fraction gives a strong Seliwanoff reaction with resorcinol and Bredereck reaction with ammonium molybdate seemed to point to the presence of a ketose.

On hydrolysis with N/5 oxalic acid, no resin was formed and a clear amber glass was obtained (OMe, 26·1%). This glass reduced Fehling's solution and reacted with sodium hypoiodite to give rise to an appreciable quantity of iodoform. It was not found possible to prepare a crystalline phenylosazone, a 2:4-dinitro phenylhydrazone or osazone or a p-nitro phenylhydrazone or osazone from this sugar.

Oxidation of the ketoside with nitric acid, followed by esterification yielded methyl oxalate, identified by

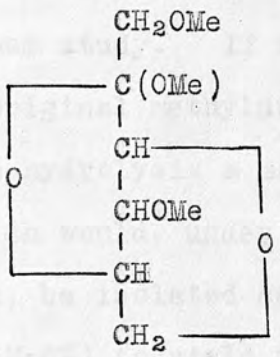
its amide and an ester which yielded an amide (m.p. 135·5° - 145°C) which was not obtained in sufficient quantity for complete analysis, but which had no methoxyl content. These oxidation experiments were repeated and in no case could any derivatives of the dimethoxy succinic acids be obtained, thus pointing to the fact that two adjacent methoxyl residues were absent.

Complete methylation of the syrupy ketoside yielded two fractions on fractional distillation, (1) a white crystalline solid (m.p. 81°C $[\alpha]_D^{14}$ + 57·3° in water) (2) a colourless mobile liquid of zero rotation. Both these fractions gave strong ketose reactions.

In order to decide whether this fully methylated substance was a fructofuranose derivative since this would agree with certain of its properties e.g. specific rotation, an attempt was made to isolate the tetra methyl fructofuronamide described by Avery, Haworth and Hirst (29). Oxidation with nitric acid under the prescribed conditions was therefore carried out but without success. Instead, oxidation followed by esterification yielded an ester ($[\alpha]_D^{14}$ + 25·6° in water) which gave a small quantity of an amide (m.p. 214°C), it was therefore not an amide of dimethoxy succinic acid, and was unfortunately obtained in insufficient quantity for analysis. The

possibility that it was an impure specimen of d-arabo trimethoxy glutaramide cannot, however, be excluded. The point must be left in abeyance, however, until larger quantities of the fully methylated product are available.

The white crystalline solid, fraction (1), appears from its analytical data to have an empirical formula $C_9H_{16}O_5$. This formula from the small percentage of oxygen and low methoxyl content would seem to be either a desoxy sugar or an anhydroketose. The latter fits in much better with the figures obtained, and from tests on a pine wood splint in comparison with digitoxose, it would appear not to be a desoxy sugar.



The formula shown is only given as a rough guide as to what the fully methylated crystalline solid might be, as there has been no proof obtained of either the position of the rings or the methoxyl groups.

Accompanying the "trimethyl anhydroketose" was a mobile liquid (OMe, 50%, $[\alpha]_D^{15}$ ± 0° in water). It was thought probable that this contained tetramethyl methyl galactoside which had eluded separation or was derived from some dimethyl galactose. That this was not the case, however, was shown by the failure to isolate the readily crystallisable tetramethyl galactose anilide on suitable treatment. Furthermore the liquid gave a strong ketose reaction, although this may be due to the "anhydroketose" dissolved in it. From these observations it is clear that "Fraction C" is a mixture of at least two constituents. It is impossible to decide whether the "anhydroketose" is present in the methylated polysaccharide or is produced in subsequent operations. This must be a matter for further study. If the anhydroketose were present in the original methylated polysaccharide, it is clear that on hydrolysis a monomethyl anhydroketose must result, which would, under the experimental conditions employed, be isolated as the corresponding methyl ketoside (OMe, 32·6%) to yield the free sugar (OMe, 17·6%) on hydrolysis. The observed methoxyl values are considerably higher than these, pointing to the fact that fraction (C) is a mixture, the other constituent containing a higher percentage of methoxyl. This may be the ketose without the anhydro-ring or some other sugar, but speculation at the present stage is clearly unprofitable.

E X P E R I M E N T A L

Preliminary preparation of the agar-agar.

Powdered agar-agar (B.D.H.) (100 gm. ash 3·3%) was washed during 5 days five times with tap water (100 l.) by decantation. After the last washing and subsequent removal of the bulk of the water, an equal volume of absolute alcohol was added to the mixture of solid and water and the mixture, after standing for a short time, was filtered. No difficulty was experienced in filtering the agar-agar in contact with alcohol through ordinary filter paper in a Buchner funnel. The agar-agar purified in this manner was kept in contact with alcohol in a flask and portions were taken as required, washed with ether and dried in air.

Analysis.

Found: C, 45·1; H, 6·2; ash 2·0;

Calc. for $(C_6H_{10}O_5)$: C, 44·4; H, 6·2%.

Treatment with N/20 hydrochloric acid at room temperature, or with N/50 hydrochloric acid at 30°C (½ hour) followed by washing and the further addition of alcohol, failed to reduce the ash content appreciably.

Acetylation of Agar-agar.

Prepared agar-agar (18 gm. dry wt.) was shaken with pyridine (100 c.c.) for 1 hour and a mixture of acetic anhydride (200 c.c.) and pyridine (100 c.c.) was added. After heating at 95-100°C for 6 hours and standing for 20 hours, the yellow solution was poured into a large vessel (10-15 litres) concurrently with cold water from the tap. In this way ample agitation of the water was obtained. The product was washed free from acid, dried, and extracted with chloroform-acetone (1:1), the solution filtered and the product precipitated by the addition of light petroleum and dried in a vacuum (22 gm.). The agar acetate softened at 230°C and had $[\alpha]_D^{15}$ - 32·1° in chloroform (c, 0·5).

Analysis.

Found: C, 49·1; H, 5·8; $CH_3 \cdot CO$, 39·0;

Calc. for $C_{12}H_{16}O_8$: C, 50·0; H, 5·6; $CH_3 \cdot CO$, 44·8%.

The acetyl content could not be increased by re-acetylation. Agar acetate (5 gm.) was dissolved in pyridine (25 c.c.) and acetic anhydride (50 c.c.), heated for 4 hours at 95°-100°C, allowed to stand overnight and then poured into a stream of water. The agar-acetate obtained, however, was found to dissolve more readily in chloroform-acetone (1:1), from which it was, as before, precipitated by the addition of light petroleum.

Preparation of Methylated Agar.

Agar acetate (14 gm.) dissolved in acetone (300 c.c.) was treated with dimethyl sulphate (70 c.c.) and 30% sodium hydroxide (180 c.c.) in 1/10th portions every 10 minutes at 56°C, followed by heating to 75°C during 30 minutes to remove the acetone. The white granular product, (OMe, 27·7%) mixed with sodium sulphate was filtered off at the pump and after being washed with hot water was redissolved in acetone and remethylated as before (OMe, 28·3%). This operation was repeated twice more. After completing this, the methylated agar was extracted several times with chloroform, from which solution it was obtained as a white powder (10 gm.) by

addition of light petroleum, the chloroform solution, before precipitation, being washed in turn with dilute acid and water, and finally being dried over anhydrous sodium sulphate. The methylated agar softened at 220°C, had $[\alpha]_D^{15}$ - 92° in chloroform (c, 0·6).

Analysis.

Found: C, 51·4; H, 7·3; OMe, 30·9%.

The Fractional Precipitation of Methylated Agar.

Methylated agar (5 gm.), dissolved in chloroform (250 c.c.) was precipitated by successive additions of light petroleum (b.p. 60-80°) to yield four fractions.

(A) 0·9 gm. $[\alpha]_D^{15}$ - 92° in chloroform (c, 0·4) OMe 31·0%.
(B) 1·2 gm. $[\alpha]_D^{17}$ - 92° in chloroform (c, 0·4) OMe 30·8%.
(C) 2·0 gm. $[\alpha]_D^{17}$ - 92° in chloroform (c, 0·4) OMe 30·9%.
(D) 0·6 gm. $[\alpha]_D^{17}$ - 92·5° in chloroform(c, 0·4) OMe 30·7%.

The Hydrolysis of Methylated Agar with Sulphuric Acid.

Methylated agar (8 gm.) was heated at 95°C with 6% sulphuric acid (170 c.c.). The substance gradually

dissolved and a brown resin was deposited, which was filtered off (0·25 gm.: OMe nil). A constant rotation ($[\alpha]_D^{20}$ + 45°) was reached in 4 hours and the hydrolysis was continued during a further hour. The hydrolysate was neutralised with A.R. Barium Carbonate, the solution filtered and the filtrate evaporated almost to dryness at 50°/10 mm.; addition of absolute alcohol precipitated a barium salt which was contaminated with reducing sugars. On redissolving in water and precipitating again with alcohol, a white powder (0·7 gm. OMe, nil) was obtained. The aqueous alcoholic residues on evaporation to dryness gave a pale yellow reducing syrup (6 gm. OMe, 44%) which still contained some of the barium salt. By a modification of the method of Freudenberg and Soff (28) it was estimated that methylated agar lost 3·5% of methyl alcohol during the hydrolysis. The apparatus consisted of two test-tubes (6" × 1 ") connected by glass tubing passing through rubber stoppers and the inlet tubes dipping into the foot of each. The first test-tube was half-filled with 7% sulphuric acid and the second test-tube in which the hydrolysis took place, contained 10 cc. 7% sulphuric acid. A tube from the latter led to a trap from which a long

tube dipped into the hydrogen iodide of a macro-Zeisel apparatus. The methylated agar was weighed out and placed in the second test-tube. Carbon dioxide from a Kipp was then bubbled through the apparatus, the two test-tubes and trap being maintained at 100°C for the duration of the experiment (3 to 4 hours), by keeping them immersed in boiling water.

Simultaneous Esterification and Glucoside Formation.

The neutralised hydrolytic product (7 gm.) from another hydrolysis, which still contained the barium salt, was refluxed with 5% methyl-alcoholic hydrogen chloride for 6 hours; the solution was neutralised with silver carbonate, filtered and evaporated at 40°/15 mm. to a non-reducing syrup (5·9 gm.) which was fractionally distilled in a high vacuum to yield the following fractions:-

(A) 0·98 gm. bath temperature 90° - 100°/0·01 mm.
(B) 3·8 gm. bath temperature 150° - 160°/0·01 mm.
(C) 0·85 gm. bath temperature 180° - 190°/0·01 mm.
Residue 0·4 gm.

Hydrolysis of methylated agar with methyl alcoholic hydrogen chloride.

Methylated agar (10 gm.) was refluxed with methyl alcoholic hydrogen chloride (400 c.c. 1·2%) for 15 hours, until the rotation was constant (+ 31·6°). The acid was neutralised with A.R. Barium carbonate, the solution was then filtered and the methyl alcohol removed under diminished pressure. The Barium chloride which was soluble to a certain extent in the methyl alcohol was removed by precipitation with ether followed by filtration. The ether was removed under diminished pressure. A yellow syrup (10·04 gm.) which was not reducing was obtained. This syrup was distilled in a high vacuum and three fractions were obtained.

(A) Colourless liquid (·1 gm.) b.p. 90°-100°/0·01 mm.

(B) 2:4:6-Trimethyl methyl galactoside (6·40 gm.) b.p. 150°-160°/0·01 mm.

(C) Light yellow syrup (2·18 gm.) b.p. 180°-190°/0·02 mm.

Methylated agar was not very easily hydrolysed by this method, in some hydrolyses, a proportion of the syrup would not distil; when this syrup was extracted

with ether, the ether removed, and the syrup (2·19 gm.) refluxed with methyl alcoholic hydrogen chloride (60 c.c. 1·2%) for a further 9 hours, the acid neutralised and the solvent removed, on distillation in high vacuo three fractions were obtained as before, viz.

(A) Laevulic ester (·1 gm.).

(B) 2:4:6-Trimethyl methyl galactoside (1·24 gm.).

(C) Yellow syrup (0·37 gm.).

Identification of (A) as methyl laevulate.

The colourless mobile liquid, which gave the iodoform reaction had n_D^{15} 1·4250, $[\alpha]_D^{15}$ ±0°.

Analysis.

Found: C, 54·8; H, 7·9; COOMe, 48·0.

Calc. for $C_6H_{10}O_3$: C, 55·3; H, 7·8; COOMe, 45·3%.

Preparation of the 2:4-dinitrophenylhydrazone.

To the mobile liquid (0·2 gm.) in a test-tube with a few drops of alcohol, was added 5 c.c. of a well shaken

suspension of 2:4-dinitrophenylhydrazine in alcohol. The test-tube was then heated, a few drops of concentrated hydrochloric acid added, and the tube again heated. On cooling a solid separated, the alcohol was then diluted with water and the orange red solid filtered off. It was recrystallised three times from chloroform and petrol-ether.

The 2:4-dinitrophenylhydrazone had m.p. 136°C alone and in admixture with the authentic 2:4-dinitrophenylhydrazone prepared in the above manner from methyl laevulate.

Preparation of Methyl laevulate.

Laevulic acid (3 gm.) was refluxed with 3% methyl-alcoholic hydrogen chloride (30 c.c.) for 6 hours. The acid was neutralised with Barium carbonate and the solution filtered. The alcohol was removed under diminished pressure at 40°C and the ester was extracted from the barium salt with ether, the ether was then removed, the ester transferred to a small distilling flask and distilled in a high vacuum. The fraction (2 gm.)

which came over at bath temperature 90°-100°/0·02 mm. was collected, and consisted of methyl laevulate.

Preparation of methyl laevulate p-nitrophenylhydrazone.

p-Nitrophenylhydrazine (0·1 gm.) was dissolved in glacial acetic acid (2 c.c.) and the ester (0·5 gm.) added, the solution was heated for 1 minute and then diluted with water (5 c.c.). On cooling a yellow solid was precipitated, which was filtered off, washed with water and dried and finally recrystallised from absolute alcohol.

Golden yellow crystals were obtained, m.p. 135°C alone and in admixture with authentic p-nitrophenylhydrazone prepared from methyl laevulate.

Analysis.

Found: C, 54·25; H, 5·65; N, 16·2;

Calc. for $C_{12}H_{15}O_4N_3$: C, 54·3 ; H, 5·7 ; N, 15·85%.

Identification of (B) as 2:4:6-Trimethyl methyl-galactoside.

This fraction, which solidified completely in the receiver during distillation, was recrystallised from light petroleum and had m.p. 70°C, $[\alpha]_D^{15}$ + 107° in water (c, 0·4).

Analysis.

Found: C, 50·0; H, 8·6; OMe, 51·1;

Calc. for $C_{10}H_{20}O_6$: C, 50·8; H, 8·5; OMe, 52·5%.

This substance also formed a crystalline hydrate on crystallisation from water, m.p. 37°C, $[\alpha]_D^{15}$ + 101° in water.

Analysis.

Found: C, 47·0; H, 8·6; OMe, 47·1;

Calc. for $C_{10}H_{20}O_6,H_2O$: C, 47·2; H, 8·7; OMe, 48·8%.

Complete Methylation and Isolation of 2:3:4:6-Tetramethyl Galactose Anilide.

The crystalline galactoside (1 gm.) was methylated completely by dissolving in a mixture of acetone (20 c.c.) and water (20 c.c.) and treating with methyl sulphate (30 c.c.) and sodium hydroxide (70 c.c. 30% solution) dropped in, in 1/10th quantities every ten minutes at 56°C. The excess alkali was neutralised with sulphuric acid and the methylated sugar extracted with chloroform. On removing the chloroform a liquid (0·6 gm.) was obtained. This was then remethylated with methyl iodide (25 c.c.) and silver oxide (30 gm.), the oxide being added in 1/4th quantities every 20 mins. and the flask being kept at 44°C for 6 hours. The contents of the flask were then extracted with four portions (50 c.c.) of boiling ether. On removing the ether, the liquid (0·6 c.c.) obtained was distilled at 0·05 mm. to yield a colourless liquid, and was then hydrolysed to the free sugar by heating with 7% hydrochloric acid (7 c.c.) at 100°C for 2 hours. The acid was neutralised with silver carbonate, the solution filtered and taken down to dryness at 50°C/15 mm. A syrupy tetramethyl gal-

actose (·6 gm.) was obtained. This was then digested for 3 hours at 100°C with freshly distilled aniline (1 c.c.) and alcohol (4 c.c.). On leaving to stand overnight a white solid was obtained. It was then filtered off and recrystallized twice from hot absolute alcohol. It had m.p. 192°C alone and in admixture with the authentic 2:3:4:6-tetramethyl galactose anilide.

Analysis.

Found: OMe, 40·8;

Calc. for $C_{16}H_{25}O_5N$: OMe, 39·9%.

The Isolation of 2:4:6-Trimethyl α-Galactose.

The trimethyl methylgalactoside (1 gm.) was hydrolysed for 2 hours at 100°C with 7% hydrochloric acid to remove the glucosidic residue. The acid was neutralised with silver carbonate, the solution was filtered and taken down to a syrup at 50°/15 mm. The sugar was extracted with ether from which solvent it was deposited in white crystals. It was recrystallised twice from a mixture of ether and petrol-ether and was obtained as

needle shaped crystals m.p. 104°-105°C, $[\alpha]_D^{18}$ + 124° in water (c, 0·4).

Analysis.

Found: C, 48·2; H, 7·9; OMe, 40·0;

Calc. for $C_9H_{18}O_6$: C, 48·7; H, 8·1; OMe, 41·9%.

Preparation of 2:4:6-Trimethyl galactose anilide.

The trimethyl galactose (0·33 gm.) was digested with aniline (0·5 cc.) and ethyl alcohol (2 cc.) for 3½ hours at 100°C and allowed to stand overnight. The white solid (0·4 gm.) which crystallised out was filtered off and recrystallised from hot ethyl alcohol, m.p. 179-180°C. $[\alpha]_D^{20}$ - 96° in acetone.

Analysis.

Found: C, 60·4; H, 8·0; N, 5·0; OMe, 32·0;

Calc. for $C_{15}H_{23}O_5N$: C, 60·6; H, 7·8; N, 4·7; OMe, 31·3%.

Osazone Formation and the Isolation of 4:6-dimethyl Galactosazone.

The above sugar (0·5 gm.) was dissolved in water (10 c.c.) phenyl hydrazine (0·5 c.c.) and glacial acetic acid (1 c.c.) added, and heated at 90°C for 1 hour. Owing to the formation of a tar the solution was transferred to another tube and heated at 90°C for a further hour. Long yellow needles (0·4 gm.) were obtained, and were recrystallised from aqueous alcohol, m.p. 158°C, $[\alpha]_D^{20}$ - 25° in alcohol (c, 0·3).

Analysis.

Found: C, 62·3; H, 6·5; OMe, 14·9; N, 15·6%.

Calc. for $C_{20}H_{26}O_4N_4$: C, 62·1; H, 6·8; OMe, 16·1; N, 14·5%.

Isolation of 2:4:6-Trimethyl δ-Galactonolactone and its crystalline amide.

The trimethyl galactose (1 gm.) in water (14 cc.) was treated with bromine (2 cc.) for 26 hours at 35°C and for 22 hours at 18°C. There was no reducing action

at the end of this time so the bromine was removed by aeration, the solution neutralised with silver carbonate and the silver precipitated from the solution by passing hydrogen sulphide gas through it. The solution was taken to dryness at 50°/15 mm. and the yellow syrup (0·7 gm.) was heated at 100°/0·01 mm. for 2 hours. It had $[\alpha]_D^{15}$ + 152° in water (c, 0·2) (Initial value) falling to + 122° (45 minutes); + 112° (2 hours); + 90° (4 hours); + 50° (16 hours, constant value).

Analysis.

Found: OMe, 40·4; 0·165 gm. required 14·9 cc. of N/20 NaOH.

Calc. for $C_9H_{16}O_6$, OMe, 42·4%; N/20 NaOH, 15·0 cc.

A crystalline amide (0·3 gm.) was formed by treating the lactone (0·3 gm.) overnight with methyl alcoholic ammonia, removing the solvent under diminished pressure at room temperature and twice recrystallising the product obtained from acetone. It formed lustrous plates m.p. 167°C, $[\alpha]_D^{12}$ + 74° in water (c, 0·3).

Analysis:

Found: C, 46·0; H, 7·8; OMe, 37·6; N, 6·2;

Calc. for $C_9H_{19}O_6N$: C, 45·6; H, 8·1; OMe, 39·2; N, 5·9%.

Weerman Reaction on the Crystalline Amide from 2:4:6-trimethyl δ-galactonolactone

To the amide (0·11 gm.) in water (0·2 c.c.) was added a solution (0·2 c.c.) of 6·9% sodium hypochlorite, and the mixture kept at zero for 1½ hours. A saturated solution of semi-carbazide hydrochloride (0·6 c.c.) and some solid sodium acetate were then added. In the case of one reaction carried out with gluconamide (0·12 gm.), employing the same quantities and conditions a copious white precipitate was formed, but in that of the amide from the trimethyl δ-galactonolactone, no appreciable quantity of precipitate was obtained.

Oxidation of Lactone with Nitric Acid.

The lactone (2 gm.) was oxidised with nitric acid (10 cc. s.g. 1·40) for 1 hour at 52°C and for 4 hours at 90°C. The nitric acid was removed by distilling under reduced pressure at 80°C, water being added to the flask from time to time. When all the nitric acid had been removed, the solution was evaporated to dryness and then refluxed for 6 hours with 3% methyl alcoholic

hydrogen chloride (100 c.c.). The acid was neutralised with silver carbonate and the alcohol removed at 80°/15 mm. A syrup (0·2 gm.) was obtained, distilled in a high vacuum and converted into the amide (0·05 gm.) by treatment with methyl alcoholic ammonia. It had zero rotation, reduced ammoniacal silver nitrate and melted to a red liquid at 165°-170°C. It was probably impure tartronamide (Found: OMe 1%).

Regeneration of 2:4:6-Trimethyl Methyl galactoside from 2:4:6-Trimethyl Galactose.

The sugar in the form of a syrup (0·1 gm.) was dissolved in 0·7% methyl alcoholic hydrogen chloride (10 c.c.) and the change in rotation observed. It rose from an initial value of + 52° to + 55° after 13 hours, + 82° after 26 hours to a constant value of + 100° in 70 hours. Neutralisation of the acid with silver carbonate and removal of the alcohol under diminished pressure at 50°C gave the crystalline trimethyl methyl-galactoside in quantitative yield.

Examination of Fraction (C).

This fraction was obtained as a pale yellow syrup, soluble in ether and chloroform. It was found difficult to free from traces of the 2:4:6-trimethyl methyl galactoside by distillation, but was purified by refluxing the distillate four times with light petroleum (60-80°), in which the trimethyl methyl galactoside was soluble.

Found $[\alpha]_D^{15}$ - 14° in chloroform (C, 0·4).

OMe, 39·9. Glucosidic residue OMe, 14·0 by a modification of Freudenberg and Soff (28).

Calculated for $C_9H_{18}O_6$ OMe 41·9%.

This syrup gave a strong Seliwanoff reaction with resorcinol and the Bredereck reaction with ammonium molybdate. Positive tests were obtained with fructose and trimethyl-methyl-fructofuranoside as controls, but negative results were obtained for galactose, glucose, 2:4:6-trimethyl galactose, fucose, digitoxose, rhamnose and methyl laevulate. Agar-agar, too, on mild hydrolysis with mineral acids gave the Bredereck reaction strongly.

The syrup was treated for 2 hours at 100°C with 6% sulphuric acid, the acid was then neutralised with bar-

ium carbonate, the solution filtered, taken down to dryness and esterified with 5% methyl alcoholic hydrogen chloride by refluxing for 6 hours. The acid was neutralised with silver carbonate and on distillation in a high vacuum of the syrup obtained after removal of the solvent, more methyl laevulate was collected, together with the unchanged syrup. This seems to indicate at least one probable source of this ester. During the hydrolysis of this syrup with 6% sulphuric acid, a resin, similar to that obtained by the hydrolysis of methylated agar itself by mineral acids, was also formed. Hydrolysis with 6% hydrochloric acid also, along with esterification and distillation gave the methyl laevulate and this resin, as well as the unchanged ketoside. It is clear therefore that this fraction is very sensitive to mineral acids.

Hydrolysis of syrup (C) with N/5 oxalic acid.

The syrup (1·25 gm.) was heated for 2 hours at 90°C with N/5 oxalic acid (20 cc.). The rotation did not change appreciably during the hydrolysis,

$[\alpha]_D^{20}$ + 16° (Initial) falling to $[\alpha]_D^{20}$ + 14° (Final)

but the solution became reducing towards Fehling's solution. The oxalic acid was neutralised with pure calcium carbonate, the solution filtered, taken down to a syrup and dried with benzene and methyl alcohol. It was extracted with chloroform, filtered to remove any inorganic impurities and on removal of the chloroform a clear amber glass (1 gm.) was obtained.

Found $[\alpha]_D^{14}$ + 27·4° in water, OMe 26·1%.

0·0656 gm. ≡ 14·54 cc. N/20 Iodine solution.
An appreciable quantity of iodoform was formed during the reaction.

It was not found possible to prepare a crystalline phenyl osazone. An attempt was therefore made to prepare the 2:4-dinitrophenylhydrazone.

The free sugar (0·2 gm.) was dissolved in a small quantity of alcohol and a few c.cs. of an alcoholic suspension of 2:4-dinitrophenylhydrazine were added. The mixture was heated for 20 minutes on a water bath and allowed to stand overnight, the solution was made acid by the addition of about a c.c. of glacial acetic acid and then diluted with water, a precipitate was obtained, which was filtered off, dried, and extracted with hot chloro-

form; on addition of light petroleum a light brown powder was precipitated.

Found: OMe, 7·1 ; N, 9·3%.

The low figure for nitrogen makes it clear that the product cannot be a dinitrophenylhydrazone or phenylosazone. Speculations on its nature are useless in view of the fact that recrystallisation was impossible.

The use of p-nitrophenylhydrazine was likewise unsuccessful.

Oxidation of ketoside with nitric acid.

The ketoside (1 gm.) was heated at 90°C for 1½ hours with nitric acid (7 cc., s.g. 1·42), brown fumes were evolved. The nitric acid was then diluted with water and distilled off in a vacuum with the continuous addition of water during 18 hours. When all the nitric acid had been removed the syrup remaining was dried in the usual manner with methyl alcohol and benzene, and esterified by refluxing with 5% methyl alcoholic hydrogen chloride for 6 hours. The acid was neutralised

with silver carbonate, the solution filtered and the solvent removed in a vacuum. The syrup (0·3 gm.) obtained was distilled at the water pump. Two fractions were obtained.

1. b.p. 50°C/15 mm. white solid m.p. 49°-54°C.
2. b.p. 170°C/15 mm. yellow liquid.

The main bulk did not distil and even after refluxing for a further period of 6 hours with 5% methyl alcoholic hydrogen chloride, neutralisation and removal of solvent it still would not distil, so it was oxidised for another 1½ hours with nitric acid (2 ccs. s.g. 1·42).

The two fractions obtained above were treated with methyl alcoholic ammonia at zero for 2 days and a white precipitate was obtained in each case. The alcohol was decanted off and the precipitate washed and dried in a vacuum.

Fraction 1 from the m.p. of both the ester (m.p. 54°C) and the amide which darkened and sublimed about 300°C proved to be methyl oxalate.

The amide from Fraction 2 melted at 135·5-145°C and the methoxyl content was nil. The quantity was not sufficient for further purification and investigation.

The nitric acid used for the oxidation of the residue was removed by continuous distillation with water in a vacuum for 18 hrs. as before and the product obtained dried with methyl alcohol and benzene. It was refluxed with 5% methyl alcoholic hydrogen chloride for 6 hours, the acid was neutralised and the solvent removed in a vacuum. The ester was then transferred to a small distilling flask and distilled at the water pump. A small quantity of methyl oxalate was formed and a colourless liquid b.p. 180°C/20 mm. was collected, in the side tube of the flask. It was washed out with ether, the ether removed and methyl alcoholic ammonia added. A white solid (m.p. 305°-312°C) was formed, but again the quantity obtained was insufficient for analysis.

Complete methylation of Fraction (C).

The syrup (1 gm.) was methylated three times with methyl iodide and silver oxide. The silver oxide being extracted four times with boiling ether at the end of each methylation. The liquid obtained from the third methylation after removal of the ether was distilled in a high vacuum. It came over at 115°C at 0·06 mm. and

was collected as a colourless liquid (0·80 gm.), n_D^{14} = 1·4505.

Found: OMe = 56·8%.

$[\alpha]_D^{15}$ = +11°.

The rotation did not change on heating with 0·1 N HCl for 2 hours at 90°C.

Oxidation of liquid obtained above.

The fully methylated liquid (0·5 gm.) was heated for 1½ hours at 90°C with concentrated nitric acid (3·5 cc. s.g. 1·42). Copious brown fumes were evolved. The nitric acid was removed by continuous distillation with water for 2 days at 50°C/15 mm. When all the nitric acid had been removed, the water was then distilled off and the syrup (0·3 gm.) obtained dried with methyl alcohol and benzene in the usual manner, it was refluxed with 5% methyl alcoholic hydrogen chloride (50 c.c.) for 9 hours. The acid was neutralised with silver carbonate, the solution filtered and the solvent

removed under reduced pressure at 50°C. The syrup obtained was methylated once with methyl iodide and silver oxide, extracted four times with ether and on removal of the ether distilled in a vacuum. A fraction (0·26 gm.) distilling at 145°-160°C/20 mm. was collected.

Found: $[\alpha]_D^{14}$ + 25·6° in water.

OMe, 50%.

The ester (0·1 gm.) was then treated for 2 days at 0°C with methyl alcohol saturated with dry ammonia, the alcohol was removed in a vacuum desiccator. A yellow syrup was left and on treating this syrup with absolute alcohol a white solid was obtained. This solid was washed with alcohol and ether and then dried in a vacuum.

M.p. 214°C at which temperature it darkened. The solid was unfortunately not crystalline and in insufficient quantity for analysis. It was certainly not an amide of one of the dimethoxy succinic acids.

Complete methylation of Fraction (C) and careful fractional distillation of product obtained.

The syrupy ketoside (2 gm.) was methylated four times with methyl iodide and silver oxide as before, the syrup extracted with ether and subjected to careful fractional distillation in a high vacuum.

Three fractions were obtained.

1. 0·4 gm. b.p. 105°-110°C $n_D^{13\cdot5}$ = 1·4540 OMe 46%.
2. 0·6 gm. b.p. 110°-120°C $n_D^{13\cdot5}$ = 1·4521 OMe 52·6%.
3. 0·2 gm. b.p. over 120°C $n_D^{13\cdot5}$ = 1·4490 OMe 56·1%.

There was a residue which consisted of a pale yellow solid left in the flask.

Fraction (1) crystallized out on standing and after inoculating fraction (2) with the crystals obtained and keeping at 0°C for a day, partial crystallization was induced. No trace of crystallization of fraction (3) could be noticed after inoculation with a crystal from fraction (1).

The crystals from (1) and (2) were freed from the syrup by placing on a piece of porous tile.

Found: (1) m.p. 81°C. $[\alpha]_D^{13}$ + 57·3° in water.

C, 53·53; H, 8·06; OMe, 46·46%.

(2) m.p. 76-79°C.

C, 52·53; H, 8·21; OMe, 43·0%.

Calc. for $C_9H_{16}O_5$

C, 52·9; H, 7·9; OMe, 45·6%.

Mixed m.p. of the crystals (1) and (2) 74°-80°C.

The tile on which the crystals were freed from the syrup was then extracted with ether and the syrup obtained distilled in a high vacuum, b.p. 110°-115°/0·02 mm.

Found: OMe, 50·0%. $[\alpha]_D^{15}$ ± 0°; n_D^{14} = 1·4551.

An attempt was made to see if this syrup contained some fully methylated galactose, by preparing the 2:3:4:6 tetramethyl galactose anilide. The syrup (0·5 gm.) was hydrolysed for 2 hours at 100°C with 6% sulphuric acid (10 cc.). A resin similar to that obtained during

the hydrolysis of both the syrupy ketoside and methylated agar with dilute mineral acids was formed. The acid was neutralised with barium carbonate and the filtrate was taken down to dryness. No anilide however was obtained on treatment of the syrup with freshly distilled aniline and alcohol in the usual way. The syrup also gave strong ketose tests as did both the crystalline fractions.

S U M M A R Y.

1. Agar-agar after suitable preliminary treatment can be acetylated easily with pyridine and acetic anhydride.

2. The Agar acetate obtained can be simultaneously deacetylated and methylated to give methylated agar.

3. Hydrolysis of the methylated agar with dilute mineral acid, followed by esterification, gives methyl laevulate, 2:4:6-trimethyl methyl-galactoside and a substance behaving as a methylated ketoside.

4. The 2:4:6-trimethyl methyl-galactoside on hydrolysis gives 2:4:6-trimethyl α-d-galactose, the structure of which is proved by

(a) Complete methylation which gives 2:3:4:6-tetramethyl galactose.

(b) The isolation of a crystalline dimethyl galactose phenyl osazone.

(c) Oxidation with bromine water forming a trimethyl -galactonolactone, which gave a crystalline amide.

(d) Oxidation with nitric acid, followed by esterification and amide formation which gave products of indefinite composition, neither trimethyl mucic acid nor dimethoxy succinic acid being obtained.

5. It would appear therefore that the galactose units are of the β-form and linked at positions 1 and 3.

6. Methylated agar can also be hydrolysed with 1·2% methyl alcoholic hydrogen chloride to give a trace of methyl laevulate, 2:4:6-trimethyl methyl-galactoside and the substance behaving as a methylated ketose.

7. Extensive examination of the latter fraction has been hindered for lack of material. Complete methylation yielded a crystalline "Trimethyl anhydro-ketose" $C_9H_{16}O_5$ but oxidative degradation has so far been unsuccessful in determining the structure of this fragment. The original "dimethyl methyl-ketoside" appears to be a mixture but oxidation with nitric acid failed to produce any dimethoxy succinic acid which is taken as evidence that the two methoxyl residues apparently present are not adjacent.

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