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Title

Further Studies on the Structure of Agar-Agar.

CONTENTS

INTRODUCTION

Further Studies on the Structure of Agar-Agar.

Considering the great advances made in the elucidation of the structure of many of the polysaccharides it is very surprising that until recently so little was known about the fundamental structure of Agar-agar. A vast amount of work has been carried out on this very complex substance, but, owing to the very conflicting nature of these earlier results, no definite conclusions could be reached as to its constitution.

It was assumed during the last century that gelose or d-galactan, the carbohydrate pectin-like basal principle of agar, gave to the latter its jellifying properties and this has proved the basis of many investigations. Payen (1) was the first to extract this substance from algae and, from its characteristic property of forming gels and its similarity to the pectins, he called it gelose, assigning to it a formula $C_6H_{10}O_5$, but in 1875 Reichardt (2) identified the carbohydrate principle as parabin ($C_{12}H_{22}O_{11}$), a substance which had previously been prepared from carrots and beetroots.

In 1882 Greenish (3) found agar to be non-reducing to Fehling's solution and not fermentable by yeasts, even after hydrolysis with sulphuric acid, and assigned to it a formula, $4C_6H_{10}O_5 \cdot H_2O$. He obtained seven carbohydrate-like compounds by various treatments of "gelose", while Morrin (4) detected oxalic and mucic acids on treatment with dilute nitric acid. A few years later Bauer (5) identified the carbohydrate residue as a galactan already obtained from lucerne seeds and various non-starchy plants ($C_6H_{10}O_5$). Cran (6), working with bacterial cultures, showed that the gelose must consist of two carbohydrates, one producing a violet colour with iodine and used as a nutrient by bacteria and the other showing no colour reaction with the same reagent.

From these early investigations it was concluded that the substance which is chemically the basal principle of agar and to which the latter owes its jellifying property was a d-galactan, although the presence of a large number of different sugars has been reported amongst the hydrolysis products of agar which has added greatly to the existing confusion.

The presence of 33% galactose was first reported by König and Bettels (7) using the mucic

acid method and was substantiated by Lüdtke (8) (30 - 40%) using the mucic acid method, and also by the isolation of galactose methyl phenylhydrazone. Arabinose and glucose were reported by Greenish (3), lactose and a mixture of glucoses by Tollens and Bourgeois (9), pentoses and methyl pentoses by several workers Reichardt (10), Seber (11), Takao (12) and Matsui (13), the latter two workers showing also the presence of a ketose, such as fructose, and Furuichi (14) stated that a uronic acid was present. Lüdtke (8) also reported on acid hydrolysis, the presence of a non-reducing hexose and an acid which appeared to be laevulinic acid, and denied the presence of a uronic acid, a finding supported by Takahashi and Shirahama (15).

During this time several workers had reported the presence of sulphur. Neuberg and Ohle (16) studying the effect of certain reducing bacteria on agar obtained hydrogen sulphide gas and detecting the presence of 1.2% of the element postulated that agar contained a sulphate residue. Samec and Ssajevič (17, 18) suggested also that agar was a sulphuric ester of gelose, the sulphur being in organic combination, shown by the above bacterial action and the sulphuric acid set free on hydrolysis,

the bonds being so strong that neither dialysis nor electro-dialysis was sufficient to separate them. It was shown in the next year by Fairbrother and Mastin (19), from work on agar-ash, that even in the presence of dilute acids all the sulphur could be liberated as sulphuric acid. When agar was heated alternately with dilute hydrochloric acid and water they obtained an ash-free gel which, however, could not be heated without hydrolysis occurring, and which did not set to a gel again on cooling; so Fairbrother and Mastin (19) postulated that agar consisted principally of the calcium salt of an acid sulphuric ester. This was corroborated by Hoffman and Gortner (20) who showed also that all the sulphur was in the form of sulphuric acid, that sols of the free agar-acid did not gelatinise on cooling and that hydrolysis occurred when agar-acid sols were heated. They stated that the gel-formation of agar was due to the gelation of a salt and not of a complex polysaccharide.

Later investigations by Takahashi and Shirahama (15) showed that native agar, on heating with water at 130° under pressure, split up into two fractions separable by their solubilities in water, called δ- and λ-hydrato kantenic acids to differentiate them from the agar-acid of Hoffman and Gortner (20).

The δ-form (0.3% Sulphur) was insoluble in water while the λ-form (2.1% Sulphur) was obtained from aqueous solution, both forms containing 33-39% of a carbohydrate. No uronic acid was found in either modification although a trace of pentose was present in the soluble form.

Neuberg and Schwietzer (21) showed that agar could be separated by simple extraction with cold water at room temperature into two fractions. The soluble portion (10%) contained the sulphate residues (5% S.) and could not be induced to form a gel while the insoluble portion (90%) was found to be free from sulphur and to possess the same power of gelation as untreated agar. Since in the experiments carried out in this laboratory the agar used had been subjected to a thorough washing with water the results are only applicable to this insoluble portion of Neuberg and Schwietzer which is considered to be the essential carbohydrate of agar.

In addition the position had been further complicated by various workers investigating the portion now known as agar-ash. For example, Forbes, Beagle and Mensching (22) showed the presence of sulphur, calcium, magnesium, sodium, potassium, chlorine and phosphorus, and even arsenic was

reported to be present (Leroide and Tassidy (23)).

It was not until 1936 that an advance was made in the elucidation of the structure of agar by Pirie, (24) who reported that he was unable to acetylate the polysaccharide by the milder methods of acetylation, using pyridine and acetic anhydride, or with acetic anhydride in the presence of sulphur dioxide, chlorine, hydrochloric acid or zinc chloride, but by the acetolysis of agar, using acetic anhydride in the presence of sulphuric acid, he obtained crystals of heptaacetyl - dl- galactose, showing that a portion (12%) of the galactose in agar existed in the l-form. He also showed that if acetylation preceded complete hydrolysis he obtained heptaacetyl - dl- galactose, whereas if the sequence was inverted, or if the agar was subjected to such mild acetylation that complete hydrolysis did not ensue, he was unable to obtain the heptaacetyl galactose but isolated crystals of the pentacetyl dl- galactose. Pirie postulated that this formation of the heptaacetyl compound indicated that in agar the dl-galactose occurs as the aldehydic or straight-chain form and not in the form of the usual furanose or pyranose ring, a condition which would explain the formation of the

pentacetyl derivative, when hydrolysis preceded acetylation. This worker also confirmed the presence of l-galactose in agar by isolating this sugar after suitable treatment, from the products of hydrolysis using galactose-trained yeasts, but the yields were low and unsatisfactory (0.8%).

Meanwhile Percival and Sim (25) in Edinburgh had succeeded in protecting the free hydroxyl groups by acetylation with acetic anhydride and pyridine to form a chloroform-soluble agar acetate (CH_3CO, 39%). Deacetylation of this compounded yielded a substance to all appearances identical with the original polysaccharide, indicating that no decomposition had taken place during the acetylation process. The regenerated compound formed a gel under the same conditions as untreated agar and it contained no sulphur. This result is in agreement with the subsequent findings of Neuberg and Schwietzer (21) i.e. that the gel formation in agar was due to a complex polysaccharide and not to the gelation of a salt, in contradistinction to the findings of Hoffman and Gortner (20).

Simultaneous deacetylation and methylation with sodium hydroxide and dimethyl sulphate yielded a chloroform-soluble methylated agar (OMe, 32%)

(Percival, Munro and Somerville (26) and Percival and Somerville (27)), which was then hydrolysed with 6% sulphuric acid followed by treatment with methyl-alcoholic hydrogen chloride to yield a syrup giving, on distillation under reduced pressure, three fractions,

(a) An optically inactive mobile ester. 16%.

(b) Crystalline trimethyl methyl-galactosides. 65%.

(c) A syrup. 14%.

Fraction (a) was recognised as laevulinic ester, and the crystalline compound (b) was found to be 2 : 4 : 6-trimethyl methylgalactoside. Proof of the structure of this compound, which gave 2 : 3 : 4 : 6-tetramethyl galactose anilide on methylation and subsequent suitable treatment, depended on oxidation methods and the comparison of properties of derivatives with the already known derivatives of 2 : 3 : 4 : - and 3 : 4 : 6-trimethyl methylgalactoside. Dr. D.J. Bell (28), working in Cambridge, recently synthesised 2 : 4 : 6-trimethyl galactose by a method which leaves no doubt as to its structure, viz.

4 : 6-Benzylidene 2-methyl β-methylgalactoside ⟶ 4 : 6-benzylidene 3-p-toluenesulphonyl 2-methyl β-methylgalactoside ⟶ 3-p-toluenesulphonyl 2-methyl β-methylgalactoside ⟶ 3-p-toluenesulphonyl 2 : 4 : 6-trimethyl β-methylgalactoside ⟶ 2 : 4 : 6-trimethyl β-methylgalactoside ⟶ 2 : 4 : 6-trimethyl α-galactose.

A comparison of specific rotations, mutarotations, melting-points and mixed melting-points, confirmed the work of Percival and Somerville (27). In this portion of the agar molecule d-galactopyranose units are therefore linked by positions 1 and 3, and not by the usual 1 : 4 linkages as in starch, glycogen and cellulose or 1 : 2 linkages as found in Inulin (29). 1 : 3 linkages have since been found in damson gum by Hirst and Jones (29) and in the galactogen of the edible snail, Helix pomatia, by Baldwin and Bell (30).

Somerville (31) postulated that owing to the negative rotations of agar derivatives, disregarding the other components of the molecule, there must be a preponderance of β-glycosidic linkages and the structure, by reasons of geometry, must consist

of either a closed ring of six units or a staggered zig-zag chain which, taken in comparison with the straight chains of the other known polysaccharides, may have some bearing on the question of gel formation.

This thesis is concerned with a further examination of fraction (C), the nature of which was not decided upon by Somerville (31) owing to lack of material although, from its general properties, it was considered to be a ketose derivative.

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PART I

THE ISOLATION AND IDENTIFICATION OF 2 : 4-DIMETHYL 3 : 6-ANHYDRO β-METHYL-ℓ-GALACTOSIDE OBTAINED FROM METHYLATED AGAR BY HYDROLYSIS AND SUBSEQUENT METHYLATION

DISCUSSION OF RESULTS

The hydrolysis of methylated agar (OMe, 32.4%) with 2% methyl-alcoholic hydrogen chloride, as shown by Somerville (1), yielded three major fractions on distillation in a high vacuum.

(A) A white crystalline compound, bath temp. b.p. 125-165°/ .01 mm.

(B) A light yellow syrup, bath temp. 145-175°/ .01 mm.

(C) A residual syrup.

(In every distillation described in this thesis the recorded temperature represents the bath temperature.)

The residual syrup, which distilled at 210°/.01mm. was rehydrolysed with 2% methyl-alcoholic hydrogen chloride yielding two fractions,

(A) A white crystalline compound, 135-145°/.01 mm.,

(B) A light yellow syrup, 160-175°/.01 mm.,

similar to the corresponding fractions from the initial distillation, showing the residue to be an intermediary stage in the hydrolysis.

The crystalline fractions consisted mainly of the 2 : 4 : 6-trimethyl methylgalactoside of

Percival and Somerville (2) and, from the rotations $[\alpha]_D^{20°}$ ca. + 60° in chloroform, are clearly mixtures of the α- and β-forms. The crystals obtained had $[\alpha]_D^{19°}$ + 62° in chloroform and $[\alpha]_D^{18°}$ + 79° in water and, on recrystallisation from light petroleum, showed m.p. 65°C and $[\alpha]_D^{20°}$ + 105° in water. A very small amount of crystalline material m.p. 105° (OMe, 51%) was also obtained and would appear to be 2 : 4 - 6-trimethyl β-methylgalactoside. Bell and Williamson (3) synthesised 2 : 4 : 6-trimethyl α-methylgalactoside $[\alpha]_D^{20°}$ + 164° in water (C, 0.9), m.p. 73-74° (large fluctuations (20°C) in the melting point were obtained on account of its very hygroscopic nature) and 2 : 4 : 6-trimethyl β-methylgalactoside m.p. 111-112° and $[\alpha]_D^{23°}$ -41° (C, 5.0).

Difficulty was encountered in freeing the syrupy fraction from the trimethyl methylgalactoside by distillation but this was effected to a great extent by refluxing many times with light petroleum until the residue gave no change in rotation on subsequent extractions. A yellow syrup, OMe, 40.4%, $[\alpha]_D^{18°}$ -22° in chloroform which gave a very strong

Seliwanoff test (4), was obtained; this syrup was distilled, giving two fractions, the properties of which, however, appeared to indicate that no significant separation had been effected.

(1) 165- 170° / .03 mm. $n_D^{?°}$ 1.4691, OMe, 40.3%, $[\alpha]_D^{17°}$ - 21° in chloroform.

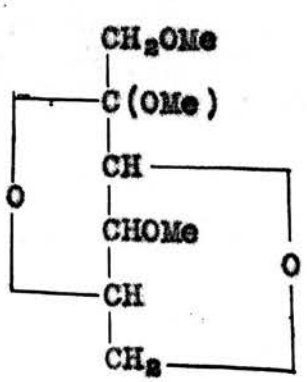
(2) 170 - 175° / .04 mm., $n_D^{20°}$ 1.4633, OMe, 40.4% $[\alpha]_D^{17°}$ - 24° in chloroform.

It has been shown later that this syrupy fraction (B) actually contains at least two constituents, which cannot be separated by extraction or distillation; namely a methylated methyl-d-galactoside and a methylated anhydro methyl-l-galactoside. This syrup ($[\alpha]_D^{18°}$ -22°) was methylated three times with methyl iodide and silver oxide, and the resulting syrup was distilled under reduced pressure. A clear colourless oil was obtained which partly crystallised on standing. The crystals were removed by tiling the mixture and the syrup, extracted from the porous tile, was remethylated. No further crystals were obtained showing that three Purdie treatments were sufficient for complete methylation.

Further yields of the crystals were obtained by methylation of the syrup remaining after re-

crystallisation of the trimethyl methylgalactosides and the syrup removed by the petroleum extractions of fraction (B), representing a presence of ca. 11.5% in methylated agar.

These crystals, first isolated by Somerville (1), were non-reducing, m.p. 82°, b.p. 85-90°/ .05 mm., $[\alpha]_D^{20°}$ + 75° in water and $[\alpha]_D^{20°}$ + 85° in chloroform (Somerville reports m.p. 81° and $[\alpha]_D^{13°}$ + 57° in water). The fact that this compound gave the Seliwanoff reaction, taken in conjunction with the high yield of laevulinic acid obtained on the hydrolysis of methylated agar with aqueous sulphuric acid, and its composition $C_6H_7O_2(OCH_3)_3$ shown by analysis, it was predicted by Somerville (1) to be a dimethyl anhydro methylketohexoside. This substance was exceedingly easily hydrolysed by dilute mineral acid, thus simulating a furanoside. On account of the small amount of material available however, the constitutional studies could not at that time be completed, but a tentative formulation as a dimethyl 3 : 6 anhydro-ketofuranoside was proposed.



Oxidation, followed by esterification and amide formation, supported this view since no amide of dimethoxy succinic acid was obtained and a pine-wood splint test, in comparison with digitoxose, appeared to remove the possibility of the presence of a 2-desoxy sugar.

Accompanying the crystalline fraction was the mobile liquid which appeared to be in the main a fully-methylated methylketoside, in that it gave a positive ketose reaction. On the assumption that this substance was related to the supposed dimethyl anhydro-methyl-ketoside and since it could be obtained in larger quantity, it was decided to investigate it first and, at the same time, to accumulate more of the crystalline material. Prolonged investigation by the well-tried oxidation methods of Avery, Haworth and Hirst (5) failed to reveal any clue to its structure, complete breakdown of the molecule resulting.

Attention was therefore turned to the crystalline dimethyl anhydro-methylhexoside. The hydrolysis with hot dilute mineral acid and with oxalic acid not leading to well defined products, the method applied by Peat and Wiggins (6), who had shown that

2 : 4-dimethyl 3 : 6-anhydro β-methylglucoside behaved like a furanoside in its ease of hydrolysis, was employed. Their method for the hydrolysis, i.e. by treatment with cold N- sulphuric acid, was attempted and it was found that the glycosidic group was smoothly eliminated at room temperature in 24 hours to yield a free dimethyl anhydro sugar $[\alpha]_D^{17^\circ}$ -23°.

Owing to the fact that a synthesis of 3 : 6-anhydro α-methyl-galactoside had been worked out by Ohle and Thiel (7), it seemed an easy matter to obtain the corresponding dimethyl derivative and since, disregarding the Seliwanoff reaction, there was a chance that the anhydro-sugar might be a galactose derivative, it was decided to attempt the preparation of the 2 : 4 -dimethyl 3 : 6- anhydro α -methyl - d - galactoside for purposes of comparison.

The synthesis was carried out in the following manner:-

d-galactose
↓
diacetone d-galactose
↓
6-p-toluenesulphonyl diacetone galactose
↓
6-p-toluenesulphonyl β - galactose
↓
6-p-toluenesulphonyl α- methylgalactoside
↓
3 : 6-anhydro α-methyl - d- galactoside.
↓

2 : 4-dimethyl 3 : 6-anhydro α-methyl d-galactoside.

The 6-p-toluenesulphonyl diacetone galactose of Freudenberg and Hixon (8) was treated with a mixture of glacial acetic acid and 50% acetic acid, forming a mixture of α- and β-forms of 6-p-toluenesulphonyl galactose, the β-form being obtained on crystallisation from water and the α-form from alcohol. The β-form was treated with 2% methyl-alcoholic hydrogen chloride, which converted it to the 6-p-toluenesulphonyl α-methylgalactoside, the tosyl group removed from the latter with hot sodium hydroxide solution, and the resulting 3 : 6-anhydro α-methyl-d-galactoside, m.p. (not recrystallised) 135-137° and $[\alpha]_D^{20°}$ + 78° in water, was treated three times with Purdie's reagents. The 2 : 4-dimethyl 3 : 6-anhydro-α-methylgalactoside was obtained as an oil, b.p. 90°/.05 mm., $n_D^{18°}$ 1.4641, $[\alpha]_D^{19°}$ + 75° in water and $[\alpha]_D^{20°}$ + 87° in chloroform, having almost identical rotations and boiling point with those exhibited by the crystalline material; it was also found that the refractive index of the superfused crystalline substance, $n_D^{69°}$ 1.4402, corresponded closely with that of the oily synthetic material at the same temperature ($n_D^{70°}$ 1.4412). It was found, moreover, that both 3 : 6-anhydro α-methylgalactoside and 2 : 4-dimethyl 3 : 6-anhydro α-methylgalactoside gave strong ketose tests according to the Seliwanoff

reaction so that the test is clearly not specific for a ketose and the possibility that the natural substance was an anhydro aldohexose could not be excluded, although it could not be the 2 : 4-dimethyl 3 : 6-anhydro β-methyl-glucoside of Peat and Wiggins (6) ($[\alpha]_D^{15°}$ - 1.7° in chloroform.) In spite of the fact that the specific rotations in chloroform and water were similar for the natural and the synthetic compounds it was impossible to induce crystallisation in the 2 : 4-dimethyl 3 : 6-anhydro α-methyl-d-galactoside on seeding with crystals of the natural material, and the reason was soon apparent in that, although hydrolysis with N-sulphuric acid was complete in 24 hours, the final rotation was $[\alpha]_D^{20°}$ + 22° instead of the -23° of the natural product. Thus, although the general properties were not inconsistent with it being a 3 : 6-anhydro aldohexose, it was clearly not derived from 3 : 6-anhydro d-galactose.

The experiments of N.W. Pirie (9) in 1936 had shown that a proportion (12%) of the galactose contained in agar was *l*-galactose, and his isolation of hepta-acetyl dl-galactose has been confirmed in this laboratory (10). Since he considered that the latter compound cannot be prepared by acetylation of the free galactose containing a pyranose ring, he postulated that the galactose existed in agar in the

straight chain form, and supported this by showing that heptaacetyl-derivatives were obtained when acetylation precedes hydrolysis breakdown, and pentacetyl compounds when the reverse is the case. It was thought that the presence of heptaacetyl -dl-galactose might be due to experimental conditions and Freudenberg and Soff (19) examined tetraacetyl methylglucosides under similar conditions of acetolysis: these authors were unable to isolate more than 2% of heptaacetyl glucose, although rotations seemed to indicate the presence of as much as 10%. Micheel and Ruhkopf (20) with their work on heptaacetyl compounds, have provided another possible course of reaction, that there is an inner aldol condensation with the formation of a cyclohexane (inositol) derivative, followed by an ethylene oxide ring closure which, on opening, would give an isomeric aldehyde. They were unable to isolate the carbocyclic compound, but, as the latter would be inactive, it would explain the presence of a racemate. It is clear, therefore, that Pirie's claim that aldehydo-galactose is present in the agar molecule must be taken with reserve but, apart from the production of l-galactose from hydrolysed agar,

after suitable treatment, using trained yeasts, he showed by the isolation of l-galactose-pentacetate that l-galactose was undoubtedly present in agar.

The fact that the rotations of the free sugars derived from the natural dimethyl anhydro methyl-hexoside (-23°) and the 2 : 4-dimethyl 3 : 6-anhydro α-methyl-d-galactoside (+ 22°) are approximately equal and opposite, although clearly possibly fortuitous, would have a significance if the natural substance were a derivative of 3 : 6-anhydro-l-galactose. This hypothesis could be readily tested by the synthesis of the only possible enantiomorph, viz.: 2 : 4-dimethyl 3 : 6-anhydro-β-methyl-d-galactoside, since the α-galactoside had proved to have properties not consistent with this view.

Owing to the elaborate preparations necessary for the synthesis of l-galactose, and although it has been found in nature among the products of hydrolysis of two plant materials (12), and flaxseed mucilage has been shown by Anderson (13) to be an improved source of the sugar, the small yields (6%), and the intricate separations required, excluded the possibility of synthesising the suspected 2 : 4-dimethyl 3 : 6-anhydro-β-methyl-l-galactoside.

The free dimethyl anhydro-sugars, synthetic (+ 22°) and natural (- 23°), unfortunately could not be induced to crystallise, and attempts to prepare crystalline anilides for comparison were complicated by the fact that tarry products were obtained at first, although subsequently crystalline derivatives were isolated. In a later stage of this research, however, during the removal of anhydride from the "ketose" fraction by hydrolysis with N-sulphuric acid (Part 2), crystals of the free 2 : 4-dimethyl 3 : 6-anhydro l-galactose were obtained giving m.p. 118°, and $[\alpha]_D^{15^\circ}$ -21° in water (c. 1.3).

At this stage of the research a private communication was received by Dr. Percival from Professor W. N. Haworth F.R.S. to the effect that a publication was about to appear from Birmingham on the subject of agar, and it was decided therefore to send a preliminary note to 'Nature' (14) outlining the work just described. While this was in the press an important letter by Hands and Peat (15) appeared in 'Chemistry and Industry' recording the isolation of the same dimethyl anhydro-methylhexoside from methylated agar by a closely similar process to that which we followed, and by the methylation of

3 : 6-anhydro α-methylgalactoside a crystalline substance had been isolated which appeared to be the enantiomorph. This was concluded on the basis of the melting-points, mixed melting-points and rotations, and an X-ray investigation also confirmed that the substances were monomeric. Further support for the view that the substance isolated from agar was derived from 2 : 4-dimethyl 3 :6-anhydro-l-galactose was furnished by the isolation of crystalline 2 : 4-dimethyl 3 : 6-anhydro-α-d-galactose, m.p. 115°, and $[\alpha]_D^{20°}$ +21° in dilute sulphuric acid (calc. for the free sugar), compared with m.p. 114°, and $[\alpha]_D^{20°}$ -22° in dilute sulphuric acid for the natural derivative, and a mixed melting-point of the two sugars of 100-107°; so the publication of Hands and Peat confirmed the possibility under consideration and their results on the major issue must receive priority.

At the same time it was obvious that the substance described in 'Nature' by Percival, Somerville and Forbes (14) as 2 : 4-dimethyl 3 : 6-anhydro-α-methylgalactoside, as an oil, b.p. 90°/.05 mm., $n_D^{18°}$ 1.4641 and $[\alpha]_D^{20°}$ + 87° in chloroform, had properties which did not agree with the substance m.p. 82°, $[\alpha]_D^{20°}$ -87° in chloroform, described as 2 : 4-dimethyl

d
l

3 : 6-anhydro-β-methylgalactoside by Hands and Peat (15), and the probability was that either the substance we described (14) was the β-form, or as seemed more likely from the specific rotation quoted by Hands and Peat, taken in comparison with the rotation of 3 : 6-anhydro-α-methylgalactoside $[\alpha]_D^{19°}$ + 84° in water (C,0.82) and 2 : 4-diacetyl 3 : 6-anhydro-α-methyl-galactoside $[\alpha]_D^{18°}$ + 59° in chloroform (C, 1.74) (7), that Hands and Peat had described the β-form and that it would necessarily follow, therefore, that the substance isolated from agar was 2 : 4-dimethyl 3 : 6-anhydro-β-methyl-l-galactoside.

It was therefore desirable to attempt the synthesis of 3 : 6-anhydro β-methyl-d-galactoside which had never been described, but the isolation of 2 : 3 : 4-triacetyl 6-p-toluenesulphonyl galactose α-l-bromide by Ohle and Thiel (7) was the obvious mode of approach, as it is well known that α-glucosyl bromides undergo Walden inversion on C_1 on treatment with silver oxide and water or silver carbonate and methyl alcohol (16).

The synthesis was carried out as below:

d-galactose

↓

diacetone galactose

↓

6-p-toluenesulphonyl diacetone galactose

↓

6-p-toluenesulphonyl galactose

↓

Tetraacetyl 6-p-toluenesulphonyl α-galactose

↓

2 : 3 :4-triacetyl 6-p-toluenesulphonyl α-d-galactose 1-bromide

↓

2 : 3 : 4-triacetyl 6-p-toluenesulphonyl β-methyl-galactoside

↓

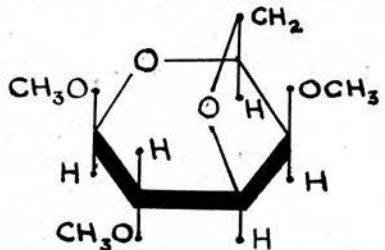
3 : 6-anhydro-β-methyl -d-galactoside

↓

2 : 4-dimethyl 3 : 6-anhydro-β-methyl-d-galactoside

Walden inversion was brought about by the treatment of the triacetyl 6-p-toluenesulphonyl α-d-galactosyl 1-bromide (m.p.147° and $[\alpha]_D^{20°}$ + 151° in chloroform) with methyl alcohol and silver carbonate, yielding a glass, $[\alpha]_D^{20°}$ -3° in chloroform, which appeared to be 2 : 3 : 4-triacetyl 6-p-toluenesulphonyl β-methyl-galactoside, followed by deacylation, yielding a crystalline derivative which, by its properties, (m.p. 118°, $[\alpha]_D^{20°}$ -114° in water and OMe, 17.0%) was clearly 3 : 6-anhydro-β-methylgalactoside. (c.f. m.p. 139° and $[\alpha]_D^{19°}$ + 84° in water obtained by Ohle and Thiel (7) for the corresponding 3 : 6-anhydro α-methylgalactoside.)

This substance was converted by methylation with methyl iodide and silver oxide into 2 : 4-dimethyl 3 : 6-anhydro-β-methylgalactoside, which crystallised spontaneously. In appearance it was identical with the product obtained from methylated agar and had an identical melting point 82°, and $[\alpha]_D^{20°}$ -77° in water and $[\alpha]_D^{20°}$ -86° in chloroform, compared with + 75° in water and + 85° in chloroform for the dimethyl anhydro methylhexoside derived from methylated agar. The mixed melting point of these substances was ca. 65° and, the analytical figures being correct, it was concluded that they were enantiomorphs and that, in consequence, the dimethyl anhydro methylhexoside derived from agar must be described as 2 : 4-dimethyl 3 : 6-anhydro-β-methyl-l-galactoside.



An attempt was made to convert the oily 2 : 4-dimethyl 3 : 6-anhydro-α-methyl-d-galactoside into an equilibrium mixture of the α-and β-forms in the hope of isolating the crystalline β-galactoside.

This was found to be surprisingly easy, for contact with cold 2% methyl-alcoholic hydrogen chloride caused a fall in specific rotation from + 98° to + 26° in 60 minutes. On neutralisation with silver carbonate and evaporation of the solvent at 25°/.15 mm., crystals of the 2 : 4-dimethyl 3 : 6-anhydro-β-methylgalactoside collected on the capillary and neck of the distillation flask, having, without recrystallisation, m.p. 81° and $[\alpha]_D^{20°}$ -76° in water.

These results were embodied in a short letter to 'Nature' (17), and there appeared simultaneously a letter by Haworth, Smith and Jackson (18), who had been investigating the properties of 3 : 6-anhydro sugars. These authors showed the extreme ease with which 2 : 4 dimethyl 3 : 6-anhydro-α-methylgalactoside could be converted into the β-form by contact with only a trace of acid, applied as a gas or in solution.

Further evidence that the 2 : 4-dimethyl 3 : 6-anhydro-methylgalactoside was derived from l-galactose was obtained by the preparation of the free sugar, both from the synthetic and natural products, the preparation of the anilides, oxidation to the lactone, and the preparation of the esters and amides of both series.

In the preparation of 2 : 4-dimethyl 3 : 6-anhydro methyl-d-galactoside for these experiments the improved method of Bell and Williamson (3) was used, in which the 6-p-toluenesulphonyl diacetone galactose is treated directly with 2% methyl-alcoholic hydrogen chloride. The crystalline compound, obtained from the solution by removal of solvent, amounted to a 93% yield of the 6-p-toluenesulphonyl α-methyl d-galactoside, compared with a 50% yield by the method of Ohle and Thiel (7).

The following tables show the relation between the properties of the natural and synthetic derivatives.

ORIGINAL STATE

Natural product from methylated agar:

Plate crystals, m.p. 82°, b.p. 85-90°/.05 mm. $n_D^{69°}$ 1.4402, $[\alpha]_D^{20°}$ + 85° in chloroform, $[\alpha]_D^{14°}$ + 75° in water.

$[\alpha]_D^{19°}$ -23° in N. sulphuric acid after 24 hours.

2 : 4-Dimethyl 3 : 6-anhydro α -methyl-d-galactoside.

Colourless oil, b.p. 90-95°/.01 mm., $n_D^{18°}$ 1.4641, $n_D^{70°}$ 1.4412, $[\alpha]_D^{20°}$ + 87° in chloroform, $[\alpha]_D^{19°}$ + 75° in water.

$[\alpha]_D^{20°}$ + 22° in N. sulphuric acid after 24 hours.

2 : 4-Dimethyl 3 : 6-anhydro β-methyl-d-galactoside.

Plate crystals, m.p. 82°, mixed m.p. with natural product 65°, $[\alpha]_D^{20°}$ -86° in chloroform, $[\alpha]_D^{20°}$ -77° in water.

FREE SUGAR

Agar derivative

Plate crystals b.p. 130°/.10 mm., m.p. 118°, $[\alpha]_D^{15°}$ -21° in water. OMe, 32.8%.

2 : 4-Dimethyl 3 : 6-anhydro-d-galactose.

Syrup, b.p. 125-130°/.05 mm., $[\alpha]_D^{20°}$ + 21° in water, OMe, 32.5%.

LACTONE

Agar derivative

Non-crystalline syrup, b.p. 135°/.01 mm., $n_D^{17°}$ 1.4629, $[\alpha]_D^{19°}$ -27° in water (67% lactone).

Crystals of acid gave $[\alpha]_D^{17°}$ -89° in chloroform.

Hydrolysis: $[\alpha]_D^{19°}$ -27° (initial) in water → -38° after 7 days at 20°C, heat at 80°C → -54° (21% lactone) after 2 days. This slow hydrolysis is in harmony with the assumption that a γ-lactone is present. Peat and Wiggins (6), however, pointed out that owing to the presence of the 3 : 6-anhydro ring, 2 : 4-dimethyl 3 : 6-anhydro δ-gluconolactone simulated a γ-lactone in the same way that the corresponding methylpyranoside simulated a furanoside.

2 : 4-Dimethyl 3 : 6-anhydro d-galactonolactone.

Non-crystalline syrup, b.p. 140°/01 mm., n_D^{19} 1.4607, $[\alpha]_D^{20°}$ + 22° in water, (lactone 53%). Crystals of unconverted acid $[\alpha]_D^{18°}$ + 83° in chloroform .

Hydrolysis: $[\alpha]_D^{20°}$ + 22° (initial) in water ⟶ + 27° after 2 days at 20°C: heat at 80°C.⟶ + 55° after 3 days, (16% lactone).

AMIDE

Agar derivative

Elongated needles, m.p. 151°, $[\alpha]_D^{16°}$ -71° in water.

2 : 4-Dimethyl 3:6-anhydro d-galactonamide.

Elongated needles, m.p. 150°, $[\alpha]_D^{19°}$ + 71.5° in water. Mixed m.p. with agar derivative 139°.

ESTER

Agar derivative

Prismatic crystals, b.p. 125°/.02 mm., $n_D^{19°}$ 1.4615, m.p. 47-49°, $[\alpha]_D^{16°}$ -64° in water, $[\alpha]_D^{10°}$ -72.5° in chloroform.

2:3:4-Dimethyl 3 : 6-anhydro methyl-d-galactonate.

Prismatic crystals, b.p. 125°/.02 mm., $n_D^{16°}$ 1.4634, m.p. 48-50°, $[\alpha]_D^{16°}$ + 63 ° in water, $[\alpha]_D^{14°}$ + 73° in chloroform.

Mixed m.p. with agar derivative 38-40°.

ANILIDE.

Agar derivative

Needle-shaped crystals m.p. 117° - insufficient material to determine a specific rotation.

2 : 4-Dimethyl 3 : 6-anhydro d-galactose anilide.

Needle-shaped crystals, m.p. 118°, $[\alpha]_D^{20°}$ + 100° in alcohol mutarotating to + 56° after 24 hours.

Mixed m.p. with agar derivative 106°.

It was unfortunate that the synthetic free sugar could not be crystallised although Hands and Peat (15) were able to describe both the natural and synthetic crystalline substances. Neither could the lactones be obtained crystalline, but the anilides, esters and amides were clearly enantiomorphs, and although the meltings points of the anilides were 3° lower than those recorded by Haworth, Smith and Jackson (18) the results here described, taken in conjunction with the findings of Hands and Peat in their independent investigations, leave no room for doubt that on the hydrolysis of methylated agar, followed by the removal of most of the 2 : 4: 6-trimethyl α-methylgalactoside and subsequent methylation, 2 : 4-dimethyl 3 : 6-anhydro β-methyl-l-galactoside can be isolated. Hands and Peat (15)

reported 11% of this material whereas Percival, Somerville and Forbes (14) recorded 16%. Many attempts have been made to repeat this early experiment but without success. At the same time it must be emphasised that the crystallisation of a substance from an oil, in which it is soluble, presents difficulties, especially with regard to the interpretation of the yields obtained. In the early experiments of Somerville an accidental choice of more favourable conditions seems to have been made. If the highest figure for trimethyl methylgalactoside is taken (this only refers to the crystalline material and it is certain that some of the β-form is present in the hydrolysate from methylated agar) i.e. 65% and 15% is allowed for the anhydro l-galactose, even then only 80% of the molecule is accounted for.

It is of obvious importance to decide whether the 3 : 6-anhydro l-galactose is preformed in agar or whether it is produced as the result of a side-reaction during methylation or hydrolysis. Anhydro sugars are formed in the laboratory by the well known method of the alkaline hydrolysis of p-toluenesulphonic esters (16, 21), and since agar is

usually quoted to be a sulphuric ester (22, 23), it might at first be thought probable that elimination of the sulphate groups had given rise to anhydride formation at some stage. As was shown by Percival and Sim (24), agar acetate containing no sulphur, could be deacetylated to give a specimen forming a rigid gel, and also that the methylated agar contained no sulphur. Furthermore, the agar used as starting material contained no appreciable amount of sulphur since it was subjected to a washing process for many days, so that it seems unlikely that 3 : 6-anhydro l-galactose is produced by the hydrolysis of a sulphuric ester.

The low acetyl content of acetylated agar (CH_3CO, 38%) and the low methoxyl content of methylated agar (OMe, 32%) compared with the corresponding values for $\left[C_6H_7O_2\ (OR)_3\right]_x$ (CH_3CO, 44.8% and OMe, 45.6%), may also be adduced as evidence that the anhydro l-galactose residues preexist in the agar molecule and are not the result of side reactions.

The precise mode of attachment is not yet clear. It is reasonably certain that they are attached by the reducing group, since agar itself shows but little reducing power and they cannot be attached by this residue alone, otherwise, on hydrolysis of methylated

agar, the crystalline 2 : 4-dimethyl 3 : 6-anhydro β-methyl-l-galactoside should be isolated directly. It is however necessary to remethylate after hydrolysis so that the anhydro residues must be attached at, at least, two points but whether 2-methyl 3 :6-anhydro methyl-l-galactoside giving a 1 : 4-link or a 4-methyl 3 : 6-anhydro methyl-l-galactoside giving a 1 : 2 link is present in the hydrolysis products is not yet decided. At any rate the 1 : 3 linkages which appear to predominate in the agar structure are not involved in this case.

It is interesting at this stage to consider the formation of laevulinic acid during the hydrolysis of agar, and although not observed by the author using non-aqueous hydrolysing agents, it has been found by other workers. In previous work on agar by Lüdtke (11) the presence of laevulinic acid was noticed in the products of hydrolysis. Percival and Somerville (2) showed that methyl laevulate was a product (16%) of the hydrolysis of methylated agar with sulphuric acid; this was established by the isolation of the crystalline p-nitro and dinitrophenylhydrazones, and Hands and Peat (15) also drew attention to this fact.

The possibility that the substance arose from a 2-desoxy sugar, was engisaged by Somerville (1) since these substances are very prone to yield laevulinic acid, but no derivatives of this type have been encountered. It is well known that ketoses give greater yields of laevulinic acid than aldoses and this, together with the strong colour reactions exhibited by agar and methylated agar and its hydrolysis products, encouraged the view that a ketose might be one of the agar building units. Hands and Peat (15) stated that they could not find a methylated ketose among the hydrolysis products of methylated agar although they did not go into detail as to the reasons for that view.

The fact that the 3 : 6-anhydro galactose derivatives gave strong colour tests for ketoses certainly throws doubt on the earlier hypothesis, as was pointed out by Percival, Somerville and Forbes (14), so it was therefore of interest to decide whether 3 : 6-anhydro galactose derivatives gave laevulinic acid on treatment with 6% sulphuric acid, as in the hydrolysis of agar and methylated agar. A preliminary experiment gave misleading results but since this work was completed it has been found in this laboratory

that a substance, giving a copious yield of iodoform on treatment with sodium hydroxide and iodine in the cold, can be produced by the hydrolysis of 3 : 6-anhydro galactose derivatives (see p.95, part III). This substance is in all probability laevulinic acid, although absolute identification has not yet been made.

It is not a simple matter to visualise the production of laevulinic acid from 3: 6-anhydro-*l*-galactose, but this also applies to its production from a ketohexose, the intermediate formation of furfural derivatives and the loss of formic acid being postulated as necessary in this case. Further work is clearly necessary if the mechanism of this reaction is to be understood.

EXPERIMENTAL

Purification of Agar-Agar.

100 G. of the polysaccharide (B.D.H. powder) were washed for several days by decantation with water (100 litres). After removing the supernatent liquid, an equal volume of absolute alcohol was added to the solid-water suspension, which was then filtered and the residue washed several times with alcohol. The purified agar was kept in contact with alcohol and portions, when required, were washed several times with ether and dried in air.

The Acetylation of Agar.

The air-dried agar (20g.) was treated with pyridine (100 cc.) for 3 hours at 100°C. and, on cooling, a mixture of acetic anhydride (100 c.c.) and pyridine (50 c.c.) added. This was heated at 100° for 24 hours and allowed to stand at room temperature for 2 days. The mixture was then poured into a stream of water with continuous stirring, the total volume being about ten litres. The product was washed free from acid and pyridine with water, dried, and extracted several times with a mixture of acetone-chloroform (1.1), the product precipitated in light petroleum (b.p. 60-80°) and dried at 100°C, under reduced pressure.

Yield 21 g. CH_3CO, 38.0%

Preparation of Methylated Agar.

Purified agar acetate (21 g.) was dissolved in acetone (450 c.c.) at 40°, and dimethyl sulphate (105 c.c.) and sodium hydroxide solution (30%) (270c.c.) were added simultaneously, with stirring, in portions at 10 minute intervals, the temperature during this reaction being raised to 56°, followed by heating to 75° to remove the acetone. A light brown solid remained in suspension and this was filtered at the pump, redissolved in acetone, and remethylated as before. The operation was repeated once more. The methylated agar was separated from the solid material by extracting several times with chloroform, the solution washed with acid and water and dried over anhydrous sodium sulphate, from which solution it was obtained as a white powder by the addition of light petroleum (b.p. 60-80°) and dried at 100°/15 mm. Yield 12 g., $[\alpha]_D^{20°}$ -85° in chloroform (C, 0.89), OMe, 32.4%. The relative solubility of methylated agar in water was examined, 0.5 g. dissolving in 20 c.c. boiling water after 3 hours, and in 40 c.c. of water (20°C.) after 8 hours , a viscous liquid being obtained in both cases.

Typical Hydrolysis of Methylated Agar with Methyl-Alcoholic Hydrogen Chloride.

Methylated agar (12 g.) was refluxed for 19 hours at 70° with 2% methylated-alcoholic hydrogen chloride (400 c.c.) until the rotation remained constant ($[\alpha]_D^{20°}$ + 31.6°). The solution was neutralised with silver carbonate, filtered, and the methyl alcohol removed under diminished pressure. The residual syrup (10.6 g.), (OMe, 39%), was fractionally distilled in a high vacuum to yield the following fractions:

(1) 3.09 g., 125-135°/.01 mm., white crystalline compound, $[\alpha]_D^{20°}$ + 66° in chloroform (C, 1.8)

(2) 2.72 g., 135-145°/.01 mm., white crystalline compound, $[\alpha]_D^{19°}$ + 62° in chloroform (C, 1.0).

(3) 1.61 g., 145-165°/.01 mm., syrup containing 50% crystals, $[\alpha]_D^{19°}$ + 14° in chloroform (C, 1.1).

(4) 1.09 g., 165-175°/.01 mm., light yellow syrup, $[\alpha]_D^{20°}$ -15° in chloroform (C, 0.6).

(5) 2.15 g., residue, $[\alpha]_D^{20°}$ -12° in chloroform (C, 0.7).

In every distillation carried out in this work the recorded temperature represents the bath temperature

A syrup (fraction 5, 2.15 g.), which formed a glass on cooling (OMe, 42%) and which could be distilled ca. 210°/.01 mm., remained in the flask. This was re-hydrolysed using 2% methyl-alcoholic hydrogen chloride and worked up as before, the following fractions being obtained:

(6) 1.40 g., 135-145°/.01 mm., white crystalline compound, $[\alpha]_D^{18°}$ + 63° in chloroform (C, 2.0).

(7) 0.55 g., 160-175°/.01 mm., light yellow syrup, $[\alpha]_D^{18°}$ ± 0° in chloroform (C, 1.2).

Fractions (3), (4) and (7) gave very strong ketose tests according to the Seliwanoff reaction(4).

Seliwanoff Test: A ketose (50 mg.) and resorcinol (10 mg.) are heated with N-hydrochloric acid (10 c.c.) for 10 mins. on a water bath (100°) when a red colouration and a red precipitate, soluble in alcohol, are obtained. Aldoses give a similar test on much longer heating so that control experiments with glucose and fructose are necessary.

Fractions (1), (2) and (6) were combined and recrystallised from light petroleum (b.p. 60-80°) to give crystalline 2 : 4 : 6-trimethyl methylgalactosides (α- and β-mixture) (6.2 g.) m.p. 65° and $[\alpha]_D^{20°}$ + 105° in water (C, 1.3), representing, together with a

further 0.5 g. obtained from fraction (3), a yield of ca. 57% based on the weight of methylated agar employed, and 64% on the weight of the hydrolysed material from which it was isolated.

A syrupy residue (0.9 g.) remained, which gave a positive Seliwanoff test. This was kept aside and combined with similar fractions from other experiments which were subsequently extracted with light petroleum and methylated, as described below, for the purpose of isolating the dimethyl anhydro methylketoside. When treated in this way 7.0 g. of this syrup yielded 1.0 g. of the crystalline dimethyl anhydro methyl-hexoside, m.p. 82°, corresponding to a yield of 0.13 g. from the experiment under consideration.

The crystalline trimethyl-methylgalactoside (0.5 g.) was removed from fraction 3 and the residual syrup (1.1 g.), together with fractions 4 and 7, extracted several times with light petroleum, using glass beads to increase the syrup-solvent interface, and decanting the supernatent liquid hot, until the residue (2.3 g.) gave a constant rptation $[\alpha]_D^{18°}$ -22° in chloroform (C, 1.1), OMe, 40.4%. A portion of this syrup, giving a strong Seliwanoff test, was distilled in a high vacuum, two fractions being collected.

Fraction (A) 165-170°/.03 mm., 0.5 g., $n_D^{17°}$ 1.4691,
$[\alpha]_D^{17°}$-21° in chloroform (C, 1.0), OMe, 40.3%
(B) 170-175°/.04 mm., 0.6 g., $n_D^{20°}$ 1.4633,
$[\alpha]_D^{17°}$-24° in chloroform (C,0.6), OMe, 40.4%).
Both fractions gave strong Seliwanoff and Bredereck tests.

Bredereck Test: 0.1 g. ketose in water (10 c.c.), add 10 c.c. of 4% ammonium molybdate solution, and two drops of glacial acetic acid. Heat in boiling water, and within 4 or 5 mins. a deep blue colour develops.

Complete Methylation of this "Ketose" $[\alpha]_D^{18°}$-22°).

The syrup (3.5 g.), obtained by combining the products of $[\alpha]_D^{20°}$-22°, OMe, 40%, from three experiments, was dissolved in methyl iodide (40 c.c.), and silver oxide (13 g.) was added to the mixture kept at 42° C. Three successive additions of silver oxide (13 g.) were made hourly and the experiment maintained at 42° for a further 3 hours. The silver residue was removed by filtration, extracted four times with ether and the solvents removed from the methylated sugar under reduced pressure. The methylation was repeated twice and the pale yellow syrup obtained was distilled in a high vacuum, three fractions being collected.

Fraction (A) 90-95°/.01 mm., 1.5 g., $n_D^{18°}$ 1.4497, $[\alpha]_D^{17°}$ + 27° in chloroform, colourless oil giving 0.45 g. crystals on standing at 0°.

Fraction (B) 95-100°/.01 mm., 1.7 g., $n_D^{18°}$ 1.4471, colourless oil + 0.35 g. crystals after seeding with a crystal from (A).

Fraction (C) 100-105°/.01 mm., 0.4 g., $n_D^{18°}$ 1.4468, $[\alpha]_D^{17°}$ -6° in water + colourless oil with a trace of crystals (0.1 g.).

The crystals were removed by drying on porous tile, the syrup being extracted from the tile by refluxing several times with ether and removing the solvent under reduced pressure. This syrup was again methylated by the Purdie method (methyl iodide and silver oxide) and the resulting syrup was distilled in a high vacuum, three fractions being collected.

Fraction (1) 95°/.01 mm., $n_D^{17°}$ 1.4487, colourless oil giving 0.1 g. crystals.

(2) 95°/.01 mm., $n_D^{17°}$ 1.4492, colourless oil with a trace of crystals (0.05 g.).

(3) 105°/.01 mm., $n_D^{17°}$ 1.4467, colourless oil + no crystals.

Analyses:

OMe on crystals 43.8%

OMe on syrup 57.6%

Both crystals and syrup were non-reducing to Fehling's solution.

1.05 g. crystals were therefore obtained from 3.5 g. "ketose" syrup ($[\alpha]_D^{18^\circ}$ -22°, OMe, 40%), representing 0.69 g. dimethyl anhydro methylketoside from the hydrolysis under consideration (2.3 g. initial material ($[\alpha]_D^{18^\circ}$-22°).

Another source of the crystalline product was found to be the syrup (0.45 g. obtained on evaporation of the light petroleum washings from the extraction of the "ketose" syrup ($[\alpha]_D^{18^\circ}$ -22°). On evaporation and methylation as described above, crystalline material amounting to 0.5 g. was obtained from several experiments. This represents a yield of 0.15 g. from the experiment under consideration.

During the course of this experiment, after removal of the crystals, an oily methylated "ketose" (OMe, 57.6%. 2.5 g., $[\alpha]_D^{20^\circ}$+ 10° in chloroform) was obtained, which was shown later (Part III) to contain ca. 16% dimethyl anhydro methylhexoside in solution, representing the presence of 0.4 g.

The yield of crystalline dimethyl anhydro

methylhexoside is therefore 0.97 g., i.e. 8%, but it is estimated that the syrup contains 0.4 g., making a total yield of ca. 11.5% based on the weight of methylated agar employed.

Identification of crystals as 2 : 4-Dimethyl 3 : 6-anhydro β-Methyl-*l*-galactoside.

Properties:- Colourless plates, m.p. 82°, b.p. 85-90°/.05 mm. non-reducing to Fehling's solution, $n_D^{69°}$ 1.4402, Seliwanoff test positive, Bredereck test - green colour $[\alpha]_D^{20°}$ + 85° in chloroform (C, 2.1), $[\alpha]_D^{14°}$ + 75° in water (C, 5.6).

Analysis:-

Found: C, 52.53 ; H, 8.06 ; OMe, 44.0.

Calc. for $C_6H_7O_2(OCH_3)_3$:C, 52.9 ; H, 7.9 ; OMe, 45.6%

Hydrolysis with N-Sulphuric Acid.

0.113 G. of the anhydride crystals was dissolved in 4 c.c. N-sulphuric acid and the change in rotation followed at room temperature.

Initial $[\alpha]_D^{19°}$ + 71.2° ; 6 hours, + 22° ; 16 hours, - 11° ; and 24 hours, -24.9°, which remained constant, the solution being strongly reducing.

A large scale hydrolysis was therefore carried out on 1.2 g. anhydride with 40 c.c. N-sulphuric acid

for 24 hours at room temperature, giving a final $[\alpha]_D^{17°}$ -23°. The solution was neutralised with barium carbonate, being warmed to prevent the formation of the soluble barium bicarbonate, filtered, and evaporated to dryness under reduced pressure, the syrup being freed from water by evaporation with alcohol and benzene.

Yield 1.1 g. OMe, 32.8% ; Calc. for $C_6H_8O_3(OCH_3)_2$, OMe, 32.6%.

Several attempts were made to crystallise this syrup using suitable solvents, etc., and a small portion was distilled in a vacuum at 130°/.10 mm. to yield a yellow syrup, but no crystals were obtained.

During the removal of anhydride from the "ketose" portion, however, (Chapter II) plate-like crystals in sufficient quantity, after crystallisation from acetone, to give a m.p. determination and a rotation were obtained m.p. 118°, $[\alpha]_D^{15°}$ -21° in water (C 0.13).

Hands and Peat (15) obtained m.p. 114° and final rotation in acid -21° for the same derivative.

Formation of the Lactone from the Free Sugar.

1.0 G. of the free dimethyl anhydro sugar in water (14 c.c.) was treated with bromine (2 c.c.) for 24 hours at 35° and allowed to stand at 20° for a further 24 hours. 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, after filtration, the silver precipitated from the solution by means of hydrogen sulphide. This silver salt decomposition was repeated twice and the filtrate taken to dryness at 50°/15 mm. Conversion to the lactone was brought about by heating at 100°/.01 mm. for 2 hours, a brown syrup and a few needle-like crystals were obtained. Yield 0.9 g. A portion of the syrup was distilled at 135°/.01 mm. yielding a non-crystalline fraction with a large amount of charring. $n_D^{17°}$ 1.4629.

The crystals obtained were recrystallised from chloroform m.p. 146-150°, $[\alpha]_D^{17°}$ -89.3° in chloroform (C, 0.28); titration with $^N/_{40}$ sodium hydroxide solution in the cold proceeded smoothly so that the crystalline substance appeared to be the unchanged acid, whereas the syrup, which required boiling during the titration, was the lactone. As the acid is converted into the lactone by heating at 100° for 2 hours, the m.p. of the acid crystals does not seem to have any great significance as the melting-point will depend on how quickly the temperature is raised above 100°. The yield of

crystals was too small for further investigation.

Hydrolysis of the Lactone-Acid Mixture.

0.0408 G. of the syrupy lactone, $n_D^{17^\circ}$ 1.4629, was dissolved in water (4 c.c.) and the rotation followed at room temperature.

Initial $[\alpha]_D^{19^\circ}$ = -27°, 1 day, -34°; 3 days, -37°; 7 days, -38°. At this stage it was thought advisable to heat the solution to 80° to accelerate the hydrolysis, and after 1 day at 80°, $[\alpha]_D^{20^\circ}$ = -48°; 2 days, -54°; and 3½ days, -54°.

This experiment was repeated using 0.048 g. lactone, in water (4 c.c.) giving a final rotation $[\alpha]_D^{20^\circ}$ -50°. The solutions were titrated with N/40-sodium hydroxide solution, using phenlophthalein as indicator, and finding the first end-point without heating, representing a rough approximation to the acid content, and the second end-point, when the pink colour remains after boiling for two minutes, giving an estimate of the lactone content.

The initial solution (-27°) contained 33% acid and 67% lactone while the final equilibrium solution was 79% acid and 21% lactone.

Formation of the 2 : 4-Dimethyl 3 : 6-Anhydro Galactonamide.

Dry methyl alcohol, (3 c.c.) saturated at 0° with

ammonia, was added to the lactone (0.5 g.) and the mixture allowed to stand at 0° for 24 hours. The alcohol and ammonia were removed under reduced pressure in a vacuum desiccator, leaving a mixture of crystals embedded in syrup. The syrup was removed by washing with ether and the residue recrystallised from acetone in the form of elongated needles. Yield 0.08 g. Additional yields of the crystals were obtained by retreating the syrup from the ether extraction with methyl-alcoholic ammonia as before.

The amide was also prepared from the ester (vide infra) by treatment in the same way with methyl-alcoholic ammonia, giving a 50% yield.

m.p. 151°, $[\alpha]_D^{16°}$ -71° in water (C, 0.73).

Analysis

Found: OMe, 28.8 ; N, 6.7

Calc. for $C_8H_{15}O_6N$; OMe, 30.2 ; N, 6.8%.

Preparation of 2 : 4-Dimethyl 3 : 6-Anhydro Methyl-galactonate.

In an attempt to obtain a larger yield of amide crystals from the lactone-acid mixture the methyl ester was formed as an intermediary compound.

0.2 G. acid-lactone mixture was treated with 30 c.c. of 3% methyl-alcoholic hydrogen chloride at

70° for 6 hours. The solution was neutralised with silver carbonate and washed and worked up in the usual way.

Distillate : 0.15 g. 125°/.02 mm. $n_D^{19°}$ 1.4615.

giving small prismatic crystals, soluble in water, chloroform, acetone and ether. The crystals gave a faint Seliwanoff test. Recrystallised from acetone gave m.p. 47-49° $[\alpha]_D^{16°}$ -64.4° in water (C, 0.59), $[\alpha]_D^{10°}$ -72.5° in chloroform (C, 0.55).

Analysis.

Found: C, 48.9 ; H, 7.3, OMe, 40.7

Calc. for $C_8H_9O_3(OCH_3)_3$; C, 49.1; H, 7.3, OMe, 42.3%.

Formation of the 2 : 4-Dimethyl 3 : 6-Anhydro *l*-Galactose Anilide.

0.4 G. free sugar, 0.2 g. freshly distilled aniline and 5 c.c. alcohol were refluxed at 95° for 1½ hours, and allowed to stand in the refrigerator for several days, to yield a small quantity (0.01 g.) of an anilide m.p. 117°, but the quantity was insufficient for a determination of the specific rotation.

The Synthesis of 2 : 4-Dimethyl 3 : 6-Anhydro α-Methyl d-galactoside.

Preparation of diacetone galactose.

80 G. finely powdered galactose were added carefully, with shaking, to a cold mixture of dried acetone (2 litres) and concentrated sulphuric acid (56 c.c.), and the mixture mechanically shaken for 24 hours. The excess of galactose was filtered off and the light yellow filtrate was neutralised with anhydrous sodium carbonate. Excess sodium carbonate and sodium sulphate were removed by filtration and, after ascertaining that the solution was neutral and adding a little barium carbonate, the solution was taken to dryness under reduced pressure. The residue was shaken well with cold water and filtered, the yellow oil (acetone condensation products) being discarded, and the aqueous solution evaporated to dryness in a vacuum. Any further condensation products were removed by heating the syrup at 100°/15 mm. and the diacetone compound distilled over in a high vacuum.

Fraction; 49 g. 160°/.01 mm. (70% Theoretical).

Recovered galactose 30 g.

Preparation of 6-p-Toluenesulphonyl Diacetone - Galactose.

The method adopted was described by Freudenberg

and Hixon (8). Pure diacetone galactose (13 g.), prepared above, was dissolved in pyridine (26 c.c.) and treated with p-toluenesulphonyl chloride (14.3 g.) by the addition of the reagent in small portions. After standing at room temperature for 27 hours the solution was poured into water (750 c.c.) and, on rubbing with a glass rod, the syrup rapidly crystallised. After one recrystallisation from methyl alcohol (Yield 14 g.) it showed m.p. 94°.

6-p-Toluenesulphonyl d-Galactose.

6-p-Toluenesulphonyl diacetone galactose (13 g.) was mixed with 80 c.c. glacial acetic acid and an equal volume of 50% acetic acid, as described by Ohle and Thiel (7), and heated at 100° on a water bath. After 1 hour 10 mins. the rotation remained constant at $[\alpha]_D^{20°} = +24°$, or + 29° calculated as the free sugar. The mixture was evaporated at 30°/15 mm. to yield crystals of the 6-p-toluenesulphonyl galactose which were removed, and the filtrate was evaporated to dryness, the resulting syrup, on addition of alcohol, giving a crystalline mixture of α- and β-forms (cf. Ohle and Thiel (7)) Yield 4.9 g. $[\alpha]_D^{20°}$ + 60°, Initial ; + 44°, 24 hours ; + 36°, 2 days ; and + 27° after 4 days, (C, 0.9) in pyridine.

Ohle and Thiel found (for the β form) $[\alpha]_D^{0^\circ}$ + 14 → $[\alpha]_D^{18^\circ}$ + 32° in pyridine (C, 1.72).

Preparation of 6-p-Toluenesulphonyl α-Methylgalactoside.

6-p-Toluenesulphonyl β-galactose (7 g.) was refluxed with 70 c.c. methyl-alcoholic hydrogen chloride according to Ohle and Thiel (7), the reaction being complete after 30 minutes and 2.4 g. of the methylgalactoside separated on cooling. Further crops were obtained by evaporating the solution, the hydrochloric acid being first neutralised with silver carbonate to prevent the tosyl group splitting off.

Yield 3.8 g. m.p. (not recrystallised) 162°

$[\alpha]_D^{20^\circ}$ + 106° in pyridine (C, 0.141).

Ohle and Thiel, after several recrystallisations from methyl alcohol obtained m.p. 170° and $[\alpha]_D^{18^\circ}$ + 103° in pyridine.

Formation of 3 : 6-Anhydro α-Methyl-d-Galactoside.

6-p-Toluenesulphonyl α-methyl-d-galactoside (7.5 g.) was dissolved in boiling alcohol (600 c.c.) and titrated hot with N-sodium hydroxide, using phenolphthalein as indicator, until the pink colour was permanent. The solution was then evaporated

to dryness under reduced pressure and the residue extracted 4 times with boiling ethyl acetate, needle-like crystals being obtained by evaporating the ethyl acetate solution almost to dryness.

Yield, 2.4 g. (63%), m.p. (uncrystallised) 135-137°, $[\alpha]_D^{20°}$ + 78° in water (C, 0.45). This substance gave a strong Seliwanoff reaction.

Preparation of 2 : 4-Dimethyl 3 : 6-Anhydro-α-Methyl d-Galactoside.

3 : 6-Anhydro α-methyl-d-galactoside (2.4 g.) was dissolved in a small quantity of acetone (7-10 c.c.) and excess methyl iodide (40 c.c.) added for the first methylation. Silver oxide (45 g.) was added in portions at hourly intervals - 6 hours at 42° in all - and worked up as before. No acetone was required for the two subsequent methylations and the product was distilled in a high vacuum.

Yield 2.32 g. - colourless oil. 90-95°/.01 mm. OMe, 44.2%, n_D^{18} 1.4641, $n_D^{70°}$ 1.4412, $[\alpha]_D^{20°}$ + 87° in chloroform (C, 0.93), $[\alpha]_D^{19°}$ + 75° in water (C, 0.13). Strong Seliwanoff reaction.

No crystals were obtained on keeping at 0° for several days, so a further Purdie methylation was carried out in case the syrup was not fully

methylated, but no crystals were obtained on distillation. The use of various solvents, seeding with crystals from the agar derivative, etc., were also found to be unsuccessful.

The above series of reactions was repeated twice.

Preparation of 6-p-Toluenesulphonyl α-Methyl-d-Galactoside.

An improved method by Bell and Williamson (3) for the formation of this compound directly from the 6-p-toluenesulphonyl diacetone galactose (93% yield), instead of by the intermediary formation of 6-p-toluenesulphonyl α-galactose, (Ohle and Thiel (7)) giving a 50% yield, was introduced.

6-p-Toluenesulphonyl diacetone α-galactose (~10 g.) was refluxed for 30 minutes with 2% methyl-alcoholic hydrogen chloride (100 c.c.). On cooling the solution to 0° a quantity of product crystallised out, which was filtered and washed with a small quantity of methyl alcohol. Further crops were obtained by the addition of methyl alcohol and concentration of the mother liquor each time.

Yield 7.5 g. (93% theoretical).

Formation of 2 : 4-Dimethyl 3 : 6-Anhydro d-Galactose.

2 : 4-Dimethyl 3 : 6-Anhydro-α-methylgalactoside (0.48 g.) was dissolved in N-sulphuric acid (40 c.c.) and the rotation followed at room temperature. $[\alpha]_D^{20°}$ + 72° (initial) falling to + 21.7° after 8 hours - unchanged after several days - giving a strongly reducing solution which was neutralised with barium carbonate and worked up as before, the syrupy residue (0.3 g.) being dried by evaporating with alcohol and benzene.

No crystals were obtained, so a small portion of the syrup was distilled under reduced pressure, care being taken to prevent charring.

Fraction : 125-130°/.05 mm., no crystals were obtained even on supercooling and treatment with various solvents. $[\alpha]_D^{20°}$ + 14° in chloroform (C, 0.28), $[\alpha]_D^{20°}$ + 21° in water (C, 1.2), OMe, 32.5%.

Preparation of 2 : 4-Dimethyl 3 : 6-Anhydro d-Galactono-lactone.

2 : 4-Dimethyl-3 : 6-anhydro-d-Galactose (0.4 g.) in water (4 c.c.) was treated with bromine (0.4 c.c.) for 24 hours at 35° and allowed to stand for 24 hours at 20° until it was non-reducing to Fehling's solution. The bromine was removed by aeration, the solution neutralised with silver carbonate and, after filtration, the silver precipitated from the solution by the passage of hydrogen sulphide. This silver salt decomposition was repeated twice and the filtrate taken to dryness at 40°/15 mm.

Conversion to the lactone was brought about by heating at 100°/.01 mm. for 2 hours.

A brown syrup and a small quantity of needle-like crystals were obtained - yield 0.3 g.

The crystals, recrystallised from chloroform, gave m.p. 138°, $[\alpha]_D^{18°}$ + 83.1° in chloroform (C, 0.73), were acid to phenolphthalein and titration with N/40-sodium hydroxide solution in the cold proved them to be unchanged acid, whereas the syrup, which required boiling during the titration, was the lactone.

The syrupy lactone distilled at 140°/.01 mm.

$n_D^{19°}$ 1.4607 and had a rotation $[\alpha]_D^{20°}$ + 22° in water (C, 0.91) (53% lactone).

Hydrolysis of this Lactone-Acid Mixture.

The acid-lactone mixture (0.0384 g.) was dissolved in water (4 c.c.) and the rotation followed at room temperature. Initial $[\alpha]_D^{20°}$ + 22° ; after 1 day, + 26° ; 2 days, + 27°. The solution was then heated at 80°. After 14 hours, $[\alpha]_D^{20°}$ = + 40° ; 2 days, + 54° ; and 3 days + 55°.

This was verified using an experiment under the same conditions;0.0347 g. lactone in 4 cc. water, giving a final rotation $[\alpha]_D^{20°}$ + 55°.

The compositions obtained were, initial (+ 22°) 53% lactone and 47% acid, while the final equilibrium was 16% lactone and 84% acid.

Formation of 2 : 4-Dimethyl 3 : 6-Anhydro d-Galacton-amide.

Methyl alcoholic ammonia (3 c.c.) was added to the lactone (0.3 g.) and allowed to stand at 0° for 24 hours. The alcohol and ammonia were removed under reduced pressure leaving a mixture of syrup and crystals. The syrup, extracted with ether, was re-treated with alcoholic ammonia to yield additional crystals of the amide.

Yield 0.1 g. Needle-shaped crystals, m.p. 150°, $[\alpha]_D^{20°}$ + 71.5° in water (C, 1.04).

Analysis.

Found : OMe, 29.3 ; N, 6.9

Calc. for $C_8H_{15}O_8N$; OMe, 30.2 ; N, 6.8%

Mixed m.p. with agar derivative 139°.

Preparation of 2 : 4-Dimethyl 3 : 6-Anhydro Methyl-d-Galactonate.

0.4 G. of lactone-acid mixture was treated with 3% methyl-alcoholic hydrogen chloride (40 c.c.) for 6 hours at 70° and worked up in the same way as the natural derivative, giving a distillate.

Fraction; 0.14 g. 125°/.02 mm., $n_D^{16°}$ 1.4634, which yielded white crystals on standing at 0°.

The ester was also prepared from 2 : 4-dimethyl 3 : 6-anhydro-d-galactonamide which had not crystallised 1 g. amide and 25 c.c. N-sodium hydroxide were kept at 90° for several hours until all the ammonia had been evolved. 25 c.c. N-hydrochloric acid were added and the solution taken to dryness at 45°/10 mm. 3% Methyl-alcoholic hydrogen chloride (150 c.c.) was then added and the solution kept at 60° for 6 hours. Neutralisation was effected using silver carbonate, the mixture filtered and the solution taken to dryness, giving a white solid (sodium chloride) and a brown syrup.

The latter was extracted with acetone and distilled in a high vacuum.

Compound recrystallised from acetone gave m.p. 48-50°. Mixed m.p. with agar derivative 38-40°. $[\alpha]_D^{16°}$ + 63.3° in water (C, 0.59), $[\alpha]_D^{14°}$ + 73.0° in chloroform (C, 0.58).

Analysis.

Found : " C, 48.9 ; H, 7.4

Calc. for $C_6H_7O_3(OCH_3)_3$; C, 49.1 ; H, 7.3%.

Formation of 2 : 4-Dimethyl 3 : 6-Anhydro d-Galactose Anilide.

2 : 4-Dimethyl 3 : 6-anhydro α-d-galactose (0.6 g.) and freshly distilled aniline (0.3 g.) were dissolved in 4 c.c. alcohol and the solution allowed to stand at 95°C. for 90 minutes, but no crystals were obtained on cooling.

2 : 4-Dimethyl 3 : 6-anhydro β-methyl-d-galactoside (0.5 g.) was dissolved in N-sulphuric acid (5 c.c.) and kept at room temperature for 24 hours. After neutralisation with barium carbonate and evaporating to dryness, the reducing syrup (0.4 g.) was treated with 0.2 g. aniline and 3 c.c. alcohol at 90° for 1½ hours. On standing, needles appeared which were recrystallised from alcohol. (Yield 0.15 g.) m.p. 118° $[\alpha]_D^{20°}$ = + 100° in alcohol (initial), + 72° (30 mins.), + 61° (12 hours), + 56° (1 day, which remained constant after three days.

Found : N, 5.7 ; OMe, 21.5

Calc. for $C_{14}H_{19}O_4N$: N, 5.3 ; OMe, 23.4%

Difficulty was encountered in this preparation in early attempts because of the formation of brown tarry products.

Mixed m.p. with the specimen prepared from agar, 106°.

Synthesis of 2 : 4-Dimethyl 3 : 6-Anhydro β-Methyl-d-Galactoside

Tetraacetyl 6-p-Toluenesulphonyl Galactose.

6-p-Toluenesulphonyl galactose (4.5 g.) was dissolved in a mixture of pyridine (25 c.c.) and acetic anhydride (6.2 c.c.) and kept at room temperature for 2 days. After pouring into water and recrystallisation from ethyl alcohol, a product (4.0 g.) m.p. 114° was obtained, $[\alpha]_D^{20°}$ + 87° in chloroform (C, 0.6).

Analysis.

Found :	C, 50.0 ;	H, 5.3
Calc. for $C_{21}H_{26}O_{12}S$:	C, 50.0 ;	H, 5.2%

Ohle and Thiel (7) quote m.p. 117° and $[\alpha]_D^{18°}$ + 89° in chloroform.

The product thus appeared to be Ohle and Thiel's (7) tetraacetyl 6-p-toluenesulphonyl galactose.

2 : 3 : 4-Triacetyl 6-p-Toluenesulphonyl α-d-Galactosyl-1-Bromide.

The above tetraacetate (3.5 g.) was treated with acetic acid saturated with hydrogen bromide at 0°. The crystals dissolved after 2 hours and the mixture was kept for 3 hours at 0°. An excess of ether was added and the ethereal solution was washed with water and sodium bicarbonate solution, and dried over anhydrous calcium chloride. The product (2.5 g.) crystallised

on removal of the ether, and had m.p. 147° and $[\alpha]_D^{20°}$ + 151° in chloroform (C, 0.7).

Found : Br, 15.0

Calc. for $C_{19}H_{23}O_{10}SBr$: Br, 15.3%.

Ohle and Thiel report Br 15.4%, m.p. 147° and $[\alpha]_D^{20°}$ = + 157° in chloroform (C, 2.02).

2 : 3 : 4-Triacetyl 6-p-Toluenesulphonyl β-Methyl-galactoside.

The above galactosyl bromide (2 g.) was shaken with anhydrous methyl alcohol (75 c.c.) and silver carbonate (12 g.) for 15 hours, the solution being then free from bromide ions. After filtration and evaporation, the product (1.5 g.) was obtained as a glass $[\alpha]_D^{20°}$ -3° (C, 1.5) in chloroform.

Found : OMe 10.5

Calc. for $C_{20}H_{28}O_{11}S$: OMe 10.75%.

For the corresponding α-methylgalactoside, Ohle and Thiel (7) found $[\alpha]_D^{18°}$ + 102.4° in chloroform (C, 0.878).

3 : 6-Anhydro β-Methyl-d-Galactoside.

5 G. of the triacetyl 6-p-toluenesulphonyl β-methylgalactoside prepared as described above were dissolved in alcohol (100 c.c.) and titrated with N-sodium hydroxide solution at 80° until a permanent

pink colour remained to phenolphthalein, a few drops of which were added to the solution, the process taking 2½ hours. The product was evaporated and extracted with ethyl acetate several times, and on removal of the solvent the product rapidly crystallised (3 g.) On recrystallisation from a mixture of ethyl acetate and light petroleum (b.p. 60/80°) it had m.p. 118°, $[\alpha]_D^{20°}$ -114° in water (C, 0.7).

Found : C, 47.5 ; H, 7.0 ; OMe, 17.0.
Calc. for $C_9H_{18}O_5$: C, 47.7 ; H, 6.9 ; OMe, 17.6%.

For the corrgesponding α-methylgalactoside Ohle and Thiel (7) obtained m.p. 139°, $[\alpha]_D^{19°}$ + 84° in water (C, 0.82).

2 : 4-Dimethyl 3 : 6-Anhydro β-Methyl-d-Galactoside.

The 3: 6-anhydro β-methyl-d-galactoside (2.6 g.) was methylated by the same method as used with the α-form (page 55), using acetone as solvent in the first Purdie methylation.

The product (2.5 g.) crystallised completely on removal of solvent and distillation was unnecessary. m.p. 80° without recrystallisation and 82° after recrystallisation from light petroleum (b.p. 60-80°). Mixed m.p. with product (m.p. 82°) prepared by the action of methyl-alcoholic hydrogen chloride on the syrupy 2 : 4-dimethyl 3 : 6-anhydro α-methyl-d-

galactoside showed no depression.

With product m.p. 81° prepared from agar - mixed m.p. 65°, $[\alpha]_D^{20°}$-77° in water (C, 0.6) ; $[\alpha]_D^{20°}$-86° in chloroform (C, 0.5).

Found	C, 52.8 ;	H, 8.1 ;	OMe, 44.5
Calc. for $C_9H_{16}O_6$	C, 52.9 ;	H, 7.9 ;	OMe, 45.6%

The above series of reactions was twice repeated.

Conversion of 2 : 4-Dimethyl 3 : 6-Anhydro α-Methyl - d-Galactoside to the β-form.

2 : 4-Dimethyl 3 : 6-anhydro α-methylgalactoside (0.28 g.) dissolved in anhydrous methyl alcohol (7 c.c.) had $[\alpha]_D^{20°}$ + 98° (C, 4.0). To this solution was added 7% methyl-alcoholic hydrogen chloride (3.c.c.) making the concentration of hydrogen chloride 2%.

Time	$[\alpha]_D^{20°}$
0	+ 98°
5 mins.	42°
60 mins.	26°
2 hours	26°
5 hours	26°
Heated at 80° for 35 mins.	14°
Stood overnight	26°
Heated at 90° for 1 hour	10°

To this solution silver carbonate was added and

after filtration and removal of the solvent, crystals sublimed on the capillary and the neck of the flask, m.p. 81° $[\alpha]_D^{20°}$ -76° in water (C, 1.0).

This experiment was repeated on 0.5 g. without any intermediate heating, the hydrogen chloride being neutralised after 2 hours. Yield of crystalline 2 : 4-dimethyl 3 : 6-anhydro β-methylgalactoside - 0.2 g.

SUMMARY

(1) The syrup obtained by the hydrolysis of methylated agar (Percival and Somerville (2)) yielded three fractions on distillation.

(1) Methyl laevulate (16%).

(2) Crystalline 2 : 4-: 6-Trimethyl methyl-galactosides (65%).

(3) A light yellow syrup giving strong ketose reactions (OMe, 40%). (14%).

(2) Fraction (3) on further methylation was shown by Somerville (1), to yield a mobile syrup (OMe, 57%) and a crystalline dimethyl anhydro methylhexoside, m.p. 81°, giving a strong ketose test. This investigator suggested that it was a dimethyl 3 : 6-anhydro methylketo-furanoside on the basis of colour reactions and ease of hydrolysis by acids. The yield of the anhydro methylhexoside appears to be not less than 11%, but a precise estimate is difficult.

(3) Hydrolysis of the crystalline material was found to take place with cold N-sulphuric acid, thus resembling the behaviour of the 2 : 4-dimethyl 3 : 6-anhydro β-methylglucoside of Peat and

Wiggins (6), and 2 : 4-dimethyl 3 : 6-anhydro α-methyld-galactoside was synthesised for comparison. Although no crystals were obtained, the specific rotations in water ($[\alpha]_D^{20°}$ + 75°) and chloroform ($[\alpha]_D^{20°}$ + 87°) of this synthetic material were similar to those of the natural product, ($[\alpha]_D^{14°}$ + 75°, and $[\alpha]_D^{20°}$ + 85° respectively), but the hydrolysis with N-sulphuric acid gave a free-sugar $[\alpha]_D^{20°}$ + 22° in comparison with a final value of $[\alpha]_D^{20°}$ -23° obtained for the corresponding agar derivative.

(4) 2 : 4-Dimethyl 3 : 6-anhydro β-methyl-d-galactoside was synthesised, by methylating the previously unknown 3 : 6-anhydro β-methylgalactoside, and shown to be the enantiomorph of the natural material, having m.p. 82°, $[\alpha]_D^{20°}$ -77° in water and $[\alpha]_D^{20°}$ - 86° in chloroform.

(5) Comparison of the free-sugars, lactones, amides, esters and anilides of the synthetic and natural products afforded additional proof that the latter was derived from ℓ-galactose, in agreement with the results of Hands and Peat (15), and the crystalline anhydro "ketohexoside" must now be described as 2 : 4-dimethyl 3 : 6-anhydro β- methyl ℓ-galactoside.

(6) It would appear that the anhydro *l*-galactose is preformed in agar on account of the low acetyl and methoxyl values of agar acetate and methylated agar. Since 2 : 4-dimethyl 3 : 6-anhydro-β-methyl *l*-galactoside is only obtained from the hydrolysis products of methylated agar after further methylation, it is clear that the anhydro -*l*- galactose residues are not attached by the reducing group alone but by at least two points in the molecule.

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PART II.

ATTEMPTS TO INCREASE THE METHOXYL CONTENT OF METHYLATED AGAR AND A STUDY OF THE HYDROLYSIS OF THIS SUBSTANCE WITH OXALIC ACID

DISCUSSION OF RESULTS

It is now fairly well established that most polysaccharides consist essentially of long chains of monosaccharide residues united by glycosidic links. On this view the molecule may terminate in two end groups or may exist as an endless loop of monosaccharide residues. In the former case methylation, followed by hydrolysis, liberates fully-methylated monosaccharides, e.g. tetramethyl-glucopyranose from cellulose (1) with different properties from the 2 : 3 : 6-trimethylglucose, which constitutes the major portion of the products of hydrolysis of the methylated polysaccharide. From a knowledge of the proportion of the fully-methylated monosaccharide in the hydrolysis products, the chain length can be calculated. The method is of general application and has already been widely used, for instance, in cellulose (Haworth and Machemer (1)), glycogen (Haworth and Percival (2)) and inulin (Haworth, Hirst and Percival (3)).

It is evident however, that a large amount of methylated agar must be hydrolysed to obtain any appreciable quantity of the end-product. Only two hexose components have been found in the products of hydrolysis of methylated agar, using methyl-alcoholic

hydrogen chloride, 2 : 4 : 6 trimethyl methyl-d-galactoside (Percival and Somerville (4)), and after further methylation, 2 : 4 dimethyl 3 : 6-anhydro β-methyl -ℓ-galactoside (This thesis, part I), so that end groups, if any, would give rise to 2 : 3 : 4 : 6-tetramethyl methylgalactoside and 2 : 4 dimethyl 3 : 6-anhydro β-methyl-ℓ-galactoside, both compounds distilling below 100°C. in a high vacuum. No low-boiling fraction, however, had been observed by Percival and Somerville (4) or by Hands and Peat (5), from the hydrolysis of methylated agar using methyl-alcoholic hydrogen chloride, possibly on account of the very small quantity which would necessarily be obtained, but a similar experiment with 25 g. of methylated agar, under the conditions described in Part I (p.40) using 2% methyl-alcoholic hydrogen chloride, showed no trace of fully-methylated compounds.

An attempt was then made to introduce further methoxyl groups into the methylated molecule (OMe, 32.1%), in case the 'end group' was resistant to methylation but it was found impossible to increase the methoxyl content. Methylated agar (10 g.) was therefore subjected to an acetylation process using pyridine and acetic anhydride and, owing to its appreciable solubility in water, the product was

precipitated in a small volume of water (1 litre), forming a gelatinous mass. The product (6.5 g.) was obtained from this residue by extraction with chloroform and precipitation in light petroleum (b.p. 60-80°), but no acetyl groups had been introduced. A further yield of product (1 g.) was obtained by an extraction of the above aqueous solution with chloroform, followed by precipitation in light petroleum. This fraction was also found to be devoid of acetyl residues. This methylated compound, however, was methylated twice, using dimethyl sulphate and 30% sodium hydroxide solution, but the final product was found to have a similar methoxyl content (32.2%) to the starting material (32.1%).

A drastic method, devised by Muskat (7), and found suitable by Hess and Lung (6) for the complete methylation of starch, was also attempted. A solution of methylated agar (1 g., OMe, 32.1%) in anisole, cooled to -40°C, was treated with a solution of sodium in liquid ammonia for 2 hours. The ammonia was evaporated and the residue was treated with methyl iodide. The excess of methyl iodide was removed under reduced pressure, and the residue extracted several times with chloroform, the methylated agar (0.55 g.) being precipitated from light petroleum.

This product was found to have OMe, 31.4%, compared with the OMe, 32.1% of the starting material. This indicates that acetylation, using pyridine and acetic anhydride, followed by three Haworth methylations, using dimethyl sulphate and sodium hydroxide, is sufficient to protect all the free hydroxyl groups in agar.

Since certain gums, for instance damson gum (Hirst and Jones (10)), are known to be decomposed simply on boiling with water with the fission of a portion of the molecule (usually a pentose), it had been thought that, by a relatively mild hydrolysis of methylated agar, it would have been possible to split off some constituent of the molecule such as the hypothetical ketose (4) (11). From the present results, however, there appears to be no evidence that agar contains any easily hydrolysable residues.

A partial hydrolysis of methylated agar was carried out using oxalic acid, and the hydrolysis was stopped after three separate intervals to see if any identifiable methylated monosaccharide could be obtained. The rotation-time curve for the hydrolysis showed a minimum value($[\alpha]_D^{20^\circ}$ -54°) owing to the slow rate of solution of methylated agar in methyl alcohol.

The hydrolysis was stopped at three points, $[\alpha]_D^{20}$ -46°, -25° and + 26° (complete hydrolysis), the solution neutralised with barium carbonate and, after glycoside formation at room temperature, worked up in the usual way. Unchanged methylated agar was removed by precipitation with light petroleum from a chloroform solution of the dried syrup and, on evaporation of the solvents, a syrup was obtained, representing the hydrolysis products. These syrups were distilled in a high vacuum but in no case was it found possible to isolate any new degradation product (which may be destroyed by mineral acid), so that this method of attack proved unsuccessful.

EXPERIMENTAL

An attempted Acetylation of Methylated Agar.

Methylated agar [10 g., OMe, 32.1%, $[\alpha]_D^{19°}$ -78° in chloroform (C, 1.5)], was suspended in pyridine (100 c.c.) and treated with a mixture of pyridine (50 c.c.) and acetic anhydride (50 c.c.), the solution being kept at 100°C. for 24 hours and allowed to stand for 2 days at 20°. Owing to the solubility of methylated agar in water, the mixture was then poured into a small volume of water (1 litre) and formed a gelatinous mass, which was washed with water and extracted several times with chloroform. The chlsroform solution was dried over anhydrous sodium sulphate and precipitated in light petroleum (b.p. 60-80°).

Yield 6.5 g. CH_3CO, nil ; OMe, 32.1%

The supernatant aqueous solution from the precipitation of the acetylated compound was extracted with chloroform, and the chloroform extract washed with sodium bicarbonate solution and several times with water. After drying over anhydrous sodium sulphate, the solution was concentrated by removal of chloroform at 35°/15 mm., and the acetylated compound precipitated

from light petroleum.

Yield 1.0 g. CH_3CO, nil.

Methylation of 'Acetylated' Methylated Agar.

The products pf the above acetylation (7.0 g.) were dissolved in acetone (150 c.c.) and treated with dimethyl sulphate (35 c.c.) and 30% sodium hydroxide (90 c.c.), added simultaneously in $\frac{1}{10}^{th}$ portions at 10 minute intervals, the temperature during the reaction being raised to 56°, followed by heating to 75° to remove the acetone. The solid residue was filtered, dissolved in acetone and remethylated as before. The solution was then filtered and the solid material extracted several times with chloroform. The chloroform solution was neutralised with dilute sulphuric acid, washed several times with water, dried by contact with anhydrous sodium sulphate and precipitated in light petroleum.

Yield 3.5 g.

OMe, 32.2%, $[\alpha]_D^{20°}$ -78.2° in chloroform (C,0.9),

CH_3CO, nil.

Attempted Methylation of Methylated Agar by Treatment with Sodium in Liquid Ammonia and Methyl Iodide in the Presence of Anisole.

A solution of methylated agar (1 g.) in anisole

(50 c.c.), cooled to -40° with carbon-dioxide-ether freezing-mixture was added slowly to a solution of sodium (1 g.) in liquid ammonia (100 c.c.) in the manner described by Hess and Lung (6). After 2 hours the solution changed in colour from blue to yellow. The ammonia was then allowed to evaporate from an open vessel and the last trace was removed by heating on an oil bath (60-70°). Methyl iodide (60 c.c.) was then added slowly and the mixture refluxed for 2 hours at 70°, the excess methyl iodide being subsequently removed under reduced pressure. The residue was extracted several times with chloroform and the product was precipitated from light petroleum (b.p. 60-80°).

Yield, 0.55 g., OMe, 31.4%, $[\alpha]_D^{20°}$ -75° in chloroform (C, 0.9).

It was found impossible to account for the small yield in this experiment, evaporating the petroleum solutions, additional chloroform extractions and methoxyl determinations on the solid residues giving no clue to the loss in weight. Several solvents were tried instead of anisole but were found to be even less successful.

Hydrolysis of Methylated Agar with Oxalic Acid.

Methylated agar (1 g.) was heated at 80° with methyl alcohol (30 c.c.) and water (10 c.c.), containing oxalic acid (0.45 g.) (Haworth, Hirst and Percival (3)), and polarimetric observations were carried out.

$[\alpha]_D^{20°}$ Initial	-24°	- only partial solution
after 13 hours	-54°	- complete solution.
20 hours	-34°	
60 hours	-14°	
120 hours	+13°	
180 hours	+26°	
240 hours	+26°	

This was in fair agreement with the final reading ($[\alpha]_D^{20°}$ + 31.6°) obtained on the hydrolysis of methylated agar using 2% methyl-alcoholic hydrogen chloride, indicating that complete hydrolysis had been effected.

This experiment was repeated, the hydrolysis being stopped after 37½ hours at 80° ($[\alpha]_D^{20°}$ -24.8°).

Neutralisation of the acid was effected with calcium carbonate, the mixture being heated to prevent the formation of calcium bicarbonate, and the filtered solution evaporated to dryness under diminished pressure. The syrup was dried by the addition of

alcohol and benzene, followed by the removal of the solvents.

Yield, 0.8 g., yellow syrup, $[\alpha]_D^{16^\circ}$ -15° in chlofoform (C, 1.0). OMe, 34.5%.

The syrup was then treated with 1% methyl-alcoholic hydrogen chloride (100 c.c.) until it was non-reducing to Fehling's solution (70 hours at 20°C.). The solution was neutralised with silver carbonate, filtered and the filtrate evaporated to dryness under reduced pressure, the resulting syrup being distilled in a high vacuum.

Fraction (1) 0.25 g., 130-140°/.02 mm., white crystalline compound, $[\alpha]_D^{20^\circ}$ + 66° in chloroform (C, 1.2). m.p. 65°C, OMe, 52.1%.

(2) 0.10 g., 160-175°/.01 mm., light yellow syrup, $[\alpha]_D^{20^\circ}$ ± 0° in chloroform (C, 0.9), OMe, 41.2%.

The semi-solid residue, which was extracted with chloroform and precipitated in light petroleum, was shown to be chiefly unchanged methylated agar (0.1 g.).

A much shorter hydrolysis was carried out under the same conditions using 0.4 g. of methylated agar,

readings being taken every 20 minutes.

$[\alpha]_D^{20^\circ}$	Initial	-24°
after	20 minutes	-34°
	40 minutes	-43°
	60 minutes	-52°
	80 minutes	-55°
	100 minutes	-58° complete solubility.
	120 minutes	-54°

The minimum in the rotation curve was due to the fall in rotation caused by the slow rate of solution of the methylated agar in methyl alcohol being counteracted by the actual hydrolysis. This hydrolysis was stopped at this point ($[\alpha]_D^{20^\circ}$ -54°), neutralised and worked up as before, giving a heavy syrup, (0.2 g.) $[\alpha]_D^{20^\circ}$-55° in chloroform (C 1.1).

This hydrolysis was therefore repeated with methylated agar (5 g.), the reaction being stopped after 3 hours ($[\alpha]_D^{20^\circ}$-46°) and the syrup worked up as before. The residual syrup wad dissolved in chloroform and fractionally precipitated by the addition of light petroleum (b.p. 60-80°), two fractions being collected, and the solvents removed from the filtrate, yielding a syrup.

Fraction (1) white powder 2.1 g., OMe, 31.2%, $[\alpha]_D^{20^\circ}$ -65° in chloroform (C, 1.1), giving a Seliwanoff test on boiling for two minutes.

(2) white powder 1.3 g., OMe, 30.8%, $[\alpha]_D^{20^\circ}$ -66° in chloroform (C, 1.0), giving a Seliwanoff test on boiling for two minutes.

(3) obtained on evaporation of the petroleum solution. Yellow syrup 1.0 g., $[\alpha]_D^{20^\circ}$ ± 0° in chloroform (C,0.6). OMe, 39.2%, Seliwanoff test on boiling for one minute.

Fractions (1) and (2) appeared to be unchanged methylated agar and fraction(3) resembled the higher boiling fraction b.p. 210°C/.01 mm. obtained on the hydrolysis of methylated agar using 2% methyl-alcoholic hydrogen chloride (page 41).

Further Hydrolysis with Oxalic Acid.

Under similar conditions, methylated agar (3 g.) was hydrolysed until the rotation remained constant ($[\alpha]_D^{20^\circ}$ + 26°) and the product, worked up in the usual way, yielded the following fractions on distillation in a high vacuum:

Fraction (1) 125-140°/.01 mm., white crystalline compound, 1.2 g., $[\alpha]_D^{19°}$ + 59° in chloroform (C, 1.0).

(2) 140-155°/.01 mm., yellow syrup containing crystals (50%), 0.7 g. $[\alpha]_D^{19°}$ + 24° in chloroform (C, 0.9).

(3) 155-175°/.01 mm., light yellow syrup, 0.6 g., $[\alpha]_D^{19°}$ -3° in chloroform (C, 0.3), OMe 40.9%.

(4) 175-220°/.01 mm., heavy syrup, 0.3 g., OMe, 39.6%, $[\alpha]_D^{19°}$ -12° in chloroform (C, 0.6).

The products therefore did not seem essentially different from those obtained by hydrolysis using mineral acid.

SUMMARY

1. Attempts at the further methylation of methylated agar by acetylation, followed by re-methylation, failed to increase the methoxyl content.
2. Methylation of methylated agar using sodium in liquid ammonia and methyl iodide, in the presence of anisole (Muskat (7)), also failed to introduce additional methyl groups, showing that three treatments with dimethyl sulphate and sodium hydroxide apparently protected all the hydroxyl groups in agar.
3. Investigation of the products of the partial hydrolysis of methylated agar, using oxalic acid, failed to reveal the presence of any easily hydrolysable component such as a ketose.

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PART III

THE EXAMINATION OF THE FULLY METHYLATED "KETOSE"

AND THE ISOLATION OF

2 : 3 : 4 : 6-TETRAMETHYL GALACTOSE ANILIDE

INTRODUCTION

The hydrolysis of methylated agar using methyl-alcoholic hydrogen chloride, yielded a fraction of high boiling point, which exhibited very strong ketose colour reactions. On further methylation, this syrup yielded a crystalline compound, which was proved to be 2 : 4-dimethyl 3 : 6-anhydro-β-methyl-ℓ-galactoside (this thesis, Part I), and a colourless mobile syrup (OMe, 56%), predicted by Somerville (1) to be a fully methylated ketose. This was in keeping with the findings of several workers, for instance, Takao (2) Matsui (3) and Furuichi (4), who all reported the presence of a ketose, such as fructose, in the hydrolysis products of agar. Somerville, however, had based his conclusion on the distinct colour reactions shown by this syrup to the Seliwanoff and Bredereck tests, but it has since been shown (Part I) that 3 : 6-anhydro galactose derivatives also give positive reactions to these tests. Somerville, also, had investigated the possibility of the fully methylated syrup containing tetramethyl methylgalactoside, but he was unable to isolate the readily crystallisable tetramethyl galactose anilide. It was assumed, therefore, that this fully methylated syrup was a

ketose, such as a fructofuranoside and as this would agree with certain of its properties, e.g. specific rotation, it was decided to attempt the isolation of tetramethyl fructofuronamide by the oxidation method of Avery, Haworth and Hirst (5). The syrup was first hydrolysed with N-oxalic acid at 80° until the rotation remained constant ($[\alpha]_D^{20°}$ + 20°, after 30 minutes). The hydrolysed syrup, (1.5 g.) exhibiting strong reducing and ketose tests, was oxidised with nitric acid and after esterification and methylation, yielded 3 fractions, of which the first two appeared to be identical and, on treatment with methyl-alcoholic ammonia, yielded the same amide (0.2 g.) (recrystallised from alcohol, m.p. 218° and $[\alpha]_D^{19°}$ + 13° in water). This amide, however, bore no resemblance to any well-known compound, although its analysis was in reasonable agreement with that of the diamide of tetramethyl mucic acid.

Fraction (3), on treatment with methyl-alcoholic ammonia, yielded a trace of a needle-shaped crystalline amide, resembling a dimethoxy succinamide in crystalline structure and melting-point, but the high boiling-point of the fraction, from which it was derived, and the analytical figures threw doubt on this finding.

Nothing resembling the tetramethyl fructofuronamide of Avery, Haworth and Hirst (5) was isolated.

As no constructive evidence was obtained from this oxidation, attention was turned to the crystalline component of the ketose fraction, and the results of this work have been described earlier in this thesis. This compound, shown to be 2 : 4-dimethyl 3 : 6-anhydro β-methyl-ℓ-galactoside, exhibited strong Seliwanoff and Bredereck tests, and it was thought possible that the positive ketose colour reactions were due to the presence of this substance, or the syrupy α-form, in solution. It was therefore decided to remove this anhydride and this was carried out by a partial hydrolysis of the syrup (using N-sulphuric acid at room temperature). The residual syrup was extracted with ether to remove unchanged material, the free anhydro sugar being almost insoluble in this solvent. It was found possible, in this way, to obtain a small quantity of crystalline 2 : 4-dimethyl 3 : 6-anhydro-ℓ-galactose, m.p. 118° (Hands and Peat (6) quote 114°), but owing to its extremely hygroscopic nature, the crystals became syrupy almost at once. This explains the difficulty encountered in attempts at crystallisation during the examination of the properties of 2 : 4-dimethyl 3 : 6-anhydro-ℓ-galactose (Part I).

The ethereal extract was evaporated to dryness

and distilled, under reduced pressure, several fractions being collected. The presence of reducing sugars in the fractions of higher boiling point showed that this method did not completely exclude hydrolysed products, and that the faint ketose tests might still be caused by anhydro-sugar impurity.

An attempt was then made to remove any tetramethyl methylgalactoside which might be present due to the incomplete removal of the 2 : 4 + 6-trimethyl methyl-galactosides, or to the presence of dimethyl methyl-galactosides in the hydrolysis products of methylated agar. A portion (1.75 g.) of the mobile syrup ($[\alpha]_D^{20°}$ + 60°, OMe 54%), after removal of the dimethyl anhydro methyl-*l*-galactoside, was hydrolysed with N-sulphuric acid at 80° until the rotation remained constant ($[\alpha]_D^{20°}$ + 56°). The reducing sugar (1.6 g.) was treated with aniline, in the presence of alcohol, to yield crystals (0.03 g.) of 2 : 3 : 4 : 6-tetramethyl galactose anilide. Several crops of crystals (0.08 g.) were obtained on prolonged cooling (0°C.). The solution, on concentration, yielded a further crop of crystals (0.24 g.). The syrupy residue was treated as described by Haworth, Hirst and Ruell (10). A reducing syrup was thus obtained which was subjected

to methylation, using silver oxide and methyl iodide. On distillation, a mobile syrup was isolated (0.12 g.), OMe 56.3% and $[\alpha]_D^{19^\circ}$ + 50° in chloroform, which gave negative results when tested for the presence of ketoses by the Seliwanoff and Bredereck reactions. Owing to the very small yield, however, no conclusion could be drawn as to the presence or absence of a ketose in the original syrup.

Another portion of the syrup (2.6 g.), from which the dimethyl anhydro methyl-galactoside had been removed by treatment with N-sulphuric acid, extraction with ether and distillation, was hydrolysed as before and the product subjected to anilide formation. In all, 0.23 g. of tetramethyl galactose anilide was recovered. The non-crystallisable residue (2.2 g.) was heated with 3% sulphuric acid and, after treatment with barium carbonate, the aniline was removed by extraction with ether. The aqueous layer, on evaporation and methylation, yielded a colourless mobile syrup (X) 1.4 g., b.p. 90-100°/.01 mm., OMe 57% $[\alpha]_D^{15^\circ}$ + 38° in chloroform, which gave no colour reactions for a ketose. This appears to show that no ketose exists in the hydrolysis products of methylated agar.

The possibility of the presence of a pentose

or a methylpentose could not be excluded at this stage; the b.p. and methoxyl content being in agreement with, for example, the trimethyl methylrhamnoside of Hirst and Macbeth (7), (OMe 55.9%, b.p. 101°/9 mm.). Moreover, the presence of a pentose and a methylpentose, in the products of the hydrolysis of agar, has been reported by several workers, e.g., Reichardt (8), Takao (2) and Matsui (3). An estimation on dried agar, by the method of Norris and Resch (9), showed that no pentose or methylpentose existed in agar.

The residual syrup (X) (1.1 g.) was, therefore, hydrolysed using more vigorous conditions, 8% aqueous hydrochloric acid at 90°, and anilide formation was carried out on the products of hydrolysis (0.9 g.). Several crops of crystals (0.35 g.) were obtained by evaporation of the solution, and were shown to consist of 2 : 3 : 4 : 6-tetramethyl galactose anilide.

From the original "fully methylated ketose syrup", therefore, the only identifiable products which have been isolated were tetramethyl galactose anilide and 2 : 4-dimethyl 3 : 6-anhydro *l*-galactose.

It has been found also recently in this laboratory that both 3 : 6-anhydro α-methyl d-galactoside and 2 : 4-dimethyl 3 : 6-anhydro α-methyl d-galactoside, when hydrolysed by sulphuric acid under the conditions used

for the hydrolysis of methylated agar by Somerville (1), were partially destroyed during the treatment to give acid products, which reacted with sodium hydroxide and iodine solution in the cold, to give an immediate precipitate of iodoform. This behaviour suggests the presence of laevulinic acid, although sufficient quantities of material have not yet been available for an exact identification. Unfortunately, an earlier experiment along these lines gave negative results, and encouraged the belief that the laevulinic acid produced from agar was due to the decomposition of another sugar, such as a ketose or a 2-desoxy pentose. If however, the laevulinic acid production is due to the decomposition of 3 : 6-anhydro-ℓ-galactose, and the amount of methyl laevulate produced is certainly of the same order as the amount of 3 : 6-anhydro-ℓ-galactose derivatives so far isolated, it is clear that, whatever other residues are present in agar, there remains no reason to assume the presence of a ketose, at any rate in large proportion.

The presence of methylated galactose derivatives in the "ketose syrup" can be due either to an incomplete separation of the trimethyl methylgalactosides or to the presence of dimethyl methylgalactosides; both of

which would eventually become tetramethyl methyl-galactosides, owing to the methylation process necessary for the isolation of the anhydro *l*-galactose fragment. Attempts to form crystalline di- or monomethylgalactosazones from the unmethylated syrup, (p. 43, OMe 40%) were successful and another method of attack must be sought if this point is to be decided.

In many polysaccharides, e.g. xylan (12), glycogen (13) (14), galactogen (15) and the araban isolated from peanuts (16), dimethylhexose or monomethylpentose residues have been isolated, and this is of great importance with regard to the theory of cross-linkages. So far, however, it has not been possible to accumulate any information along these lines for agar, owing to the experimental difficulties.

EXPERIMENTAL

Hydrolysis of the Fully-Methylated "Ketose".

A specimen of the clear colourless syrup (0.4 g., $n_D^{9°}$ 1.4492, OMe 56%, $[\alpha]_D^{20°}$ + 16°), obtained after the removal of the 2 : 4-dimethyl 3 : 6-anhydro-β-methyl-*l*-galactoside crystals from the fully-methylated "ketose" fraction of the hydrolysis products of methylated agar, was heated with N-oxalic acid (20 cc. aqueous solution) and the temperature was maintained at 80°C.

$[\alpha]_D^{20°}$ = + 16° ; + 20° after 30 minutes; + 20° after 1 hour, and + 20° after 12 hours, the solution being then strongly reducing to Fehling's solution. The oxalic acid was neutralised by heating with calcium carbonate, the solution filtered and the residual yellow syrup dried and distilled under reduced pressure, b.p. 140-145°/.02 mm., to yield a yellow syrup, (0.09 g.), $n_D^{20°}$ 1.4592, $[\alpha]_D^{20°}$ + 26° in water (C, 1.0), OMe 48%, which gave a strong Seliwanoff test and was strongly reducing to Fehling's solution. The aniline acetate test showed the presence of furfural and the syrup decolourised a neutral potassium permanganate solution.

This experiment was repeated using the above

syrup (2 g.), under the same conditions but stopping the hydrolysis after 30 minutes, to yield a yellow syrup (1.6 g.), b.p. 140-145°/.01 mm., $n_D^{19°}$ 1.4553, $[\alpha]_D^{19°}$ + 19° in water (C, 0.6), OMe 52%, strongly reducing to Fehling's solution and giving positive furfural and Seliwanoff ketose tests.

Oxidation of this Hydrolysed Product.

The above reducing syrup (1.5 g.) was treated with concentrated nitric acid (10 c.c.) at 90° for 1½ hours in the manner described by Avery, Haworth and Hirst (5). The mixture was diluted with water and the excess nitric acid removed by distillation at 50°/ 16 mm., water being added continuously during two days. The water was then removed and the syrup dried by several treatments with alcohol and benzene, the solvents being evaporated under reduced pressure. Methyl-alcoholic hydrogen chloride (3%, 90 c.c.) was then added and the mixture kept at 80° for 4 hours. The solution was neutralised with silver carbonate, filtered and the filtrate evaporated to dryness at 40°/15 mm. The residual syrup was methylated, using methyl iodide (50 c.c.) and silver oxide (30 g.), and the fully methylated syrup (1.4 g.) distilled.

Fraction (1) 0.5 g., b.p. 90-95°/.01 mm., colourless syrup, $n_D^{17°}$ 1.4412, $[\alpha]_D^{20°}$ -21° in methyl alcohol (C,1.1), OMe 50%, COOMe 39.0%, non-reducing to Fehling's solution.

(2) 0.5 g., b.p. 95-105°/.01 mm., colourless syrup, $n_D^{17°}$ 1.4460, $[\alpha]_D^{19°}$ -23° in methyl alcohol (C, 0.6), OMe 49%, COOMe 39.4%, non-reducing to Fehling's solution.

(3) 0.15 g., b.p. 160-180°/.01 mm., heavy colourless syrup, $[\alpha]_D^{19°}$ ± 0° in methyl alcohol (C, 0.3), OMe 55%.

Fraction (2) was treated with methyl-alcoholic ammonia (5 c.c. saturated at 0°C.) and kept at 0° for 48 hours. The ammonia and the methyl alcohol were removed under reduced pressure, giving a small amount of crystalline material (0.1 g.) which was recrystallised from alcohol, m.p. 218°, $[\alpha]_D^{19°}$ + 13° in water (C, 0.3).

Analysis

Found : C, 44.3 ; H, 7.3 ; N, 10.6%

Calc. for $C_{10}H_{20}O_6N_2$; C, 45.4 ; H, 7.5 ; N, 10.6%.

Ester determination on Fraction (2) ; COOMe, 39.4,

Calc. for $C_{12}H_{22}O_8$; COOMe, 40.1%.

Similar crystals (0.1 g.) m.p. 218°, were obtained on treatment of Fraction (1) in the same way.

Fraction (3) on treatment with methyl-alcoholic ammonia yielded long needle-shaped crystals (0.05 g.), m.p. 268-280°, which resembled d-dimethoxy succinamide, m.p. 276-282°, and a mixed melting-point with an authentic specimen showed no marked depression (262-278°); the difference in rotation, however, threw doubt on this finding, viz. $[\alpha]_D^{19°}$ + 53° in water (C, 0.6) compared with $[\alpha]_D^{18°}$ + 89° in water (C, 0.7) for dimethoxy-d-succinamide.

Analysis.

Found : C, 38.5 ; H, 6.5 ; N, 17.6

Calc. for $C_6H_{12}O_4N_2$; C, 40.9 ; H, 6.8 ; N, 15.9%.

Methoxyl on ester, found ; 55%

Calc. for $C_8H_{14}O_6$; 60%.

It is obvious, therefore, that if this crystalline compound consists of d-dimethoxy succinamide, it must contain a large amount of impurity.

Removal of 2 : 4-Dimethyl 3 : 6-Anhydro-Methylgalactoside from the "Ketose" fraction.

The methylated "ketose" (8.1 g., $[\alpha]_D^{20°}$ + 10° in chloroform, OMe 56%), was treated with N-sulphuric acid

(50 c.c.) for 24 hours at room temperature, the solution neutralised with barium carbonate and the filtrate evaporated to dryness under reduced pressure, the syrup being dried by treatment with alcohol and benzene, and the residue was extracted with ether, leaving a white solid (0.7 g.). This solid, m.p. 118°, OMe, 32.8% (calc. for $C_8H_{14}O_5$, OMe 32.6%), and $[\alpha]_D^{15°}$ -21° in water (C, 1.3), was extremely hygroscopic and became syrupy on standing. It was strongly reducing to Fehling's solution and gave a strong Seliwanoff test, its properties thus being in accordance with those of 2 : 4-dimethyl 3 : 6-anhydro-*l*-galactose, which we were unable to crystallise on former occasions (Hands and Peat (6) quoted m.p. 114° for this substance).

The solvent was removed in a high vacuum.

Fraction (1) 0.93 g., b.p. 85-90°/.01 mm., colourless oil, $n_D^{11°}$ 1.4651, faint Seliwanoff test, non-reducing to Fehling's solution.

(2) 2.75 g., b.p. 95-105°/.01 mm., colourless oil, $n_D^{11°}$ 1.4570, faint Seliwanoff test, non-reducing to Fehling's solution.

(3) 1.40 g., b.p. 105-115°/.01 mm., colourless oil, $n_D^{11°}$ 1.4602, positive Seliwanoff test, slightly reducing.

(4) 1.05 g., b.p. 115-125°/.01 mm., colourless syrup, $n_D^{11°}$ 1.4796, strong Seliwanoff test, strongly reducing. OMe, 39.2%.

(5) Residue 1.1 g., strongly reducing, Seliwanoff test positive.

Fractions (1), (2) and (3) were combined and subjected to two methylations with methyl iodide (30 c.c.) and silver oxide (39 g.) for 11 hours at 42°, and worked up in the usual way.

Fraction (1) 2.01 g., b.p. 90-100°/.01 mm., colourless mobile syrup, $n_D^{10°}$ 1.4531, OMe, 54%, $[\alpha]_D^{16°}$ + 60.2° in chloroform (C, 0.8), faint Seliwanoff test, non-reducing to Fehling's solution.

(2) 1.79 g., b.p. 100-110°/.01 mm., colourless mobile syrup, $n_D^{11°}$ 1.4520, OMe, 56%, $[\alpha]_D^{16°}$ + 60° in chloroform (C, 0.5), faint Seliwanoff test, non-reducing.

(3) 0.46 g., b.p. 110-125°/.01 mm., colourless syrup, $n_D^{10°}$ 1.4556, $[\alpha]_D^{18°}$ + 44.6° in chloroform (C, 0.4), faint Seliwanoff test, non-reducing.

Removal of Tetramethyl Methylgalactopyranoside from this Syrup through the Formation of 2 : 3 : 4 : 6-Tetramethyl Galactose Anilide.

Hydrolysis with N-Sulphuric Acid.

Fraction (1) (0.15 g., $n_D^{10°}$ 1.4531) was dissolved in N-sulphuric acid (10 c.c.) and the temperature maintained at 80°. $[\alpha]_D^{16°}$ + 60°; + 58°. after 1 hour; + 56° after 2 hours; and + 56°, after 6 hours.

The solution at this stage was strongly reducing. 5 N-sulphuric acid (5 c.c.) was added and the mixture heated for a further 3 hours at 80°, but no change in specific rotation was observed.

A similar hydrolysis was carried out using 1.75 g. of syrup ($n_D^{10°}$ 1.4531) in N-sulphuric acid (50 c.c.) at 80° for 4 hours, $[\alpha]_D^{16°}$ + 55°, unchanged on further heating. The solution was neutralised with barium carbonate and worked up, yielding a strongly reducing brown syrup (1.6 g.).

Formation of Tetramethylgalactose Anilide.

The reducing syrup (1.6 g.) was dissolved in a mixture of alcohol (10 c.c.) and pure re-distilled aniline (0.8 g.), and heated at 90° for 1½ hours. On cooling, silky needle-shaped crystals were obtained (0.03 g.), m.p. 195°. The solution was filtered

and the filtrate allowed to stand at 0°C. for two days, yielding more crystals (0.04 g.). A third batch of crystals (0.02 g.) was obtained after several days at 0°C. The alcoholic solution was evaporated under reduced pressure yielding a further 0.02 g. of crystals, and was then evaporated to dryness, the crystals (0.24 g.) being separated on a porous tile and washed with a small quantity of alcohol. These crystals, (0.35 g.) recrystallised from alcohol, had m.p. 197°-198° and gave a mixed melting-point 196° with an authentic sample of 2 : 3 : 4 : 6-tetra-methylgalactose anilide, m.p. 197°.

Analysis

Found: C, 60.8; H, 7.97; N, 4.55; OMe, 38.7.

Calc. for $C_{18}H_{29}O_6N$; C, 61.7; H, 8.04; N, 4.50; OMe, 39.9%.

Recovery of Unchanged Methylated Compounds from the Anilide Mixture.

After removal of the tetramethyl galactose anilide crystals, the porous tile was extracted several times with acetone and the aniline removed by the method of Haworth, Hirst and Ruell (10). The solution was rendered faintly acid to Congo red paper with hydrochloric acid and then steam distilled for 3½ hours, until no aniline was obtained in the distillate. The

residual mixture was neutralised with silver carbonate and the filtrate evaporated to dryness under diminished pressure. The syrup was dried by treatment with alcohol and benzene and the solvents removed in a vacuum from the dark solution. The residue was extracted with methyl alcohol, yielding a faintly reducing light brown syrup. This syrup was subjected to methylation with silver oxide (20 c.c.) and methyl iodide (26 g.) for 11 hours at 42°, and distilled under reduced pressure, to yield a mobile syrup, 0.12 g., b.p. 95-105°/.01 mm., $n_D^{13°}$ 1.4524, OMe, 56.3%, $[\alpha]_D^{19°}$ + 50° in chloroform (C, 1.1), non-reducing and giving negative tests with the Seliwanoff and Bredereck reagents.

Another portion of the syrup (2.6 g.), after removal of the dimethyl anhydro methyl-galactoside in the manner described above, was hydrolysed using N-sulphuric acid at 80° for 4 hours and the reducing sugar (2.4 g.), treated with aniline (1.2 g.) and alcohol (10 c.c.) at 90° for 1½ hours. In all, 0.23 g. of tetramethyl galactose anilide was obtained and the residue, extracted from the porous tile with acetone, was treated in the following manner. The anilide mixture (2.2 g.) was heated with 3% sulphuric acid

(50 c.c.) at 90° for 8 hours, and then warmed with excess barium carbonate, which neutralised the sulphuric acid, and, at the same time, decomposed the aniline sulphate. The aniline was removed under reduced pressure to yield more crystals of tetramethyl galactose anilide which had evidently resisted decomposition. The aqueous layer was evaporated to dryness under reduced pressure, to yield a brown syrup (1.4 g.) which reduced Fehling's solution but gave negative ketose tests. This syrup was methylated with silver oxide (39 g.) and methyl iodide (30 g.c.) for 12 hours at 42° and the product distilled in a high vacuum, to yield a mobile syrup, 1.20 g., b.p. 90-100°/.01 mm., $n_D^{12°}$ 1.4545, OMe, 57.0%, $[\alpha]_D^{15°}$ + 38° in chloroform (C, 0.4), non-reducing and giving no ketose colour reactions.

This methylation and distillation were undertaken partly for the purposes of purification and partly to attempt to increase the methoxyl content to the value for a fully methylated hexose.

Isolation of more Tetramethyl Galactose Anilide.

The above syrup (1.1 g.) was then hydrolysed at 90° using 8% aqueous hydrochloric acid (25 c.c.). $[\alpha]_D^{20°}$ + 38° fell to + 35° after 3 hours and remained unchanged on further heating. The solution, neutral-

ised with silver carbonate and worked up in the usual way, yielded a brown syrup (0.9 g.), which was very strongly reducing to Fehling's solution.

Aniline (0.5 g.) and alcohol (4 c.c.) were added, and the mixture maintained at 90° for 1½ hours. On cooling, needle shaped crystals (0.05 g.) separated out, m.p. (without recrystallisation) 194-195°; mixed m.p. 192-195° with a known sample of tetramethyl-galactose anilide showed no depression. The rotation $[\alpha]_D^{16°}$ -68° in acetone (C, 0.5) was in accordance with $[\alpha]_D^{20°}$ -71° in acetone (C, 0.2), found by Munro (11). Further batches of crystals (0.3 g., m.p. 189-192°) were obtained on evaporation of the mother liquor, showing that the fully methylated syrup contained a large proportion of 2 : 3 : 4 : 6-tetramethyl methyl-galactoside.

Pentose and Methylpentose Estimation on Agar.

Dried agar (0.5 g.), with sodium chloride (22 g.) (Norris and Resch (9)), was heated for 2½ hours at 175-180°C. with 12% hydrochloric acid (100 c.c.) in an apparatus fitted with a dropping funnel and a condenser. During the course of the experiment 12 additions of 30 c.c. portions of 12% hydrochloric acid were made. The standard Phloroglucinol solution (40 c.c.) was added to the distillate and the

total volume made up to 400 c.c. with 12% hydrochloric acid. No precipitate was obtained after standing for several days, showing the absence of pentoses and methylpentoses.

Attempted Isolation of a Crystalline Galactosazone.

The 'ketose' fraction (1.6 g.) from the hydrolysis of methylated agar (p. 43, OMe 40%) was hydrolysed at 80° for 3 hours with 5% sulphuric acid, neutralised and worked up in the usual way. The reducing syrup (1.5 g.) was heated with phenylhydrazine hydrochloride (1.2 g.), sodium acetate (1.9 g.) and sodium bisulphite (0.2 g.) at 100° until the solution became cloudy (90 minutes). The solution was cooled, filtered and the residue washed with water until free from phenylhydrazine. Attempts were made to crystallise this product but without success. The compound, however, was precipitated from a chloroform solution by petroleum (b.p. 60-80°), and the solid obtained was purified by subsequent precipitations. The solid, m.p. 82° and OMe 6.3% [$C_{18}H_{21}O_3N_4(OCH_3)$ contains 8.3%] must, therefore, be purified by another method before its structure can be elucidated.

SUMMARY

1. Oxidation of the fully methylated 'ketose' (p.84) by the method of Avery, Haworth and Hirst (5), failed to produce any conclusive evidence as to its constitution.

2. Hydrolysis of this syrup and suitable treatment yielded a crystalline anilide, namely 2 : 3 : 4 : 6-tetramethyl galactose anilide, showing the syrup to consist mainly of tetramethyl methyl-galactoside. No other crystalline anilides were encountered.

3. A pentose and methylpentose estimation on agar showed the absence of these residues in the polysaccharide.

4. Osazone formation on the 'ketose' fraction from the products of hydrolysis of methylated agar failed to produce any identifiable products so that, although the presence of dimethyl galactoses was suspected, no evidence for this has yet been secured.

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