LVIII. THE PREPARATION OF HEPTAACETYL-*dl*-GALACTOSE BY THE ACETOLYSIS OF AGAR.

By NORMAN WINGATE PIRIE.

From the Biochemical Laboratory, Cambridge.

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AGAR is more resistant to acetylation than many other polysaccharides and it can be recovered unchanged after treatment by the gentle methods of acetylation that have been described recently. Treatment with pyridine and acetic anhydride under various conditions or with acetic anhydride in the presence of SO₂ and Cl₂, HCl, KCNS or ZnCl₂ are all alike ineffective, whereas the original acetylation method of Franchimont [1879] works well with this material. Nothing is gained by the use of perchloric rather than sulphuric acid as a catalyst.

With sulphuric acid as catalyst the rate and extent of the action depend on the temperature and acid concentration; with 1 volume of sulphuric acid in 6 or 7 of acetic anhydride there is little action at room temperature even in 3 days, whilst about 80 % of the agar dissolves in 2 days at 38° and in 2 hours at 55°. At higher temperatures all the agar dissolves but there is very serious blackening of the solution and no water-insoluble products can be isolated afterwards. The agar that remains unattacked after 2 days at 38° or 2 hours at 55° has the properties of untreated agar; it will make a gel in the usual way and when acetylated again under the same conditions it dissolves and gives similar acetylation products. From this point of view therefore agar is relatively homogeneous.

From the products of the acetolysis of agar a crystalline substance was isolated which proved to be heptaacetyl-*dl*-galactose; a method for the synthesis of this substance has meanwhile been published by Micheel *et al.* [1935].

The diacetyl derivatives of most of the common aldehydes have been prepared by the direct acetylation of the aldehydes under conditions similar to those used here with agar; attempts were therefore made to prepare heptaacetyl-*d*-galactose by acetylation of galactofuranose and galactopyranose derivatives. These attempts were unsuccessful but heptaacetyl-*d*-galactose could readily be isolated from the products of acetylation of aldehydogalactose derivatives. It appears therefore that under the conditions of acetylation the oxygen-containing ring in an ordinary galactose derivative is not opened and that it is necessary by some other means to get the sugar into the open chain form before acetylation of the aldehyde group can take place. The acetylation of some sugars in this way are described in the following paper. The fact that a heptaacetyl sugar can be obtained from agar may be adduced as evidence that in it the sugar exists, in part at any rate, in the aldehyde form. It is interesting in this connection to note that it has proved impossible to isolate heptaacetyl-galactose from the acetylation products of galactose methylphenylhydrazone although Wolfson and Christman [1931] have brought forward evidence that this is a real hydrazone derived from aldehydogalactose and has not, like some other supposed hydrazones, a ring structure. It may be relevant to mention in this connection that galactose methylphenylhydrazone gives a very small yield of mucic acid on oxidation with nitric acid.

EXPERIMENTAL.

Heptaacetyl-dl-galactose.

Commercial powdered agar contains up to 20 % of water; this can be removed by heating the agar on a boiling water-bath *in vacuo*. Shredded agar is less easy to dry and it is not recommended as material for acetylation because, presumably on account of the smallness of the exposed surface, it is more slowly dissolved by the acetylation mixture.

To 10 g. of dried agar an ice-cold mixture of 40 ml. acetic anhydride and 6 ml. conc. H_2SO_4 is added. The mixture is shaken well and left at room temperature for an hour or two before being put in an incubator at 38° . The action is exothermic but no precautions are necessary when working on this scale; if larger quantities are used a thermometer should be kept in the acetylation mixture. The temperature should not be allowed to exceed 45° or the action may become violent. The mixture soon goes nearly black, it is shaken occasionally and taken out and cooled after about 44 hours. When cold it is rather viscous but it will filter through a sintered glass funnel. The residue of unacetylated agar is washed with 10–20 ml. of acetic acid. If dried it weighs 1–2 g. and, although somewhat discoloured, it has the properties of untreated agar, *i.e.* it will form a rigid jelly and when acetylated gives heptaacetyl-*dl*-galactose in the usual yield.

The filtrate and washings are poured into 300 ml. of water containing 35 g. of hydrated sodium acetate and some ice. After standing with occasional stirring for 2–3 hours the brown fluid and putty-like solid are distilled together to dryness *in vacuo*. Some water is added and the mixture is again distilled to remove the rest of the acetic acid.

50 ml. of water and 50 ml. of chloroform are added and the flask shaken until all the lumps of sodium sulphate and acetylation products have dissolved, the chloroform layer being then removed in a separating funnel. With the help of a strong beam of light the interface between the two dark-coloured layers can be fairly easily seen. The chloroform layer is washed once again with water and distilled to dryness *in vacuo*. 30–40 ml. of hot water are added and the gum is shaken into an emulsion. On cooling it resolidifies and the water can be poured off. The gum is dissolved in 30–40 ml. of hot alcohol and partially decolorised with charcoal; it is generally impossible to remove all the colour at this stage. The solution may also be decolorised by addition of half a volume of ether to the warm alcoholic solution. A brown flocculent precipitate separates which must be filtered off quickly for the heptaacetyl-*dl*-galactose is apt to precipitate soon. The latter will crystallise from the filtrate in a few hours, generally without seeding, in the form of a felt of needles sometimes embedded in jelly. After recrystallisation the yield is 1–2 g.

The melting-point is very much affected by impurities and after several recrystallisations and thorough drying it reaches 132° . Recrystallisation is most easily carried out from 30 parts of methyl or ethyl alcohol or from 150 parts of boiling water. In many other solvents the compound is inconveniently soluble and from pyridine, benzene, acetone and cyclohexane it retains solvent obstinately; after drying *in vacuo* at room temperature the solid may retain 1–2 % of solvent for several days but the amount is always lower than a molecular proportion. The solvent can be driven off by heating *in vacuo* to 100° or by boiling with water. In some solvents, benzene for example, the solvated form is less soluble than the solvent-free; the latter therefore dissolves quickly in a small amount of solvent and then, after a few minutes, a precipitate of the

solvated form separates. This property somewhat limits the choice of solvent for measuring the depression of freezing point.

The optical rotation of a pure sample in 10% solution in chloroform was $< 0.02^\circ$ in a 2 dm. tube. Since some galactose derivatives are known which have very low specific rotations [Wolf from, 1930; Levene and Meyer, 1927], a sample was hydrolysed by refluxing for 15 min. with N H_2SO_4 and the *dl*-galactose isolated by removing the sulphuric acid with baryta, concentrating and crystallising the sugar by the addition of alcohol. The yield was 80% and the product contained 98% of reducing sugar, had no measurable optical rotation and gave on oxidation with nitric acid as much mucic acid as the same weight of *d*-galactose. Furthermore the mother-liquor, on oxidation with nitric acid gave more mucic acid. This showed that *dl*-galactose and probably this alone occurred in the acetylated material.

When hydrolysed with $5N$ H_2SO_4 and then distilled with the continuous addition of water an amount of titratable acid corresponding to an acetyl content of 61–62% was found in the distillate. These distillates were free from both carbon dioxide and formic acid and it was therefore necessary to assume that the substance did in fact contain more than one acetyl group for each carbon atom. The synthesis of the corresponding derivative of *d*-galactose confirmed the possibility of the existence of orthodiacetyl derivatives of the sugars. If these acetylated sugars are saponified by heating with alkali before distillation, large and variable amounts of carbon dioxide are formed; acid hydrolysis is therefore preferable.

The sugar content whether measured by the total carbohydrate method of Tillmans and Philippi [1929] or by the method of Hagedorn and Jensen was 36%; in each case the ester was hydrolysed in the course of the estimation. When oxidised with nitric acid 509 mg. gave 133 mg. of mucic acid. This value is that to be expected, from Van der Haar's [1917] tables, for a heptaacetyl-galactose.

A 4% solution (by volume) in acetic acid gave F.P. depression of 0.30° and a 4% solution (by volume) in bromoform a depression of 0.40° . These correspond to mol. wt. 486 and 496 respectively. (Found (Weiler): C, 49.04; H, 5.85; acetyl, 62.3%. $C_{26}H_{28}O_{14}$ with 7 acetyl groups requires: C, 48.76; H, 5.73; acetyl, 61.2%.)

The analytical figures are a very unsatisfactory index of purity, since all likely contaminants would have a similar percentage composition. The melting point is better but the optical rotation is the best criterion. The uncrystallisable acetylated agar that remains in the alcoholic solution from which heptaacetyl-*dl*-galactose has been isolated can be obtained as a pale yellow powder that gradually softens to a gum having $[\alpha]_D$ about $+24^\circ$ in chloroform. The last trace of optical activity can always be removed from heptaacetyl-*dl*-galactose by crystallisation from acetone but the product obtained in this way requires intensive drying to remove the acetone.

Micheel *et al.* [1935] prepared heptaacetyl-*dl*-galactose by acetylating tetraacetylaldehyde-*d*-galactose-6-iodohydrin and explain the loss of optical activity by postulating the intermediate formation of a symmetrical *cyclohexane* derivative which then breaks down to give a *dl*-galactose derivative. The isolation of a derivative of *dl*-galactose cannot therefore be taken as conclusive proof of its preexistence in a complex molecule and it becomes necessary to obtain supplementary evidence.

There is nothing intrinsically improbable in the occurrence of *dl*-galactose in agar for it is already known as a constituent of Chagual gum [Winterstein,

1898] nori [Oshima and Tollens, 1901] and quince gum [von Lippmann, 1922] whilst *l*-galactose has been found in flax seed mucilage by Anderson [1933]. Hydrolysis for 1 hour with $N/10$ H_2SO_4 at 100° is sufficient to liberate 50 % of the reducing groups of agar as estimated by the Hagedorn-Jensen method; at this stage no free galactose has been liberated and the hydrolysate is neither fermented by galactose-trained yeast nor will it give a hydrazone with methylphenylhydrazine. Hydrolysis for 1 hour with N H_2SO_4 on the other hand gives a maximum yield of methylphenylhydrazone and a maximum evolution of carbon dioxide with galactose-trained yeast, the yields in each case corresponding to a galactose content, in terms of dry agar, of 28–30 %.

Lüdtke [1929] has isolated galactose methylphenylhydrazone from hydrolysed agar and I have followed his method in part; it is however unwise to heat the mixture of neutral hydrolysate, alcohol and methylphenylhydrazine to 37° as he does because this leads to the formation of a tarry material besides the hydrazone. The condensation is complete after 10–12 hours at room temperature. This hydrazone is decomposed with acetaldehyde by the method of Collatz and Neuberg [1932] and the sugar solution is allowed to crystallise with the addition of alcohol. The first crops of crystals are pure *d*-galactose but the later fractions crystallise with more difficulty and have a low optical rotation although they still contain 100 % of sugar. These later fractions and the mother-liquor are fermented with galactose-trained yeast for 3–5 days. Pure *l*-galactose can now be isolated by filtering off the yeast and adding alcohol to the concentrated filtrate. From 25 g. of agar only 200 mg. of *l*-galactose have so far been isolated in this way, corresponding to a yield of 1.6 % of *dl*-galactose. The yield of heptaacetyl-*dl*-galactose on the other hand may be as high as 20 %, corresponding to 7.2 % of *dl*-galactose. This discrepancy is serious and it cannot be claimed that in this case none of the *dl*-galactose has arisen by secondary actions during the acetylation. It will however be shown later that acetylation of hexoses is not generally accompanied by such a process.

The uncrystallisable gum from an acetylation carried out in this way has an acetyl content of 55–57 % and contains about half the carbohydrate of the agar used. The proportion of galactose in this acetylated agar is larger than in the original agar; there is little or no destruction of the galactose under these conditions and the loss of total carbohydrate on acetylation is mainly at the expense of the unknown constituents which make up 70 % of the molecule.

Agar which has been slightly hydrolysed, *e.g.* by boiling for 40 min. with $N/10$ H_2SO_4 , can be acetylated very much more easily than intact agar. Under these conditions of hydrolysis no free galactose is liberated and the hydrolysate, after acetylation with 1:20 H_2SO_4 -acetic anhydride for 24 hours, seems to contain no heptaacetyl-*dl*-galactose. If however this acetylated agar is subjected to further acetylation, heptaacetyl-*dl*-galactose can be isolated in the usual way and in the usual yield. Similarly, if the small amounts of acetylager that can be obtained by treating agar for a short time or at a low temperature with the usual acetylation mixture are subjected to more vigorous acetylation, the heptaacetyl derivative can be isolated from them.

Agar which has been completely hydrolysed, *e.g.* by boiling for 1–2 hours with N H_2SO_4 , can also be easily acetylated but in this case no heptaacetyl-*dl*-galactose can be isolated. It is easy however to isolate α -pentaacetyl-*dl*-galactose and α -pentaacetyl-*d*-galactose by the same technique. These can be separated by fractional crystallisation from alcohol, the former being the less soluble.

Pentaacetyl-*dl*-galactose was identified by its acetyl content, 55.4 %, by the fact that it gave the expected yield of mucic acid after oxidation with nitric

acid and by its optical inactivity even after hydrolysis. It was presumably in the α -form since Micheel *et al.* [1935] give 111° as the melting point of the corresponding β -derivative whereas the substance prepared from agar melts at 144° . Furthermore it has been made by a method which, when used with *d*-galactose, gives rise to the α -pentaacetate.

These results are compatible with the hypothesis that in agar at least the *dl*-galactose occurs in a form other than the usual furanose or pyranose ring and that the heptaacetyl derivative results from preparations in which acetylation precedes complete hydrolysis. If the sequence is inverted or if the agar is subjected to such mild acetylation that complete hydrolysis does not ensue, heptaacetylgalactose cannot be isolated, in the first case because the sugar has cyclised and can only undergo pentaacetylation and in the second case because it is still attached to the other constituents of the polysaccharide. In the second case it can still be liberated by acetolysis. Further evidence that in agar the galactose occurs in an open-chain form will be presented in a later publication.

SUMMARY.

Heptaacetyl-*dl*-galactose is the only crystalline substance that can be isolated from the acetolysis products of agar. By the acetylation of hydrolysed agar α -pentaacetyl-*d*-galactose and α -pentaacetyl-*dl*-galactose can be prepared. The isolation of *l*-galactose from hydrolysed agar is described.

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