29. *The Chemistry of Galactogen from* Helix Pomatia. l-*Galactose as a Component of a Polysaccharide of Animal Origin.*

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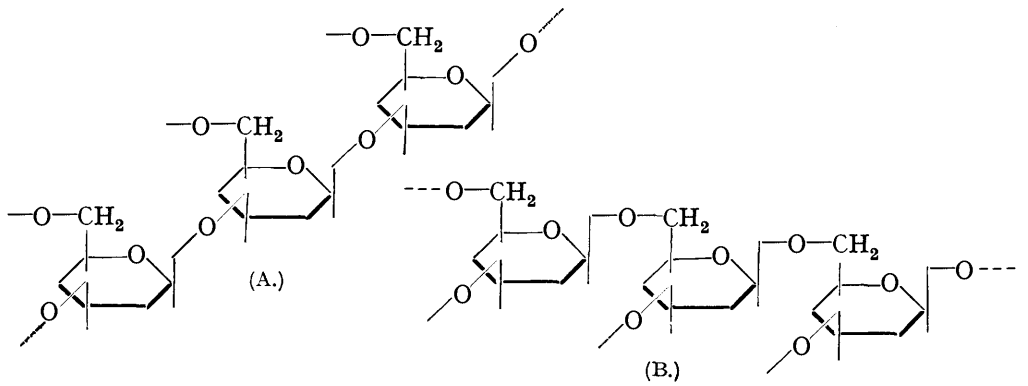
The results discussed in the present communication lead to the following main conclusions: (i) galactogen consists entirely of galactose radicals; (ii) the main part of the galactogen molecule consists of a chain of " backbone " radicals, each carrying a side-chain radical in the manner indicated in formulæ (A) and (B); (iii) for every three backbone radicals there are four side-chain radicals; (iv) the backbone radicals, and together with them three out of every four side-chain radicals, consist of residues of *d*-galactose; (v) one in every four side-chain radicals is derived from *l*-galactose; (vi) the " unit " of structure in galactogen probably comprises seven galactose radicals, and the colloidal properties of the polysaccharide may be due to the aggregation of a number of these 7-radical units.

Galactogen thus appears to be unique in containing radicals derived from both *d*- and *l*-galactose. The latter sugar, so far as we are aware, has not hitherto been detected in material of animal origin.

In a preliminary study (Baldwin and Bell, J., 1938, 1461) we found that methanolysis of approximately fully methylated galactogen gives rise to dimethyl and tetramethyl methyl-galactosides in roughly equimolecular proportion. We identified the former as 2 : 4-dimethyl methyl-*d*-galactoside and, having at the time no evidence to the contrary, the latter was identified as 2 : 3 : 4 : 6-tetramethyl methyl-*d*-galactoside. We suggested two provisional formulations of the galactogen molecule possessing as their main feature a " backbone " consisting of triply substituted galactose radicals, each of which carried as " side chain " a glycosidically linked galactose radical. The backbone radicals might be linked in either of two ways, (A) in the 1 : 3 position, the side chains being attached glycosidically at C_6, or (B) in the 1 : 6 position with side chains at C_3, but we have as yet no evidence which points specifically to either of these possibilities.



May (*Z. Biol.*, 1934, **95**, 277) observed that, if galactogen is hydrolysed with hot dilute mineral acid, the final $[\alpha]_D$ of the hydrolysate is + 53·6° (calculated in terms of hexose) as against the + 80·5° expected for an equilibrium mixture of α- and β-*d*-galactopyranoses. Estimations of the galactose contents of these hydrolysates indicated that complete

hydrolysis had been achieved and that no other sugar was present. May concluded that he had to deal with β-*d*-galactopyranose, for which $[\alpha]_D = +53°$, stabilised in some manner hitherto unknown. Carefully repeating May's experiment, we found a final $[\alpha]_D$ value of + 56·5° (corr. for ash and calc. in terms of hexose). This is in close agreement with the value of + 57·5° which may be calculated for a mixture of *d*- and *l*-galactopyranoses in the proportion of 6 : 1. Other experiments gave values in similarly good agreement with such a ratio.

Using a " galactose-trained " yeast, we attempted to remove *d*-galactose from hydrolysates of galactogen in the hope of isolating some of the *l*-sugar, but in this were unsuccessful. Later, however, we obtained evidence for the presence of 2 : 3 : 4 : 6-tetramethyl methyl-*l*-galactosides in material obtained by the methanolysis of methylated galactogen.

We accordingly resumed our search for *l*-galactose in acid hydrolysates of the parent polysaccharide, this time with the aid of purely chemical procedure. Galactogen was hydrolysed by aqueous hydrochloric acid; from the hydrolysate several crops of *d*-galactose crystallised. The uncrystallisable syrup remaining had only a small positive rotation; from it we prepared by the procedure of Moore and Link (*J. Biol. Chem.*, 1940, **133**, 293) 2-dl-*galactobenziminazole* identical in its properties with material similarly prepared from authentic *dl*-galactose. This isolation of a derivative of *dl*-galactose proves without doubt that the *l*-sugar occurs as a component of the polysaccharide.

At the time of our original experiments we had no criteria wherewith to establish the homogeneity of a small sample of 2 : 3 : 4 : 6-tetramethyl αβ-methyl *d*-galactoside, the few records of physical data in the literature being incomplete and in poor agreement (cf. Bell, J., 1940, 1543). Our sole guide was the preparation of the crystalline anilide, which, in the instance referred to, afforded no evidence of the presence of any but *d*-galactose derivatives. In order to obtain the necessary data one of us (Bell, *loc. cit.*) undertook an investigation of the $n_D^{16°}/[\alpha]_D$ relationship in mixtures of 2 : 3 : 4 : 6-tetramethyl α- and β-methyl-*d*-galactosides along the lines of that already established for the glucose series by Hirst and Young (J., 1938, 1247).

When, in the light of these new data (as already mentioned above) we examined the " pentamethyl " fractions from methylated galactogen, we found that they possessed specific rotations considerably smaller than was to be anticipated from their refractive indices. Analytically, however, the materials were pentamethyl hexose. Having then shown that *l*-galactose radicals were actually present in galactogen itself, we considered the possibility of their methylated derivatives being constituents of the " pentamethyl " fractions. That this was indeed the case was established by hydrolysing the " penta " material and then carefully methylating the product with Purdie's reagents, whereby a large proportion of the β-galactoside was formed. By a procedure involving fractional crystallisation and fractional distillation, we were able to isolate a fraction which was almost optically inactive. This, on appropriate treatment, yielded an almost optically inactive anilide which could be satisfactorily compared with an authentic synthetic specimen of 2 : 3 : 4 : 6-*tetramethyl* dl-*galactose anilide.*

No evidence could be obtained that *l*-galactose radicals occurred in any parts of the galactogen molecule except those which yield a tetramethylated radical on methylation. Hence we believe that all the *l*-galactose radicals are to be found among the " side chains " and the " backbone " consists exclusively of the *d*-sugar. From investigation of the specific rotation of the " penta " material we believe that the *d*/*l* ratio of the two stereoisomers is 3 : 1.

Having discussed the discovery of *l*-galactose as a component of galactogen, we shall now consider the analysis of the total products of methanolysis. The final figures obtained are in Table I (figures in parentheses indicate that the amounts were deduced by approximation). Taken together these products amount to 8·362 g., thus accounting for 94% of the mixed glycosides originally taken for analysis.

Of these products the " penta " and " tri " substances must have originated respectively in the singly substituted side-chain radicals and the triply substituted backbone radicals of the parent polysaccharide. As will be pointed out, we consider that the mixed " tetra " substances originated in potentially " penta "-yielding (*i.e.*, side-chain)

TABLE I.

Composition of methanolysis fractions.

Fraction.	"Penta," g.	"Tetra," g.	"Tri," g.	Total, g.
V	2·545	—	—	2·545
VI	1·430	—	—	1·430
VII	(0·408)	(0·240)	—	0·648
VIII	—	(0·276)	(0·069)	0·345
C	—	(0·092)	(0·584)	0·676
A	—	—	0·590	0·590
IV	—	—	2·128	2·128
Totals	4·383	0·608	3·371	8·362

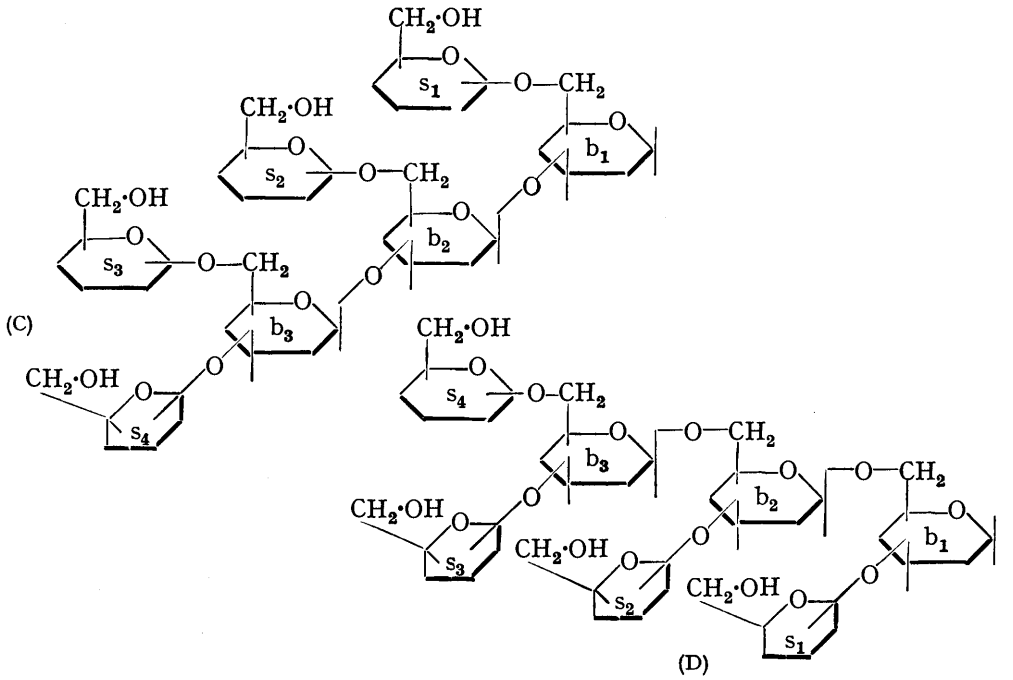
groups which had not been completely methylated. The "tetra" material, of which 0·608 g. was found in all, would correspond to 0·644 g. of "penta" material, and the total amount of the latter must therefore be provisionally considered as 5·027 g. If the "penta" total is thus taken to be 5·027 g. and the "tri" total to be 3·371 g., the molecular ratio of these fractions is 2·01 : 1·52 or 4 : 3 as a very close approximation. That is, the ratio No. of "side-chain" radicals/No. of "backbone" radicals is 4 : 3, which gives the simplest expression for a structure of the galactogen molecule, unless the total number of component radicals is large.

As we have already indicated, the proportion of *d*- and *l*-isomers among the main "penta" fractions V and VI is exactly 3 : 1. In fraction VII this ratio could not be directly evaluated, but there is evidence that both isomers were present and that the proportions were not widely different. As for the "tetra" material, which accounts for only about 10% of the total we have to consider, we do not know for certain whether both stereoisomers were or were not present, or in what proportion. In these circumstances the most reasonable course to follow appears to be to assume that the *d*/*l* ratio of 3 : 1 prevailing in the main fractions (derived from side-chain radicals) prevails also in the subsidiary fractions. As has been already stated, consideration of the final $[\alpha]_D$ attained in acid hydrolysates of galactogen also indicates, for the whole molecule, a *d*/*l* ratio of 6 : 1. Then, since for every three backbone radicals four side-chain radicals are present, one of the latter being derived from *l*-galactose, we reach the conclusion that the molecular unit of galactogen contains a total number of radicals equal to 7 or to some multiple of 7 and, moreover, that one out of every seven radicals is derived from *l*-galactose, the remainder arising from the *d*-sugar.

The basic structures represented in formulæ (A) and (B) suggest several possible arrangements of the galactose radicals. The backbone radicals might constitute a closed loop. In this case the side-chain/backbone ratio would be 1 : 1 instead of the 4 : 3 found. (The 1 : 1 ratio would also occur if the backbone radicals form a very long open chain, each member of which carries a single side chain.) One of the backbone radicals must therefore carry two side chains. The radical in question must be terminally situated in the backbone, for if a second side chain were attached to any intermediate backbone radical, we should expect to find a monomethyl methylgalactoside among the products of methanolysis of the methylated polysaccharide, but we have not found such a product. If, moreover, the terminal backbone radical carried only a single side chain, it would give rise to a trimethyl methylgalactoside, and in this case we should be able to distinguish between (A) and (B), since the terminal backbone radical of the former would be expected to yield 2 : 3 : 4-trimethyl methylgalactoside and that of the latter to yield the 2 : 4 : 6-compound. The small amounts of trimethyl methylgalactosides actually found consisted, however, of more than one isomer.

We must thus conclude, provisionally, that it is the terminal radical of the backbone to which the additional side chain is attached, so, if we picture the molecular unit of galactogen in the simplest possible terms compatible with the evidence, *i.e.*, as containing seven galactose radicals in all, it may be formulated as in (C) or (D). In these formulæ b_1—b_3 represent the backbone radicals and s_1—s_4 those constituting the side chains. With the exception of one of the latter, all these radicals are derived from *d*-galactose.

We have no further evidence regarding the position occupied by the single *l*-galactose radical, beyond the fact that it is one of the side chains.



In both these formulæ a reducing group is represented as being present in the initial backbone radical, b_1, but galactogen possesses only the feeblest of reducing power. The presence of such a reducing group in a 7-radical unit might, however, by analogy with the case of starch, be expected to lead to the polymerisation of a number of such units to form larger colloidal aggregates. Hirst and Young (J., 1939, 1471) have written : " consideration of the conditions of the disaggregation process leads to the conclusion that in the starch molecule, the repeating units . . . are linked to a non-terminal glucose residue of another unit by primary valencies of the glycosidic type." If we suppose, for example, that in the case of galactogen aggregation is brought about by the formation of linkages between the " reducing group " of one 7-radical unit and a hydroxyl group of a side-chain radical of a second such unit, the particular hydroxyl group involved being selected at random, then methylation and subsequent methanolysis would yield " penta," " tetra," and " tri " products in proportions which would vary according to the number of times the inter-unit linkage was repeated in the parent molecule. The proportions can readily be calculated and for the simplest case of polymerisation, in which only two 7-radical units are concerned, the theoretical ratios are 7 : 1 : 6.

It so happens that our actual yields of " penta," " tetra," and " tri " materials correspond to molecular ratios of 1·75 : 0·256 : 1·52, or very nearly 7 : 1 : 6, but this must be a coincidence. Nevertheless it serves to remind us that the " incompletely methylated side-chain radicals," to which we have so far attributed the formation of the " tetra " products, may have failed to undergo complete methylation for one of two reasons, either (*a*) because the original methylation procedure was not sufficiently vigorous or (*b*) because they were in fact involved in inter-unit linkages of the kind just suggested and therefore not susceptible of methylation in any case. Definitely to establish the nature of the inter-unit linkages and the number of these present in the complete galactogen molecule would require material far in excess of the amounts at present at our disposal, but we hope to be in a position to carry out further work on the problem at some future time.

Experimental.

Throughout the work solvents were evaporated under reduced pressure. Polarimetric observations were made in a 2 dm. tube.

Isolation of 2-dl-*Galactobenziminazole from a Galactogen Hydrolysate.*—7·475 G. of galactogen were hydrolysed with 1% hydrochloric acid on the boiling water-bath for 7 hrs. The hydrolysate was neutralised with silver carbonate, filtered, treated with hydrogen sulphide, filtered through charcoal and kieselguhr, and evaporated nearly to dryness. The residue was dissolved in boiling 90% alcohol, decolorised with charcoal, and again filtered. From the solution six crops of crystalline material were obtained: these had the following properties after recrystallisation, and consisted of practically pure *d*-galactose:

Crop No.	Wt., g.	Initial $[\alpha]_D$ (extrapolated).	Final $[\alpha]_D$.	M. p.	M. p. mixed with *d*-galactose.
1	1·670	+144°	+77·7°	162°	162—163°
2	0·629	+141	+76·3	160	160—163
3	0·200	+141	+77·2	160	160—162
4	0·325	+140	+74·0	161	161
5	0·285	+132	+74·9	161	160—162
6	0·273	+148	+75·3	162	160—162

The residual syrup was decolorised with charcoal in aqueous solution and the residue remaining after filtration and evaporation of the filtrate was dissolved in boiling 97% alcohol. Some syrup separated on cooling; this was rejected and the material remaining in the mother-liquor was recovered by evaporation to dryness. The residue showed $[\alpha]_D$ + 11·5°, corresponding to a mixture of approximately 57% of *d*-galactose with 43% of the *l*-isomer (or about 86% of *dl*- and 14% of *d*-galactose).

This material was submitted to the procedure described by Moore and Link (*loc. cit.*) for the preparation of benziminazole derivatives of hexoses. The product crystallised from hot water in prisms (Found: C, 53·3; H, 6·4; N, 10·0. $C_{12}H_{16}O_5N_2$ requires C, 53·7; H, 6·0; N, 10·4%). In crystalline form and m. p. it differed from the derivative of *d*-galactose (Moore and Link, *loc. cit.*). It was optically inactive in 5% aqueous citric acid (*c*, 2·5) and had m. p. 233°, not depressed by authentic 2-dl-*galactobenziminazole* (m. p. 233°) prepared from *dl*-galactose (Found: C, 53·5; H, 6·3; N, 10·4%). From the material obtained from galactogen, a second crop of crystals was obtained by evaporation of the mother-liquor from the first crop, but this was almost entirely the *d*-compound; the *dl*-derivative is sufficiently insoluble to crystallise almost completely in the first crop.

Preparation of Methylated Galactogen.—Our galactogen was prepared from albumin glands taken from hibernating specimens of *H. pomatia* by the method already described (Baldwin and Bell, *loc. cit.*). Acetylation again proved very difficult of accomplishment, but the final product could not be noticeably fractionated by precipitation with light petroleum from its solution in chloroform.

Methylated galactogen was prepared by treatment of the acetate with 30% caustic soda solution and methyl sulphate, the method being essentially the same as that already described. After 16 treatments with these reagents a methoxyl content of 41·8% was attained, and the product then purified by repeated precipitation from solution in chloroform by the addition of light petroleum.

Methanolysis of Methylated Galactogen.—Preliminary fractionation of the products. 9·0 G. of the methylated polysaccharide were boiled for 10 hrs. with 500 ml. of methyl alcohol containing 5 g. of dry hydrogen chloride. After neutralisation with lead carbonate the solution was filtered and evaporated to dryness, and the residue exhaustively extracted with ether containing 10% of acetone. All organic material was in this way extracted. The extract was boiled with charcoal, filtered, and evaporated to dryness; the pale yellow syrup (8·90 g.) obtained was fractionally distilled in a high vacuum, giving fractions I—IV and a still residue, *A*. The properties of these and the later fractions are summarised in Table II.

Fractions I and II were united and redistilled, giving two fractions, V and VI, and a still residue, *B*. The last was united with the partly crystalline fraction III and the whole redistilled to give fractions VII and VIII and a still residue *C*.

Investigation of the "penta"-fractions (V *and* VI): *Isolation of sub-fractions containing tetramethyl* l-*galactose.* Fraction V obviously consisted wholly of pentamethyl hexose, but consideration of the $n_D^{16°}/[\alpha]_D$ relationship (Bell, *loc. cit.*) showed that tetramethyl α- and β-methyl-*d*-galactopyranosides could not be the sole constituents, since the $n_D^{16°}$ found corresponded to an $[\alpha]_D$ of about + 90°, the value actually observed being, however, + 47·5°.

TABLE II.

Properties of the Methylated Fractions.

Fraction.	Wt., g.	B. p./mm.	$n_D^{16°}$.	$[\alpha]_D$ in water.	% OMe.
I	3·177	120—130°/0·08	1·4513	—	—
II	1·719	130—140/0·08	1·4535	—	—
III	1·155	140—166/0·08	1·4644	—	—
IV	2·128	166—175/0·08	—	—	—
A	0·590	—	—	—	—
V	2·545	110/0·001	1·4490	+47·0° (c = 5); +47·5° (c = 8·7)	60·7
VI	1·430	110/0·001	1·4494	+55·6° (c = 2·7)	61·0
B	—	—	—	—	—
VII	0·648	120/0·005	1·4560	—	53·0
VIII	0·345	145/0·005	1·4600	—	49·5
C	0·676	—	—	—	—

Similarly fraction VI, also wholly " pentamethyl," had $[\alpha]_D$ + 55·6°, much lower than the + 118° anticipated from the $n_D^{16°}$ observed. On the assumption that these fractions are derived from a mixture of the *d*- and the *l*-form of galactose, fraction V would correspond to a mixture of 76% of the *d*- and 24% of the *l*-, and fraction VI would correspond to a mixture of 73% of *d*- with 27% of the *l*-component. These fractions were further investigated in the following manner.

They were united and 2·697 g. hydrolysed with 40 ml. of N-hydrochloric acid to give the free sugars. The hydrolysates were neutralised with barium carbonate, filtered, and evaporated to dryness. The free sugar was extracted from the residue with a small quantity of light petroleum (b. p. 60—80°), from which, after evaporation of the solvent, 2·029 g. of a colourless syrup were obtained. This material had $[\alpha]_D$ in water + 37·2° (c = 4·8). 2 : 3 : 4 : 6-Tetramethyl *d*-galactose has $[\alpha]_D$ + 117·8° at equilibrium. If, therefore, our material consisted of a mixture of the *d*- and the *l*-derivative, its $[\alpha]_D$ would correspond to a mixture of 66% of the *d*- and 33% of the *l*-isomer. Presumably some of the *d*-material had been left behind in the barium residues on account of its greater relative insolubility, as compared with the *dl*-compound.

The free sugar was now carefully methylated with Purdie's reagents, and the product very slowly distilled in a high vacuum. Two fractions were collected and found to have identical properties. Both crystallised. They were united, and the crystals drained on porous tile for 3 hrs. at 0° and then for 12 hrs. at room temperature. Three recrystallisations at about 3° from light petroleum (b. p. below 40°) gave 42 mg. of fine needles having the properties of pure 2 : 3 : 4 : 6-tetramethyl β-methyl-*d*-galactoside, namely, m. p. 47—49°, $[\alpha]_D$ in water + 18·9° (c = 1·4) (cf. the constants given by Bell, *loc. cit.*). A mixture with authentic material showed no depression of m. p.

The still residue from the last distillation also crystallised, and after draining on porous tile and recrystallisation from light petroleum 77 mg. of needles, m. p. 43—44°, were obtained. This sub-fraction (*X*) had very small optical activity, however, showing $[\alpha]_D$ in water + 1·2° (c = 2·6), corresponding to a mixture of about 53% of *d*- and 47% of *l*-tetramethyl αβ-methyl-galactosides.

The material absorbed by the tiles used in the above operations was extracted with ether. After evaporation of the solvent, the crystals which remained were drained on porous tile and twice recrystallised; the m. p. was then 39—41°. The substance had $n_D^{16°}$ 1·4486 and $[\alpha]_D$ in water + 25·4° : from the $n_D^{16°}/[\alpha]_D$ relationship this corresponded to a mixture of 72% of the *d*- and 28% of the *l*-isomer. Crystallisation from more than the minimum quantity of solvent, kept at 20°, yielded a second sub-fraction (*Y*), m. p. 42°, $[\alpha]_D^{20°}$ + 0·5° (water; c = 1·0).

No further purification of *X* and *Y* was possible on account of the exceptional solubility of the pentamethyl hexose in all solvents. United, these sub-fractions amounted to about 100 mg., which were hydrolysed by hot 5% hydrochloric acid to the free sugar, and then subjected to anilide formation in the usual way. The fine needles (70 mg.) obtained, when recrystallised from alcohol or ethyl acetate, had m. p. 179—181° and $[\alpha]_D$ in acetone (uncatalysed), − 2° (c = 2) (Found : C, 61·7; H, 8·3; N, 4·4; OMe, 39·3%). A mixture with 2 : 3 : 4 : 6-tetramethyl *d*-galactose anilide melted at 179—197°. A mixture with synthetic 2 : 3 : 4 : 6-tetramethyl *dl*-galactose anilide (prepared as described below) showed no depression.

2 : 3 : 4 : 6-*Tetramethyl* dl-*galactose anilide* was prepared in the following manner. 1·2 G. of pure *dl*-galactose were boiled for 6 hrs. with 25 ml. of methyl alcohol containing 1% of dry hydrogen chloride. The product (1·2 g.), a glass, isolated in the usual way, possessed no reducing properties and was optically inactive. It was methylated once by the methyl sulphate

method and once with Purdie's reagents, 0·9 g. of a colourless syrup being obtained. This had the properties of tetramethyl methyl-*dl*-galactoside, *viz.*, $n_D^{16°}$ 1·4502; $[\alpha]_D$ in water, zero ($c = 3·21$); OMe, 60·0%. This product was hydrolysed by boiling with 5% hydrochloric acid, and the free sugar isolated in the usual way. It was optically inactive in water ($c = 5·0$) and had OMe, 51·0%. 0·7 G. of this sugar was boiled with 0·7 g. of aniline and 10 ml. of 97% alcohol for 5 hrs. On evaporation a crystalline residue remained. The new substance was recrystallised from alcohol or ethyl acetate until a constant m. p. of 179—180° was attained; yield, 0·45 g. A mixture with 2 : 3 : 4 : 6-tetramethyl *d*-galactose anilide melted at 179—197°. The substance was optically inactive in acetone ($c = 2·5$) (Found: C, 61·7; H, 8·7; N, 4·6; OMe, 39·2. $C_{16}H_{25}O_5N$ requires C, 61·7; H, 8·0; N, 4·5; OMe, 39·9%).

There appears to be no doubt that the anilide obtained from the crystalline sub-fractions *X* and *Y* derived from fractions V and VI consists of tetramethyl *dl*-galactopyranose anilide, mixed with a small proportion of the *d*-isomer. Since Haworth and Leitch (J., 1913, **113**, 188) record $[\alpha]_D$ in acetone (uncatalysed), — 83·3° for the latter substance, the value found, — 2°, corresponds to the presence of about 2—3% of the *d*-anilide. The evidence afforded by the m. p. and mixed m. p. determinations agrees with this conception.

Investigation of fraction IV. 2·010 G. of the hard crystalline mass to which this distillate rapidly set were hydrolysed by boiling with N-hydrochloric acid in the usual manner. After the acid had been neutralised with silver carbonate, the solution was filtered before and after passage of hydrogen sulphide and evaporated to dryness. The bulk of the residual syrup, 1·75 g. (95% yield), crystallised at 37° from moist ethyl acetate–alcohol, yielding several crops of 2 : 4-dimethyl galactose monohydrate, m. p. 104—106°, $[\alpha]_D$ in water (at equilibrium) + 85·0° ($c = 4·5$). Smith (J., 1939, 1724) gives + 85° and + 87·5° and later (J., 1940, 1035) + 86° for the specific rotation of this compound.

The dried syrupy residues remaining after the above crystallisations weighed 400 mg. and had $[\alpha]_D^{22°}$ in water + 93·4° ($c = 3·1$). The above value of + 85·0° for the monohydrate corresponds to + 92° for the anhydrous sugar. This indicated that no appreciable amount of derivatives of *l*-galactose occurs among the " trimethyl " fraction.

300 Mg. of the syrup were boiled with 0·4 ml. of aniline and 5 ml. of alcohol for 4 hrs. The crystalline residue obtained on evaporation of the solution to dryness was recrystallised from alcohol (yield, 110 mg.); it had m. p. 216°, not depressed by authentic 2 : 4-dimethyl *d*-galactose anilide, $[\alpha]_D$ in pure, dry pyridine, — 180° ($c = 1·5$). 300 Mg. of authentic 2 : 4-dimethyl *d*-galactose, under identical conditions of anilide formation, yielded 285 mg. of pure anilide, $[\alpha]_D$ in pyridine, — 181°; Hirst and Jones (1939) give a value of — 174°. Fraction IV therefore consisted almost wholly of 2 : 4-dimethyl αβ-methyl-*d*-galactoside. No contaminant could be detected.

Investigation of residue A. This consisted of 590 mg. of a hard brown glass containing some crystals. It was boiled for 4 hrs. in methyl alcohol containing 1% of dry hydrogen chloride. After neutralisation with silver carbonate, filtration, etc., the residue crystallised from ether containing a little acetone to yield 490 mg. of 2 : 4-dimethyl αβ-methyl-*d*-galactosides (OMe, 41%). This was confirmed by hydrolysis to the free sugar, followed by anilide formation, 400 mg. of product being obtained, m. p. alone or mixed with authentic anilide, 215°; $[\alpha]_D$ in pyridine, — 181°.

From the crystallisation of the mixed dimethyl galactosides a dark coloured residual syrup (120 mg.) remained. Beyond showing that it approximated in composition to a trimethyl hexose, we did not further investigate it. Residue *A* thus consisted essentially of 2 : 4-dimethyl methyl-*d*-galactosides (or polymerisation products).

Investigation of fraction VII. This fraction (648 mg.) had OMe 53%, a value which might be approximately afforded by either of the following mixtures of methylated hexosides: (*a*) 30% of " pentamethyl " with 70% of " tetramethyl " (*b*) 63% of " pentamethyl " with 37% of " trimethyl." In an endeavour to decide between these possibilities, 600 mg. of the material were hydrolysed to the free sugar in the usual way, and the crude product extracted with ether. The syrup (490 mg.) obtained by removal of the solvent was subjected to anilide formation and 500 mg. of fine needles were obtained on crystallisation from alcohol. Although these were superficially identical with the anilide of 2 : 3 : 4 : 6-tetramethyl *d*-galactose, the m. p. was considerably lower, *viz.*, 178°. No other crystalline substance could be detected. The methoxyl content of the crystals (Found: OMe, 39·1. Calc. for tetramethyl hexose anilide: OMe, 39·8%) indicated that this anilide was derived from a tetramethyl hexose, and since the presence of derivatives of *l*-galactose had been discovered in the " pentamethyl " fractions it seemed likely that the product under discussion was a mixture of the *d*- and the

l-tetramethyl galactose anilide. Haworth and Leitch (*loc. cit.*) state that the specific rotation in acetone of 2 : 3 : 4 : 6-tetramethyl *d*-galactose anilide is initially − 83·3°, rising to + 40·7°. Our material showed $[\alpha]_D$ in acetone, − 59·7°, remaining constant for 60 minutes, but on catalysis with a trace of hydrogen chloride it rose to a constant value of + 28·2° in 10 minutes. The initial value corresponds to a ratio of *d*/*l*-components of 86 : 14 and the final value to a ratio of 87 : 13. From the yields of tetramethyl *d*- and *l*-galactose anilides obtained in this experiment it would appear that fraction VII consists of tetramethyl methyl-*d*- and -*l*-galactosides (63% or 0·408 g. approx.) together with trimethyl methylgalactoside (37% or 0·240 g. approx.), and possibly a little dimethyl methylgalactoside.

Investigation of fraction VIII. This amounted to 345 mg., of which 320 mg. were hydrolysed to the free sugars and condensed with aniline in the usual way. The crude product consisted in part of very fine needles (50 mg.); recrystallised from ethyl acetate, these had m. p. 180°. A mixture with authentic 2 : 4 : 6-trimethyl *d*-galactose anilide (Hirst and Jones, J., 1939, 1482) showed no depression, whereas a mixed m. p. with material from fraction VII was 155—165°. The crystalline form of the anilide is very characteristic; no sign of the plate-like crystals of the 2 : 3 : 4-isomer (Hirst and Jones, *loc. cit.*) could be detected. 2 : 3 : 6-Trimethyl galactose does not form a crystalline anilide, and the derivative, if any, of 3 : 4 : 6-trimethyl galactose is unknown. Our material showed mutarotation in acetone (catalysed by a trace of hydrogen chloride) from $[\alpha]_D$ − 96·1° to + 40·0° in 120 minutes ($c = 1{\cdot}32$); Hirst and Jones report values of from − 92° to + 38° for 2 : 4 : 6-trimethyl galactose anilide.

Apart from a few mg. of 2 : 4-dimethyl *d*-galactose anilide, no identifiable substance was obtained from the residues remaining after the above crystallisation. The methoxyl content (49·5%) of fraction VIII, together with its position in the distillation sequence, indicated that it must consist approximately of 80% of " tetramethyl " and 20% of " trimethyl " material, *i.e.*, about 276 mg. and 69 mg. respectively. Since a 70% yield of strongly crystallising anilide may be expected from 2 : 4 : 6-trimethyl galactose, and our yield was only 50 mg. instead of the 235 mg. to be expected, only some 20% of the tetramethyl fraction consisted definitely of 2 : 4 : 6-trimethyl methyl-*d*-galactoside.

It seems probable that the small amounts of " tetramethyl " material present in fractions VII and VIII consisted of a mixture of several isomers, although only one of these could be identified. It is therefore likely that the heterogeneous " tetramethyl " substances had their origin in incomplete methylation of some of the side-chain radicals of the original galactogen, rather than in the presence in that polysaccharide of a typical " end-group." Such a group would be expected to give rise to a single trimethyl methylgalactoside.

Investigation of residue C. This was partly crystalline and weighed 676 mg. It was repeatedly extracted at room temperature with large volumes of light petroleum (b. p. 60—80°). The residue (490 mg.) had $n_D^{16°}$ 1·4757 and OMe 40·1%. This was hydrolysed to the free sugar, from which a crystalline anilide was obtained in good yield. This proved to be 2 : 4-dimethyl *d*-galactose anilide, m. p. 214—216°, alone or mixed with authentic material. This portion must thus have consisted almost entirely of 2 : 4-dimethyl αβ-methyl-*d*-galactosides. The petroleum extracts were evaporated to dryness and yielded 184 mg. of a syrup with $n_D^{16°}$ 1·4658 and OMe 46%. These figures and the position in the distillation sequence suggest that it consisted of approximately equal parts of " tetra " and " tri " materials. Hydrolysis to the free sugar, followed by condensation with aniline, failed to yield any identifiable material.

Residue *C* thus approximated in composition to a mixture of 92 mg. of trimethyl methylgalactoside (probably mixed *d*- and *l*-isomers) with 584 mg. of 2 : 4-dimethyl methyl-*d*-galactoside.

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