115. *The Constitution of Larch ε-Galactan.*

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Hydrolysis of methylated larch ε-galactan yields 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, 2 : 3 : 4-tri-*O*-methyl-D-galactose, and 2 : 4-di-*O*-methyl-D-galactose in approximately equal quantities, together with smaller amounts of 2 : 3 : 4-tri-*O*-methyl-L-arabinose, 2 : 5-di-*O*-methyl-L-arabinose, 2 : 4 : 6-tri-*O*-methyl-D-galactose, and 2-*O*-methyl-D-galactose. Partial acid hydrolysis of the polysaccharide affords two disaccharides, 6-*O*-β-D-galactopyranosyl-D-galactose and 3-*O*-β-D-galactopyranosyl-D-galactose, and a series of higher oligosaccharides. Partial acid hydrolysis of the fragment remaining after degradation of the periodate-oxidised polysaccharide with phenylhydrazine affords 3-*O*-β-D-galactopyranosyl-D-galactose, and only small amounts of the second disaccharide. It is concluded (i) that the larch polysaccharide is a highly branched arabogalactan in which the majority of L-arabinose residues are accommodated as 3-*O*-β-L-arabopyranosyl-L-arabofuranosyl side-chains, and (ii) that the framework of the molecule consists of chains of 1 : 3-linked β-D-galactopyranose residues, the majority of which carry side-chains containing an average of two 1 : 6-linked β-D-galactopyranose residues.

It has been shown previously [1] that the water-soluble ε-galactan from European larch wood (*Larix decidua*) is a complex polysaccharide containing D-galactose and L-arabinose residues in the approximate ratio of 6 : 1. Evidence was presented which indicated that the material examined was a mixture, the main component being a galactan containing only minimal quantities of arabinose residues, and there also was present either an arabogalactan or an araban and a second galactan. Hydrolysis of the methylated polysaccharide derived from the main component gave equimolecular proportions of 2 : 3 : 4 : 6-tetra-, 2 : 3 : 4-tri-, and 2 : 4-di-*O*-methyl-D-galactose, indicating a highly branched molecule for which several possible repeating units could be suggested. More recently Jones [2] has isolated 3-*O*-β-L-arabopyranosyl-L-arabinose from the products of mild acid hydrolysis, indicating the presence in the polysaccharide of some arabinose residues in the less common pyranose form. We now report the results of a further investigation of this material.

ε-Galactan, isolated from larch sawdust by aqueous extraction, gave on hydrolysis galactose (85%) and arabinose (12%). No evidence of heterogeneity in this sample was found by Heidelberger [3] in a study of the precipitation reactions of the polysaccharide with various pneumococcal sera. Furthermore, an ultracentrifugal examination, kindly carried out by Dr. C. T. Greenwood, indicated the presence of only one molecular species. This latter result is in contrast to the findings of Mosimann and Svedberg,[4] whose ultracentrifugal measurements indicated the presence of two distinct components in the ε-galactan from European larch.

ε-Galactan was converted into its fully methylated derivative, but we could find no evidence of heterogeneity of the type encountered in the earlier investigation.[1] Hydrolysis of the methylated polysaccharide gave 2 : 3 : 4 : 6-tetra-, 2 : 3 : 4-tri-, and 2 : 4-di-*O*-methyl-D-galactose in approximately equimolecular proportions, together with smaller amounts of 2 : 3 : 4-tri- and 2 : 5-di-*O*-methyl-L-arabinose, and 2-*O*-methyl-D-galactose. Since the physical constants found for the tri-*O*-methyl-D-galactose did not correspond exactly to those of pure 2 : 3 : 4-tri-*O*-methyl-D-galactose, a search was made for other trimethyl ethers of galactose. Careful fractional crystallisation of the aniline derivatives afforded a small quantity of the aniline derivative of 2 : 4 : 6-tri-*O*-methyl-D-galactose. The proportions of the two trimethyl ethers were estimated by determining the formaldehyde formed on periodate oxidation of the derived hexitols; the 2 : 3 : 4-isomer yields

[1] Campbell, Hirst, and Jones, *J.*, 1948, 774.
[2] Jones, *J.*, 1953, 1672.
[3] Heidelberger, *J. Amer. Chem. Soc.*, 1955, **77**, 4308.
[4] Mosimann and Svedberg, *Kolloid Z.*, 1942, **100**, 1.

formaldehyde whereas the 2 : 4 : 6-isomer does not. The results indicated the presence in the mixture of 2 : 3 : 4- (92%) and 2 : 4 : 6-tri-*O*-methyl-D-galactose (8%). Since this work was completed Jones and Perry [5] have reported the use of a similar procedure for the estimation of the relative proportions of mixtures of methylated sugars.

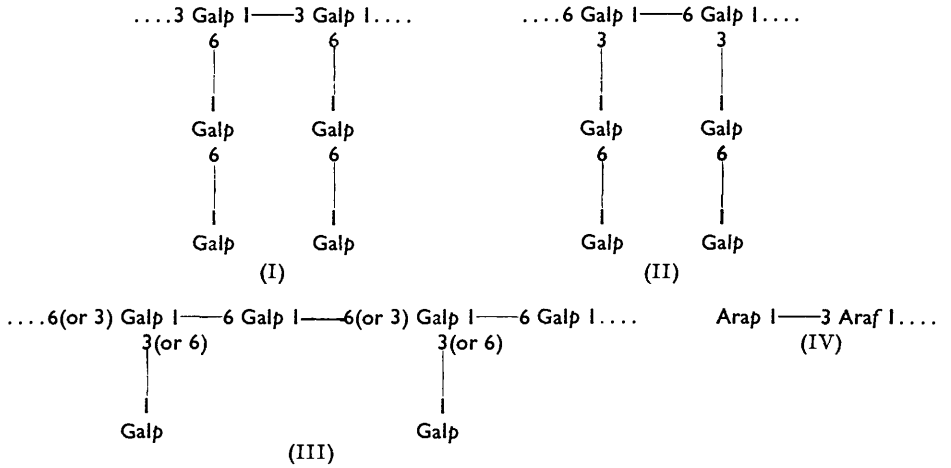
These results are in general accord with the previous findings [1] in respect of the main framework of galactose residues. The small quantity of galactose residues linked solely through $C_{(1)}$ and $C_{(3)}$ were not previously detected. Although the 2-*O*-methyl-D-galactose may have arisen from incomplete methylation of the polysaccharide, the absence of other monomethyl ethers of galactose suggests that this sugar may have structural significance, arising from a small proportion of doubly-branched galactose residues. 2 : 3 : 4-Tri-*O*-methyl-L-arabinose and 2 : 5-di-*O*-methyl-L-arabinose were isolated in approximately equimolecular amounts. In the absence of more than trace amounts of other arabinose derivatives it is clear that these sugars must represent fragments of an arabogalactan, and that on this evidence there is no indication of the presence in larch wood of polysaccharides composed solely of arabinose residues. Only traces of 2 : 3 : 5-tri-*O*-methylarabinose could be detected amongst the products of hydrolysis of the methylated polysaccharide and it is doubtful if terminal arabofuranose units are present to any significant extent in the polysaccharide. Since 3-*O*-β-L-arabopyranosyl-L-arabinose has been isolated from the products of partial acid hydrolysis of the polysaccharide,[2] it is now certain that the majority of arabinose residues are accommodated in 3-*O*-β-L-arabopyranosyl-L-arabofuranosyl side-chains, the furanose linkages being more easily cleaved on acid hydrolysis. Although this disaccharide has been isolated as an acid reversion from L-arabinose,[6] it is accompanied under these conditions by two other arabinose-containing disaccharides, neither of which was detected in the larch polysaccharide hydrolysate. Furthermore, in this instance, the disaccharide is destroyed when the hydrolysis is prolonged, whereas under the extreme conditions employed when acid reversion products are isolated in large amount [6] it is probable that an equilibrium is established between monomer and the various polymeric products.

On the basis of the methylation results various structures may be advanced for the repeating unit of the polysaccharide. Structures (I), (II), and (III) are representative of those containing an ordered arrangement of galactose residues; these structures do not take into account the mode of attachment of arabinose residues in side-chains (IV) to galactose. A partial distinction between the various possible structures has been made after the application of Barry's method of degradation [7] to the polysaccharide.

The periodate-oxidised ε-galactan was degraded by treatment with phenylhydrazine and acetic acid, and the products were separated into a mixture of compounds of low molecular weight and a polysaccharide residue. The products of low molecular weight were fractionated on alumina, and glyoxal bisphenylhydrazone and glycerosazone were isolated crystalline. Traces of galactosazone and arabinosazone were detected chromatographically, but the structural significance of these trace amounts is doubtful in view of the slightly acid conditions used in the degradation. Chromatography of the products of partial acid hydrolysis of the polysaccharide residue showed galactose, arabinose, and a series of galactose-containing oligosaccharides. The various structures represented by formula (III) can now be rejected since these contain only isolated galactose residues unattacked by periodate. The polysaccharide must contain, therefore, a resistant backbone of galactose residues with the majority of galactose residues, which are attacked by periodate, being accommodated in the side-chains of the molecular structure (I or II). A distinction between structures (I) and (II) has been made on the basis of evidence obtained from a study of the oligosaccharides isolated on partial acid hydrolysis of ε-galactan and of the periodate-oxidised polysaccharide after degradation with phenylhydrazine.

[5] Jones and Perry, *J. Amer. Chem. Soc.*, 1957, **79**, 2787.
[6] Ball, Jones, Nicholson, and Painter, *T.A.P.P.I.*, 1956, **39**, 438.
[7] Barry and Mitchell, *J.*, 1954, 4020.

The oligosaccharides derived from ε-galactan on partial hydrolysis were separated on charcoal–Celite by elution with water containing increasing quantities of ethanol. Two disaccharides, readily distinguishable by paper chromatography, were isolated pure. The 1 : 6-linked galactobiose was differentiated from the 1 : 3-linked isomer by the fact that the latter only gave formaldehyde on periodate oxidation. Galactobiose I had an optical rotation indicative of a β-linkage, and the structure of the disaccharide was established since hydrolysis of the methylated derivative afforded 2 : 3 : 4 : 6-tetra- and 2 : 3 : 4-tri-*O*-methyl-D-galactose. Galactobiose II, isolated crystalline, had similar physical constants to those recorded for 3-*O*-β-D-galactopyranosyl-D-galactose, isolated from the graded hydrolysis of *Acacia pycnantha* gum.[8] The mode of linkage in the disaccharide was confirmed by the isolation of 2 : 3 : 4 : 6-tetra- and 2 : 4 : 6-tri-*O*-methyl-D-



galactose on hydrolysis of the methylated derivative. Three galactose-containing trisaccharides were also isolated, and, although insufficient quantities of these compounds were available for full structural determinations, preliminary experiments provided evidence of their probable structures. Galactotriose I, isolated crystalline, gave only 6-*O*-galactosylgalactose and galactose on partial acid hydrolysis and is probably *O*-D-galactopyranosyl-(1 → 6)-*O*-D-galactopyranosyl-(1 → 6)-D-galactose. Galactotriose II gave both the 1 : 3- and the 1 : 6-linked galactobiose on partial hydrolysis, but since the derived glycitol gave galactose and no reducing disaccharide on partial hydrolysis, it is probable that the branched trisaccharide is 3 : 6-di-*O*-galactopyranosylgalactose. Galactotriose III gave 3-*O*-galactosylgalactose and galactose on partial hydrolysis and is probably *O*-galactopyranosyl-(1 → 3)-*O*-galactopyranosyl-(1 → 3)-galactose. Such a 1 : 3-linked galactotriose could only arise from a polysaccharide in which the repeating unit (I) is an important part of the molecular structure.

The degraded polysaccharide, remaining after treatment of the periodate-oxidised ε-galactan with phenylhydrazine and acetic acid, was subjected to graded acid hydrolysis, the products were fractionated on charcoal, and the disaccharide fractions were examined. 3-*O*-β-D-Galactopyranosyl-D-galactose, isolated crystalline, was the major component of this fraction and only small quantities of the 1 : 6-linked disaccharide were detected chromatographically. It follows from this observation that the backbone of the polysaccharide, resistant to attack by periodate, is composed of chains of 1 : 3-linked β-D-galactopyranose residues. The significance of the small amount of 6-*O*-galactosylgalactose detected in this experiment is not yet clear. It is possible that the backbone of the molecule may contain a small proportion of 1 : 6-linkages. Alternatively, if the arabinose containing side-chains are attached to the outer chains of the molecule, *e.g.*, through

[8] Hirst and Perlin, *J.*, 1954, 2622; Perlin, *Analyt. Chem.*, 1955, **27**, 396.

position 3 of galactose, some galactose residues in the outer part of the molecular structure would be unattacked by periodate and would give rise to the 1 : 6-linked disaccharide on partial hydrolysis of the degraded polysaccharide.

We can now summarise the main conclusions from these experiments. The ε-galactan from European larch is a highly branched arabogalactan in which the majority of arabinose residues are accommodated as 3-*O*-β-L-arabopyranosyl-L-arabofuranosyl side-chains (IV) linked to the framework of galactose residues. There is no evidence yet for the presence in larch wood of a polysaccharide composed solely of arabinose residues. The arrangement of galactose residues is best represented by the repeating unit (I), in which each D-galactopyranose residue in a 1 : 3-linked chain carried through position 6 an average of two 1 : 3-linked D-galactopyranose residues. In this respect, the molecular structure is strikingly similar to that now known to be present in gum arabic.[9] Although these experiments have indicated the essential homogeneity of ε-galactan, it is possible that, as in the xylan group,[10] several closely-related molecular species with larger or smaller proportions of arabinose residues may occur together.

In many respects the European larch ε-galactan is similar to the arabogalactan from Western larch (*Larix occidentalis*), which was extensively studied by White.[11] This polysaccharide also contained galactose and arabinose units in the ratio of 6 : 1, and it was shown by methylation that the D-galactopyranose residues were linked in a similar manner. There was also evidence from studies of a methylated degraded polysaccharide that at least a part of the backbone of the molecule is composed of chains of 1 : 3-linked D-galactopyranose residues. The polysaccharide, however, differed from ours in that L-arabinose residues were found only as end groups in the furanose form. Recently, further evidence for the similarity of the two polysaccharides has come from the isolation of 3-*O*-β-L-arabopyranosyl-L-arabinose and 6-*O*-β-D-galactopyranosylgalactose from the mild hydrolysis of the Western larch arabogalactan.[12] It is probable that the former disaccharide arises from cleavage of an arabofuranosyl linkage, but Bouveng and Lindberg [12] suggest that the second disaccharide also arises from scission of a furanosyl linkage. In preliminary experiments on the European larch ε-galactan it was shown that under the hydrolysis conditions required to release 3-*O*-arabopyranosylarabinose, appreciable quantities of the 1 : 6-linked galactobiose were also formed. Furthermore, attempts to prepare an arabinose-free galactan by selective hydrolysis under mild conditions failed as extensive breakdown of the polysaccharide to galactose-containing oligosaccharides occurred before all the arabinose residues were removed. Since we could find no evidence for the presence in the polysaccharide of galactofuranose residues, it is clear that in this case the rates of hydrolysis of arabofuranosides and galactopyranosides are not markedly different.

Experimental

Paper chromatography was on Whatman No. 1 filter paper, the following solvent systems (v/v) being used: (A) butan-1-ol–ethanol–water (40 : 11 : 19); (B) ethyl acetate–pyridine–water (10 : 4 : 3); (C) benzene–ethanol–water (169 : 47 : 15; upper layer); (D) butan-1-ol–formic acid–water (500 : 115 : 385; upper layer); (E) butan-2-one saturated with water.

The larch ε-galactan was prepared at the Forest Products Research Laboratory, Princes Risborough, by extraction of the sawdust with water, purification by passage through columns of Amberlite resins IR-120 and IRA-400, and precipitation from aqueous solution with ethanol. The polysaccharide yielded on hydrolysis galactose (85%) and arabinose (12%), the proportions of the sugars being estimated by Hirst and Jones's [13] method. Preliminary chromatographic studies showed that when the ε-galactan was heated at 100° with 0·01N-hydrochloric acid,

[9] Dillon, O'Ceallachain, and O'Colla, *Proc. Roy. Irish Acad.*, 1953, **55**, *B*, 331; 1954, **57**, *B*, 31; Smith and Spriestersbach, Amer. Chem. Soc. Meeting, Minneapolis, Sept., 1955, Abs. Papers, 7D.
[10] Hirst, *J.*, 1955, 2974.
[11] White, *J. Amer. Chem. Soc.*, 1941, **63**, 2871; 1942, **64**, 302, 1507, 2838.
[12] Bouveng and Lindberg, *Acta Chem. Scand.*, 1956, **10**, 1515.
[13] Hirst and Jones, *J.*, 1949, 1659.

arabinose, galactose, 3-*O*-arabopyranosylarabinose, and galactobiose I were released. On prolonged hydrolysis the arabinose-containing disaccharide disappeared and there was no indication of its formation under these conditions as an acid reversion product. An attempt was made to prepare a degraded galactan devoid of arabinose residues by mild acid hydrolysis (0·01N-hydrochloric acid at 100°), but even after heating for 20 hr. the precipitable degraded polysaccharide still contained arabinose residues, and galactose and galactose-containing oligosaccharides could be detected in the supernatant liquid.

Methylation of ε-Galactan.—ε-Galactan was methylated extensively with methyl sulphate and sodium hydroxide to give a methylated polysaccharide, $[\alpha]_D^{18}$ −49° (*c* 1·2 in chloroform), $[\alpha]_D^{18}$ −27° (*c* 1·1 in methanol) [Found: OMe, 44·1; dimethylaraban, 5·2% (based on the yield of furfuraldehyde on distillation with 12% hydrochloric acid under standard conditions [14])]. Fractionation of the methylated polysaccharide failed to yield materials differing significantly in dimethylaraban content.

Hydrolysis of Methylated ε-Galactan and Separation of Methylated Sugars.—The methylated polysaccharide (10 g.) was suspended in 2N-sulphuric acid (150 ml.) and kept at room temperature until dissolution was complete. Water (150 ml.) was added and the solution was warmed slowly so that the methylated polysaccharide remained in solution and was then heated at 100° for 7 hr. The cooled solution was neutralised with barium carbonate and on concentration gave a syrupy mixture of methylated sugars (9·8 g.). A portion (5 g.) of the hydrolysate was separated on cellulose (80 × 3·5 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3) saturated with water as eluant to give ten fractions; a further fraction was obtained by elution of the cellulose with water.

Analysis of hydrolysate of methylated ε-galactan.

Fraction	Material eluted (g.)	R_G in solvent A *	Paper chromatography sugar *	Sugar given after demethylation *
1	0·006	0·96	2 : 3 : 5-trimethylarabinose	arabinose
2	0·437	0·96 (*t*)	2 : 3 : 5-trimethylarabinose	arabinose (*t*)
		0·90	2 : 3 : 4 : 6-tetramethylgalactose	galactose
3	0·701	0·90	2 : 3 : 4 : 6-tetramethylgalactose	—
4	0·033	0·90	2 : 3 : 4 : 6-tetramethylgalactose	—
		0·86	2 : 5-dimethylarabinose	—
		0·83	2 : 3 : 4-trimethylarabinose	—
5	0·211	0·86	2 : 5-dimethylarabinose	arabinose
		0·83	2 : 3 : 4-trimethylarabinose	
6	0·022	0·86	2 : 5-dimethylarabinose	arabinose
		0·83	2 : 3 : 4-trimethylarabinose	
		0·72	trimethylgalactose	galactose
7	0·970	0·72	trimethylgalactose	galactose
8	0·057	0·72	trimethylgalactose	galactose
		0·59 (*t*)	unknown sugar	arabinose (*t*)
		0·52 (*t*)	unknown sugar	
9	1·315	0·46	2 : 4-dimethylgalactose	—
10	0·260	0·30	2-methylgalactose	—
11	0·101	—	galactose (*t*), arabinose (*t*) + methylated uronic acid	—

* *t* = trace

Identification of Sugars from Hydrolysis of Methylated ε-Galactan.—*Fraction* 2. The syrup consisted almost entirely of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, paper chromatography indicating only a trace of tri-*O*-methylarabinose. The syrup crystallised on nucleation and after recrystallisation from ether–light petroleum had m. p. and mixed m. p. 68° (Found: OMe, 52·5. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%). The sugar was further characterised as 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine, m. p. and mixed m. p. 192°.

Fraction 3. The sugar, after recrystallisation, had m. p. and mixed m. p. (with 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose) 68°, $[\alpha]_D^{19}$ +142° (5 min.) ⟶ +117° (3 hr., const.) (*c* 1·1 in water)

[14] Dorée, "The Methods of Cellulose Chemistry," Chapman and Hall, London, 1947; Bott and Hirst, *J.*, 1932, 2621.

(Found: OMe, 53·1. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%), and its identity was confirmed by conversion into the aniline derivative, m. p. and mixed m. p. 192°.

Fraction 5. The syrup had OMe, 41·0%, $[\alpha]_D^{20}$ +82° (*c* 1·2 in water), and chromatography showed two components travelling at the same rates as 2 : 3 : 4-tri-*O*-methyl and 2 : 5-di-*O*-methyl-L-arabinose, with traces of methylated galactoses. The mixture of sugars (270 mg.), obtained from a separate experiment, was separated on filter sheets, solvent C being used, to give fractions 5*a* (111 mg.) and 5*b* (154 mg.), each of which contained only traces of the second component. Fraction 5*a*, $[\alpha]_D^{20}$ +120° (*c* 2·2 in water) (Found: OMe, 46·1. Calc. for $C_8H_{16}O_5$: OMe, 48·4%), was identified by conversion into 2 : 3 : 4-tri-*O*-methyl-L-arabonophenylhydrazide, m. p. 157° (Jones [2] quotes m. p. 159°). Fraction 5*b*, $[\alpha]_D^{18}$ −3° (*c* 1·5 in water) (Found: OMe, 33·9. Calc. for $C_7H_{14}O_5$: OMe, 34·8%), was identified by conversion into 2 : 5-di-*O*-methyl-L-arabonomide, m. p. 131°.

Fractions 4 *and* 6. The combined fractions were shown by chromatography to contain the same two sugars as fraction 5, together with smaller quantities of methylated galactoses. A portion (41 mg.), separated on filter sheets by using solvent C, yielded chromatographically pure 2 : 3 : 4-tri-*O*-methyl- (11 mg.) and 2 : 5-di-*O*-methyl-arabinose (22 mg.).

Fraction 7. The crystalline sample, which travelled on the chromatogram at the same rate as 2 : 3 : 4- and/or 2 : 4 : 6-tri-*O*-methyl-D-galactose, had $[\alpha]_D^{20}$ +125° (5 min.) ⟶ +104° (2 hr., const.) (*c* 1·1 in water) and after dehydration at 60° over phosphoric oxide under reduced pressure had $[\alpha]_D^{20}$ +109° (equil.) (*c* 1·1 in water) [Found (after dehydration): OMe, 41·3. Calc. for $C_9H_{18}O_6$: OMe, 41·9%]. Recrystallisation from acetone–ether–light petroleum gave a substance, m. p. (unaltered on further recrystallisation) 56°, $[\alpha]_D^{20}$ +139° (5 min.) ⟶ +109° (2 hr., const.) (*c* 1·1 in water) (Found: OMe, 38·2. Calc. for $C_9H_{18}O_6,H_2O$: OMe, 38·7%). {2 : 3 : 4-Tri-*O*-methyl-D-galactose monohydrate is reported to have m. p. 80°, $[\alpha]_D$ +152° ⟶ +114° (in water) and 2 : 4 : 6-tri-*O*-methyl-D-galactose to have m. p. 104—105°, $[\alpha]_D$ +124° ⟶ +93° (in water).} A sample (100 mg.) was dehydrated and refluxed with ethanolic aniline for 5 hr.; recrystallisation of the product from acetone yielded the characteristic plates of 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-galactosylamine, m. p. and mixed m. p. 166° (a mixture with the characteristic needles of 2 : 4 : 6-tri-*O*-methyl-*N*-phenyl-D-galactosylamine, m. p. 171°, had m. p. 144°). Concentration of the mother liquor yielded a mixture, m. p. 143°, of needles and plates but it was not possible to separate the two components.

Fraction 9. The chromatographically pure sugar was recrystallised from acetone containing 1% of water and had m. p. and mixed m. p. (with 2 : 4-di-*O*-methyl-D-galactose monohydrate) 102°, $[\alpha]_D^{20}$ +136° (5 min.) ⟶ +86° (2 hr., const.) (*c* 1·1 in water) (Found: OMe, 27·1. Calc. for $C_8H_{16}O_6,H_2O$: OMe, 27·2%). The identity of the sugar was confirmed by conversion into 2 : 4-di-*O*-methyl-*N*-phenyl-D-galactosylamine, m. p. and mixed m. p. 215°.

Fraction 10. The sugar (recrystallised from glacial acetic acid) had m. p. and mixed m. p. (with 2-*O*-methyl-D-galactose) 148—149°, $[\alpha]_D^{20}$ +53° (5 min.) ⟶ +85° (2 hr., const.) (*c* 0·9 in water), and the derived 2-*O*-methyl-*N*-phenyl-D-galactosylamine had m. p. 163°.

Fraction 11. The main component behaved chromatographically as a methylated uronic acid with low mobility in neutral solvents but having R_G 0·84 in solvent D and giving a characteristic cherry-red colour with aniline oxalate.

*Re-examination of Tri-*O*-methylgalactose Fraction.*—Recrystallisation of the tri-*O*-methylgalactose fraction (from a separate experiment) from acetone–light petroleum afforded 2 : 3 : 4-tri-*O*-methyl-D-galactose hydrate, m. p. 73—76° (some sintering from 65°). Tri-*O*-methylgalactose (0·6 g.; anhydrous) was heated with aniline (0·3 ml.) in ethanol (20 ml.) for 1 hr. After removal of solvent the crystalline product was fractionally crystallised from acetone and acetone–light petroleum. The first fractions were composed solely of the aniline derivative (plates), m. p. and mixed m. p. 163—165°, of 2 : 3 : 4-tri-*O*-methyl-D-galactose. Subsequent fractions contained two crystalline forms and mechanical separation of the needles, followed by two recrystallisations from acetone–light petroleum, afforded the aniline derivative (needles), m. p. 166—167°, mixed m. p. (with sample, m. p. 178°) 169—171°, of 2 : 4 : 6-tri-*O*-methyl-D-galactose ; the m. p. was depressed on admixture with the aniline derivative of 2 : 3 : 4-tri-*O*-methyl-D-galactose.

*Estimation of the Relative Proportions of Tri-*O*-methylgalactoses.*—Methyl ethers of D-galactose were converted into the corresponding methyl ethers of D-galactitol. The sugar (200 mg.) in water (10 ml.) was added to potassium borohydride (60 mg.) in water (5 ml.). The solution stood for 14 hr. at room temperature, excess of borohydride was destroyed by the

addition of acetic acid, the solution was de-ionised by passage through columns of ion-exchange resins, Amberlite IRA-400 and Zeo-Karb 225, and concentrated to a crystalline residue. These materials were used without further purification in the periodate oxidation experiments. 2 : 4-Di-*O*-methyl-D-galactose afforded 2 : 4-*di*-O-*methyl*-D-*galactitol*, which after recrystallisation from ethanol–light petroleum had m. p. 133—134°, $[\alpha]_D^{20}$ +16° (*c* 0·3 in water) (Found: OMe, 30·1. $C_8H_{18}O_6$ requires OMe, 29·5%). The mixture of tri-*O*-methylgalactoses afforded the corresponding mixture of galactitols, recrystallisation from ethanol–light petroleum giving 2 : 3 : 4-*tri*-O-*methyl*-D-*galactitol*, m. p. 119°, $[\alpha]_D^{20}$ +6° (*c* 0·8 in water) (Found: OMe, 42·0. $C_9H_{20}O_6$ requires OMe, 41·5%).

The formaldehyde formed on periodate oxidation of the methylated hexitols was determined with chromotropic acid by O'Dea and Gibbons's method.[15] 2 : 4-Di-*O*-methyl-D-galactitol gave theoretical amounts of formaldehyde (cf. D-glucose). The formaldehyde formed on oxidation of the mixture of tri-*O*-methyl-D-galactitols corresponded to the presence therein of 92% of the 2 : 3 : 4-isomer, and, by difference, of 8% of the 2 : 4 : 6-isomer.

Degradation of Periodate-oxidised ε-Galactan with Phenylhydrazine.—The polysaccharide (1·7 g.) was oxidised with sodium metaperiodate solution (120 ml.; 0·2M) for 144 hr. (consumption of periodate was complete, corresponding to the uptake of 8·7 moles of periodate per 6 residues of galactose and 1 residue of arabinose). The solution was treated with lead acetate to remove iodate and periodate, and then with dilute sulphuric acid to precipitate excess of lead. The solution of oxygalactan (160 ml.) was treated with phenylhydrazine (3·5 ml.) in 10% acetic acid (10 ml.), and the precipitate was washed with water and dried to a yellow powder (1·5 g.). The oxygalactan phenylhydrazine derivative was suspended in ethanol (40 ml.), phenylhydrazine (5 ml.) in glacial acetic acid (8 ml.) and water (15 ml.) was added, and the mixture was refluxed for 4 hr. Removal of the ethanol under reduced pressure yielded crystals A (1·7 g.) which were separated and washed. The filtrate was extracted with benzene and ether to give a dark red solution B, and concentration of the aqueous solution yielded a red gum C (0·6 g.).

The crystalline precipitate A was dissolved in benzene and adsorbed on alumina (80 g.). Elution with benzene (250 ml.) gave fraction Ai (1·0 g.) which on recrystallisation from benzene yielded glyoxal bisphenylhydrazone, m. p. and mixed m. p. 168°. Elution with 1 : 1 benzene–ether (350 ml.) gave fraction Aii (0·1 g.), recrystallisation from benzene yielding first *N*-acetylphenylhydrazine, m. p. and mixed m. p. 128°, and then glycerosazone, m. p. and mixed m. p. 130°. Elution with ether (175 ml.) gave fraction Aiii (0·25 g.), which on recrystallisation from benzene yielded *N*-acetylphenylhydrazine. Elution with 9 : 1 ethanol–water gave fractions Aiv (0·3 g.) (containing *N*-acetylphenylhydrazine), Av (0·04 g.), and Avi (0·01 g.). Circular-paper chromatography showed fraction Av to contain glycerosazone and a trace of galactosazone, and fraction Avi to contain glycerosazone, arabinosazone, and galactosazone.

The benzene and ether extracts (B) yielded more *N*-acetylphenylhydrazine on concentration (fraction Bi). The residual syrup was dissolved in benzene and adsorbed on alumina (100 g.). Elution with ether (500 ml.) yielded fraction Bii (0·05 g.), shown by chromatography to contain only glyoxal bisphenylhydrazone. Elution with 9 : 1 ethanol–water gave fractions Biii (1·06 g.) and Biv (0·07 g.). Fraction Biii on recrystallisation from benzene–light petroleum yielded *N*-acetylphenylhydrazine and chromatographic examination of the mother liquor showed glyoxal bisphenylhydrazone and glycerosazone. Chromatographic examination of fraction Biv showed arabinosazone, galactosazone, and material which did not move on the chromatogram. Hydrolysis of fraction Biv yielded galactose and arabinose.

The red gum (C) consisted of the backbone of the polysaccharide remaining unattacked by periodate, hydrolysis of which gave arabinose and galactose. Partial acid hydrolysis yielded a series of galactose-containing oligosaccharides.

Partial Acid Hydrolysis of ε-Galactan.—ε-Galactan (10 g.) was heated (boiling-water bath) in 0·2N-sulphuric acid for 3 hr. The cooled solution was neutralised with Amberlite resin IR-4B, concentrated to 100 ml., and poured into ethanol (400 ml.). The precipitated degraded polysaccharide was separated at the centrifuge and concentration of the supernatant liquid gave a syrup (D) (4·46 g.). The degraded polysaccharide was re-hydrolysed by N-sulphuric acid (200 ml.) for 1 hr. at 100° and gave syrup (E) (3·76 g.) and a gum (1·8 g.) insoluble in ethanol–water (4 : 1). Chromatography showed that syrups (D) and (E) contained similar mixtures of galactose, arabinose, and a series of oligosaccharides. The combined syrups (8·21 g.) were

[15] O'Dea and Gibbons, *Biochem. J.*, 1953, **55**, 580.

dissolved in water and poured on charcoal–Celite (1 : 1; 300 g.). Elution with water gave fraction 1 (6·12 g.) containing galactose and arabinose. Elution with water containing 2·5% of ethanol gave fraction 2 (368 mg.) containing a disaccharide (galactobiose I), R_{Gal} 0·40 in solvent B, and traces of other sugars. Elution with water containing 5% of ethanol gave fraction 3 (260 mg.) containing a disaccharide (galactobiose II), R_{Gal} 0·60 in solvent B. Elution with water containing 7·5% of ethanol gave fraction 4 (40 mg.), containing an oligosaccharide having R_{Gal} 0·18 and traces of other sugars, and fraction 5 (160 mg.), having a major component with R_{Gal} 0·25. Elution with water containing 10% of ethanol gave several small fractions (total, 58 mg.) containing mixtures of oligosaccharides having R_{Gal} 0·25, 0·15, and 0·10, which were not examined. Elution with water containing 15% of ethanol gave fraction 6 (128 mg.), having a major component with R_{Gal} 0·30.

Examination of Oligosaccharides.—Fraction 2. Chromatographically pure galactobiose I was isolated by separation on filter sheets with solvent B; the syrup had $[\alpha]_D^{17}$ +41° (*c* 1·18 in water). Oxidation of the disaccharide (12 mg.) with periodate [16] gave no formaldehyde. The major portion of the syrup (247 mg.) was dissolved in water (5 ml.), and methyl sulphate (1 ml.) and sodium hydroxide (1·5 ml., 30%) were added dropwise during 2 hr. Two more additions of methyl sulphate (7 ml.) and sodium hydroxide (10 ml., 30%) were made during a period of 6 hr. The reaction was completed by heating the solution on the boiling-water bath for 30 min., and the methylated disaccharide (243 mg.) was isolated by continuous extraction with chloroform for 12 hr. Hydrolysis of a sample (1 mg.) of the methylated disaccharide gave only tetra- and tri-*O*-methylgalactose. Methylated galactobiose I (93 mg.) was heated with N-hydrochloric acid (10 ml.) at 100° for 4 hr., and, after neutralisation with silver carbonate, gave a syrup (68 mg.) which was separated on a filter sheet with solvent E yielding fractions *a* (32 mg.) and *b* (25 mg.). Fraction *a* was characterised as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose and fraction *b* as 2 : 3 : 4-tri-*O*-methyl-D-galactose by conversion into the aniline derivatives, m. p. and mixed m. p. 192—193°, and m. p. 159—160° and mixed m. p. (with sample of m. p. 163—165°), 160—163° respectively.

Fraction 3. Galactobiose II crystallised readily and after recrystallisation from ethanol–water had m. p. 175—177°, $[\alpha]_D^{17}$ +78° (10 min.) ⟶ +63° (120 min., equil.) (*c* 0·67 in water). Hirst and Perlin [8] record m. p. 159—160°, $[\alpha]_D$ +62° (in water) for 3-*O*-β-D-galactopyranosyl-D-galactose monohydrate. Periodate oxidation [16] of a sample (12 mg.) gave formaldehyde, identified as the dimedone compound, m. p. 188—190°. Galactobiose II (100 mg.) was methylated as described above to give the methylated disaccharide (106 mg.). Hydrolysis of methylated galactobiose II (106 mg.) with N-hydrochloric at 100° for 4 hr., followed by neutralisation with silver carbonate, gave a syrup (75 mg.) which was separated on a filter sheet with solvent E yielding fractions *c* (34 mg.), *d* (25 mg.), and *e* (7 mg.). Fraction *c* was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose and fraction *d* as 2 : 4 : 6-tri-*O*-methyl-D-galactose by conversion into the aniline derivatives, m. p. and mixed m. p. 185—187°, and m. p. and mixed m. p. 168—169°, respectively. Fraction *e* contained di-*O*-methylgalactose, probably arising from incomplete methylation of the disaccharide.

Fraction 4. A chromatographically pure sugar, having R_{Gal} 0·18 in solvent B, m. p. 157—160°, $[\alpha]_D^{18}$ +16° (equil.) (*c* 0·38 in water), crystallised from ethanol–water. Partial acid hydrolysis of the sugar gave galactose, galactobiose I, and unchanged sugar.

Fraction 5. A sample of the major component, R_{Gal} 0·25 in solvent B, was separated. The sugar gave on partial acid hydrolysis galactose, galactobiose I, galactobiose II, and unchanged sugar. Partial hydrolysis of the derived glycitol (borohydride reduction) gave galactose and no other reducing sugars.

Fraction 6. A sample of the major component, R_{Gal} 0·30 in solvent B, was separated chromatographically. Partial acid hydrolysis of the sugar gave galactose, galactobiose II, and unchanged sugar, and partial hydrolysis of the derived glycitol gave galactose and galactobiose II.

Partial Acid Hydrolysis of Gum (C).—Gum (C), prepared from ε-galactan (6·5 g.) by phenylhydrazine degradation of the periodate-oxidised polysaccharide as described above, was heated with N-sulphuric acid (100 ml.) at 100° for 1 hr. The cooled solution was neutralised with Amberlite resin IR-4B and extracted with ether, the extract concentrated, and ethanol (2 vol.) added. A small precipitate was removed at the centrifuge and concentration of the supernatant liquid gave a syrup (1·82 g.) which was dissolved in water and poured on charcoal–

[16] Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1476.

Celite (1 : 1; 100 g.). Elution with water gave a mixture (0·80 g.) of galactose and arabinose. Elution with water containing 2·5% of ethanol gave a syrup (33 mg.) containing galactose, arabinose, and a small amount of galactobiose I, R_{Gal} 0·40. Elution with water containing 5% of ethanol gave the crystalline galactobiose II (60 mg.), R_{Gal} 0·60, which after recrystallisation from ethanol–water had m. p. 176—179° and mixed m. p. 175—178°, $[\alpha]_D^{18}$ +78° (5 min.) ⟶ +62° (60 min., equil.) (*c* 0·51 in water).

The authors thank the Norwegian Council for Science and The Humanities (Norges Almenvitenskapelige Forskingsråd) for the award of a maintenance grant to one of them (E. R.) and the Rockefeller Foundation and Imperial Chemical Industries Limited for grants.

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