280. *Studies on Seed Mucilages. Part I. A Preliminary Examination of the Mucilaginous Polysaccharide of the Seeds of* Plantago lanceolata.

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Extraction of the seed with water and suitable treatment yielded an " acid polysaccharide " of equivalent *ca.* 1100, composed of pentosan 72%, methyl pentosan 11%, uronic anhydride 15%, together with galactose. Hydrolysis indicated that *d*-xylose constituted the main building stone of the molecule.

Acetylation, followed by methylation, simultaneous hydrolysis and glycoside formation yielded four main fractions, which were shown to be (1) trimethyl methlyxylopyranosides (30%), (2) 3 : 4-dimethyl methylxylopyranosides (28%), (3) a mixture of the latter with 2 : 4 : 6-trimethyl methylgalactosides and glycosides of lower methoxyl content (22%), and (4) a mixture containing 2 : 4 : 6-trimethyl methylgalactosides, a partly methylated methyluronoside and unidentified glycosides (17%).

Attention is drawn to the branched-chain nature of the polysaccharide with its " end groups " of xylopyranose residues, the unusual arrangement of xylopyranose residues linked by 1 : 2-β-linkages and the presence of a small proportion of galactopyranose units linked as in agar.

PRECIPITATION with alcohol of the mucilaginous solution obtained when the seeds of *Plantago lanceolata* (rib-grass) are steeped in water yielded a polysaccharide with an ash content, unchanged on prolonged dialysis, of 7% (as sulphate), of which calcium and potassium sulphates are the main constituents. When the original mucilaginous solution was poured into acidified alcohol, an almost ash-free polysaccharide was obtained which had an acid equivalent of *ca.* 1100 and showed $[\alpha]_D^{16°}$ — 60° in aqueous solution. The approximate composition was found to be pentosan 72%, methyl pentosan 11%, and uronic anhydride 15%. Hydrolysis with oxalic acid, followed by neutralisation with calcium carbonate, yielded a calcium salt (30%), the analysis of which indicated that it was mainly the salt of an aldobionic acid containing the methylpentose residue, together with a syrup which crystallised almost completely as α-*d*-xylose. The non-crystallisable residue was devoid of arabinose but contained a small proportion of galactose. This result is different from that reported for the mucilage isolated by Anderson and Fireman (*J. Biol. Chem.*, 1935, **109**, 437) from the seed of *Plantago psyllium*, since arabinose, xylose, and *d*-galacturonic acid were present, but neither galactose nor a methyl pentose was detected in the products of hydrolysis of their mucilage.

More vigorous hydrolysis of the polysaccharide with sulphuric acid yielded a uronic acid which, although it was not obtained crystalline, yielded mucic acid on oxidation with bromine water and with nitric acid and suffered a reversal of rotation in contact with 1% methyl-alcoholic hydrogen chloride at room temperature in a manner closely similar to that displayed by *d*-galacturonic acid; it is considered highly probable, therefore, that this is the uronic acid present.

On acetylation the acid mucilage yielded an acetate, of which (A) (40%) was soluble in acetone, having $[\alpha]_D^{17°}$ — 72° and $CH_3{\cdot}CO$ 41%, as against $CH_3{\cdot}CO$ 36% for the insoluble residue (B) (60%). The properties and acetyl content of (B) were unchanged on reacetylation, and it may be that this fraction contains a resistant residue [the mucilage itself on hydrolysis always yields a dark insoluble residue, 7% (cf. Anderson and Fireman, *loc. cit.*)]. Although the crude acetate, the insoluble acetate (B), and the soluble acetate (A) on deacetylation yielded methylated polysaccharides with almost identical properties (OMe 35%, $[\alpha]_D^{18°}$ — 100°), viscosity measurements in *m*-cresol indicated that the molecular size of (A) and of the methylated product prepared from it was approximately half that of the methylated polysaccharides obtained from the other two acetates. Further work on large quantities of material is necessary before assigning a reason for this difference. Fractional precipitation of the methylated polysaccharide obtained from the crude acetate yielded fractions showing slight variations in methoxyl content (33—35%) and specific rotation ($[\alpha]_D^{20°}$ — 95° to — 109°), but simultaneous hydrolysis and glycoside formation

did not indicate any noticeable differences in composition, although these may become apparent when larger-scale operations are completed.

Repeated fractional distillation of the products obtained by the hydrolysis of the methylated polysaccharide with methyl-alcoholic hydrogen chloride yielded four main fractions (Table I).

TABLE I.

	B. p.	OMe, %.	$n_D^{17°}$.	Yield, %.
Fraction I	90—95° (bath temp.)/0·02 mm.	59	1·4406	(30)
,, II	100—120 ,, /0·02 ,,	48	1·4555	(28)
,, III	120—140 ,, /0·03 ,,	44	1·4675	(22)
,, IV	140—190 ,, /0·04 ,,	39	1·4727	(17)

Fraction I on hydrolysis gave crystalline trimethyl α-*d*-xylopyranose and was therefore a mixture of trimethyl methylxylopyranosides.

Fraction II proved to be a dimethyl methylxyloside from its properties and from the fact that further methylation and hydrolysis yielded quantitatively crystalline trimethyl xylopyranose. That the dimethyl xylose obtained from this fraction by hydrolysis was not 2 : 3-dimethyl xylose was shown by its failure to form the crystalline anilide which this sugar readily forms (Hampton, Haworth, and Hirst, J., 1929, 1739) and which was prepared for purposes of comparison. Oxidation yielded a crystalline *lactone*, the rate of hydrolysis of which was so rapid as compared with that of 2 : 3-dimethyl-γ-xylonolactone that it was evidently a δ-lactone, indicating that position 4 was occupied by a methoxyl residue. The corresponding amide also, in contrast to 2 : 3-dimethyl xylonamide, readily gave hydrazodicarbonamide on treatment with sodium hypochlorite, followed by semicarbazide (Weerman, *Rec. Trav. chim.*, 1917, **36**, 16). It is clear, therefore, that the dimethyl xylose must possess an α-hydroxy-group, so it must be 3 : 4-dimethyl-xylopyranose. Confirmation of this view was obtained by oxidation with nitric acid, since after esterification an ester was obtained which proved to be an active *methyl dimethoxyglutarate*, of which the corresponding amide also gave a positive " Weerman " test. Further methylation yielded *i*-xylotrimethoxyglutaric ester, characterised as the crystalline amide and compared with an authentic specimen prepared from trimethyl xylopyranose. More vigorous oxidation, followed by esterification and amide formation, yielded, in addition, a small quantity of *l*-dimethoxysuccinamide [*d*-(−)threodimethoxysuccinamide], a further proof of the existence of methoxyl residues on C_3 and C_4. Fraction II consists, therefore, of 3 : 4-dimethyl methylxylosides.

Fraction III proved to be a mixture of partly methylated methyl-xylosides and -galactosides. Complete methylation and hydrolysis yielded mainly trimethyl xylopyranose, but after the bulk of this had been removed by crystallisation, the residue gave, on treatment with aniline, tetramethyl galactopyranose anilide. This was traced to the presence of 2 : 4 : 6-trimethyl galactose, since the sugar derived from III on treatment with aniline gave the crystalline anilide of this sugar. By oxidation of fraction III with bromine water a δ-lactone was obtained very similar in properties to 3 : 4-dimethyl δ-xylonolactone, and the corresponding amide gave a positive " Weerman " test. From this fact it appears that 3 : 4-dimethyl methylxylosides together with 2 : 4 : 6-trimethyl methylgalactosides are present in fraction III. The methoxyl content indicates, however, that less highly substituted methylglycosides remain to be characterised in this fraction.

Fraction IV was also shown to be a complex mixture, from which on hydrolysis and anilide formation 2 : 4 : 6-trimethyl galactose anilide was isolated. It also appears that this fraction contains the ester of a partially methylated uronic acid together with at least one other constituent, neither of which has yet been isolated.

The main facts which emerge from this examination so far as it has proceeded are: (1) the abnormally high proportion of " terminal " groups of xylopyranose units which indicates the branched-chain nature of the molecule (cf. arabic acid; Smith, J., 1939, 1774; this vol., pp. 74, 79, 1035). (2) The unusual appearance of 3 : 4-dimethyl xylose units among the products of hydrolysis of the methylated mucilage, which are evidently joined by 1 : 2-β-linkages, since the polysaccharide, the acetate and methylated derivatives have strong negative rotations, although the main product of hydrolysis of the polysac-

charide is *d*-xylose. (3) Galactopyranose units are present in the molecule linked by positions 1 and 3 as in agar (Percival and Somerville, J., 1937, 1615), damson gum (Hirst and Jones, J., 1939, 1482), and certain other naturally occurring polysaccharides, although the proportion of galactose is by no means high. The mode of linkage of the uronic acid and the nature of the methyl pentose present, however, must be the subject of further study.

Experimental.

Preparation of the Mucilage.—Rib-grass seeds (500 g.) were soaked in water (10 l.) with occasional stirring for 24 hours. After filtration through muslin the thick filtrate in 500 c.c. portions was poured into alcohol (1 l.) with vigorous stirring. The stringy product was dehydrated in alcohol and ether (yield, 25 g.) [Found: ash (direct), 5%; (as sulphate), 7% containing Na, 2·3; K, 14·5; Ca, 18·8; SO_4, 53·2%].

When the mucilaginous solution, prepared as above, was poured into alcohol containing hydrogen chloride (0·7%), followed by repeated trituration with alcohol, the acid polysaccharide used in the subsequent operations was obtained. It showed $[\alpha]_D^{16°}$ − 60° in water (*c*, 0·4) [Found: equiv., by titration, 1100; uronic anhydride, 15·2% (Dickson, Otterson, and Link, *J. Amer. Chem. Soc.*, 1930, **52**, 1174; Hirst, Young, and Campbell, *Nature*, 1938, **142**, 912); pentosan, 72% (Marshall and Norris, *Biochem. J.*, 1937, **31**, 1053); methyl pentosan, 11% (Ellet and Tollens, *Ber.*, 1905, **38**, 492)].

Hydrolysis of the Mucilage with Oxalic Acid.—The mucilage (16·2 g.) was heated for 20 hours at 100° with oxalic acid (100 c.c., 3%). The insoluble residue (1·14 g.) was removed and the remaining solution, after neutralisation with calcium carbonate, filtration and concentration to 50 c.c. at 45°/15 mm., was treated with alcohol to yield a calcium salt " X " (5·4 g.). The filtrate and washings on evaporation gave a syrup " Y " (7·8 g.).

" X " appeared to be mainly the calcium salt of an aldobionic acid containing the methyl pentose. It showed $[\alpha]_D^{17°}$ + 89° in water (*c*, 0·5) [Found: Ca, 5·0; methyl pentose, 40·2; uronic acid, 45; pentose, nil. Calc. for $(C_{12}H_{21}O_{11})_2Ca$: Ca, 5·5; methyl pentose, 45·4; uronic acid, 54·3%].

After several weeks " Y " crystallised almost completely; the crystals removed on treatment with acetic acid showed m. p. 142°, not depressed on admixture with α-*d*-xylose, $[\alpha]_D^{17°}$ + 80° in water (*c*, 0·7), + 18° after 24 hours. The presence of *d*-xylose was confirmed by the formation in good yield of the osazone, $[\alpha]_D^{18°}$ − 44° in alcohol (*c*, 0·4), m. p. 158°, unchanged on admixture with an authentic specimen of *d*-xylosazone; and, on oxidation, followed by suitable treatment, by the isolation of the characteristic boat-shaped crystals of cadmium bromide–cadmium xylonate. The acetic acid filtrate after the removal of " Y " was concentrated to yield a syrup, 0·5 g. of which yielded on oxidation with nitric acid 0·18 g. of mucic acid, m. p. 223°, unchanged on admixture with an authentic specimen. Attempts to prepare arabinose diphenylhydrazone were abortive, although under the same conditions a mixture of xylose (0·1 g.) and arabinose (0·01 g.) gave arabinose diphenylhydrazone (0·026 g.).

Hydrolysis with Sulphuric Acid.—The mucilage (15 g.) was heated at 100° for 24 hours with sulphuric acid (15%). An insoluble residue (1·9 g.) was removed and the filtrate, after neutralisation with barium carbonate and precipitation with alcohol, gave a *barium* salt (1·8 g.) and then on evaporation a syrup (6·9 g.). Extensive decomposition took place during the hydrolysis.

The barium salt had $[\alpha]_D^{16°}$ + 22° in water (*c*, 0·6) [Found: Ba, 29·5; uronic acid, 61·7; pentosan and methyl pentosan, nil. $(C_6H_{11}O_7)_2Ba$ requires Ba, 23·3; uronic acid, 66·3%]. The salt was evidently contaminated with some other fission product but reprecipitation failed to purify it further. To the acid (0·1 g.) obtained by removal of the barium as sulphate, nitric acid (5 c.c., 50%) was added, and the solution evaporated to 1 c.c. during 2 hours. After the addition of water and standing, mucic acid separated (0·7 g.), m. p. 223°, unchanged on admixture with an authentic specimen. From another portion of the acid, mucic acid, m. p. 223°, was readily obtained by treatment with bromine water for 24 hours at 16°.

Furfuronoside Formation.—A portion of the acid syrup was treated with methyl-alcoholic hydrogen chloride (1%) at 15°. $[\alpha]_D^{15°}$ + 45° (initial value; *c*, 0·4); + 42·5° (3 hours); ± 0° (36 hours); − 19·7° (48 hours); − 42·4° (80 hours, constant value). *d*-Galacturonic acid in methyl-alcoholic hydrogen chloride (1%) showed $[\alpha]_D^{17°}$ + 50° (initial value; *c*, 0·6); + 46·3° (3 hours); + 25° (12 hours); + 0° (24 hours); − 31° (39 hours); − 51° (48 hours, constant value).

Acetylation of the Mucilage.—The dry mucilage (30 g.) was dispersed by vigorous shaking with pyridine (350 c.c.); acetic anhydride (250 c.c.) was then added in 50 c.c. portions with

shaking. After being heated at 95° on the water-bath for 3 hours, the mixture was set aside for 48 hours with occasional shaking. The acetate was then precipitated in a large excess of water as a cream-coloured solid (35 g.), which was repeatedly extracted with acetone–chloroform (1 : 1) to yield an insoluble residue " B " (20 g.), and a soluble acetate " A " (14 g.), which was isolated by precipitating the concentrated extract with light petroleum (b. p. 60—80°). " A " had $[\alpha]_D^{18°}$ — 72° in acetone (*c*, 0·3) (Found : " A ", $CH_3{\cdot}CO$, 41·0%; " B ", 36·0%). Further acetylation of " B " failed to raise this value.

Methylation of " A ."—The acetate (5 g.) in acetone (200 c.c.) was treated in the usual way with methyl sulphate (100 c.c.) and sodium hydroxide solution (250 c.c., 30%) in one-tenth portions every 10 minutes at 40—45°. After removal of the acetone the solution was heated at 80° for 1 hour and the light brown solid which separated was filtered hot. This solid was dissolved in acetone and remethylated as before; the solid which separated was washed with boiling water, remethylated twice in the same way, and finally extracted with boiling chloroform, dried over sodium sulphate, concentrated to small bulk, and precipitated with light petroleum (b. p. 60—80°) as a white powder (2 g.), $[\alpha]_D^{18°}$ — 104° in chloroform (*c*, 0·7) (Found : OMe, 35%).

" B " was methylated in the same way to yield a product (OMe, 34%), $[\alpha]_D^{18°}$ — 99° in chloroform (*c*, 1·1), and a similar result was obtained on methylating the crude acetate.

Fractional precipitation from chloroform solution by light petroleum (b. p. 60—80°) indicated that the methylated polysaccharides so obtained were essentially homogeneous.

Viscosity Determinations.—These were carried out in *m*-cresol with the results shown in Table II, where $\eta_{sp.}$ is the specific viscosity, *c* is the concentration of substance in g./100 c.c. of *m*-cresol, and *c'* is the concentration in g.-mols. of acetylated or methylated anhydroxylose residues per litre, assuming the repeating unit to be $C_5H_8O_4$.

TABLE II.

Substance.	*c*.	Temp.	$\eta_{sp.}$	$\eta_{sp.}/c$.	$\eta_{sp.}/c'$.
Soluble acetate " A "	0·311	18°	0·262	0·842	18·2
	,,	25	0·236	0·759	16·4
Methylated product from " A "	0·313	18	0·363	1·160	18·6
	,,	25	0·331	1·058	16·9
Methylated product from " B "	0·307	18	0·782	2·550	40·8
	,,	25	0·714	2·326	37·2
Methylated product from crude acetate	0·314	18	0·793	2·526	40·4
	,,	25	0·728	2·320	37·1

Typical Hydrolysis of the Methylated Polysaccharide.—A portion (8·14 g.) of methylated mucilage (OMe, 34·7%; $[\alpha]_D^{16°}$ — 104°) was boiled with methyl-alcoholic hydrogen chloride (200 c.c.; 3%) until the rotation became constant ($[\alpha]_D^{18°}$ + 90°; 17 hours). After neutralisation with silver carbonate and filtration the liquid was concentrated to yield a non-reducing syrup (8·32 g.), which on vacuum distillation gave four fractions. The first two were refractionated from a Claisen flask with a vacuum-jacketed column, giving finally :

Fraction I 2·52 g., b. p. 90—100°/0·02 mm. (bath temp.), $n_D^{17°}$ 1·4406
,, II 2·31 g., b. p. 100—120°/0·02 mm. ,, ,, $n_D^{14°}$ 1·4555
,, III 1·86 g., b. p. 120—140°/0·03 mm. ,, ,, $n_D^{14°}$ 1·4675
,, IV 1·40 g., b. p. 140—190°/0·04 mm. ,, ,, $n_D^{14°}$ 1·4727

Still residue 0·23 g.

Fraction I.—This had $[\alpha]_D^{20°}$ + 46° in chloroform (*c*, 0·5) (Found: OMe, 58·7. Cac. for $C_9H_{18}O_5$: OMe, 60·1%). A portion (0·5 g.) was hydrolysed at 100° for 1 hour with 2% nitric acid (15 c.c.), the solution neutralised with barium carbonate and evaporated to dryness, and the residue repeatedly extracted with boiling ether to give a syrup which rapidly crystallised completely (0·45 g.). The crystalline material had m. p. 89°, not depressed by authentic trimethyl xylopyranose, $[\alpha]_D^{20°}$ + 55° in water (*c*, 0·7), falling to + 20° (Found : OMe, 47·4. Calc. for $C_8H_{16}O_5$: OMe, 48·4%).

Methylation of Fraction II.—Fraction II (1·0 g.), $[\alpha]_D^{18°}$ + 69° in chloroform (*c*, 1·0) (OMe, 47·7%), was twice methylated by treatment with silver oxide and methyl iodide to yield a mobile oil (1·09 g.), $n_D^{17°}$ 1·4410. This (1·07 g.) was hydrolysed in the usual way with 2% nitric acid to give a syrup (0·87 g.), which crystallised on standing, m. p. 89°, unchanged by authentic trimethyl xylopyranose, $[\alpha]_D^{18°}$ + 53° in water (*c*, 1·0), falling to + 20° (Found : OMe, 47·7%).

Isolation and Examination of a Dimethyl Xylose from Fraction II.—Hydrolysis of II (0·5 g.)

with nitric acid as for fraction I yielded a viscous syrup (0·42 g.), $n_D^{14°}$ 1·4750, $[\alpha]_D^{16°}$ + 27° in water (*c*, 1·0) (Found : OMe, 33·0. Calc. for $C_7H_{14}O_5$: OMe, 34·8%).

Seven attempts to prepare a crystalline anilide failed (cf. trimethyl xylopyranose, which behaves similarly). Control experiments with 2 : 3-dimethyl xylose showed that under the same conditions this sugar readily formed the anilide, m. p. 143° (Hampton, Haworth, and Hirst, *loc. cit.*).

Lactone Formation.—The dimethyl xylose (0·6 g.) in water (9 c.c.) was treated with bromine (1 c.c.) at 40° for 2 days until reducing action had ceased. Bromine was then removed by aeration, the solution neutralised with silver carbonate, silver ions removed by hydrogen sulphide, the solution concentrated, and the syrupy product finally heated at 100°/15 mm. for 3 hours, followed by exhaustive extraction with ether. The syrup so obtained was distilled at 140°/0·03 mm. (bath temp.) to yield a *dimethyl xylonolactone* (0·35 g.), $n_D^{14°}$ 1·4600, which crystallised on standing, m. p. 67° (Found : OMe, 35·2. $C_7H_{12}O_5$ requires OMe, 36·1%), $[\alpha]_D^{18°}$ + 41° (5 minutes in water, *c* 0·4); + 36° (2 hours); + 31° (4 hours); + 31° (6 hours, constant value). 0·083 G. required 8·7 c.c. of N/20-sodium hydroxide for complete neutralisation. $C_7H_{12}O_5$ requires 9·4 c.c. The end-point of the titration was characteristic of a δ-lactone.

Dimethyl Xylonamide.—On treatment with methyl-alcoholic ammonia a syrupy *amide*, $[\alpha]_D^{14°}$ + 54° in water (*c*, 0·8), was obtained (Found : OMe, 30·7. $C_7H_{15}O_5N$ requires OMe, 32·1%).

The amide (0·12 g.) was dissolved in water (2 c.c.), the standard sodium hypochlorite solution (Weerman, *loc. cit.*) (1·4 c.c.) added, and the mixture kept at 0° for 3 hours. The slight excess of hypochlorite was destroyed with sodium thiosulphate and on the addition of semicarbazide hydrochloride and sodium acetate a dense precipitate of hydrazodicarbonamide (0·45 g.) rapidly formed, m. p. 257° (decomp.), unchanged by an authentic specimen. A control experiment on gluconamide (0·05 g.) yielded hydrazodicarbonamide (0·013 g.), but 2 : 3-dimethyl xylonamide (m. p. 131°) gave no precipitate even on long standing.

Oxidation with Nitric Acid.—Fraction II (0·9 g.), dissolved in nitric acid (10 c.c.; *d*, 1·4), was heated at 50° until the evolution of brown fumes had ceased (1—2 hours); the temperature was then raised slowly and kept at 90° for 4 hours. After dilution with water nitric acid was removed by the continuous addition and removal of water under diminished pressure. The syrup finally obtained was esterified by boiling with methyl-alcoholic hydrogen chloride (50 c.c.; 3%) for 6 hours, the acid neutralised with silver carbonate, and the syrupy ester (0·8 g.) distilled : " C," 0·3 g., b. p. 120—125°/0·04 mm. (bath temp.), $n_D^{13°}$ 1·4459, $[\alpha]_D^{15°}$ + 45° in methyl alcohol (*c*, 0·6); OMe, 55·2%. " D," 0·45 g., b. p. 130—150°/0·05 mm. (bath temp.), $n_D^{13°}$ 1·4458, $[\alpha]_D^{15°}$ + 41° in methyl alcohol (*c*, 0·8) (Found : OMe, 53·7. $C_9H_{16}O_7$ requires OMe, 52·5%).

" D " (0·1 g.) was treated with methyl-alcoholic ammonia; the resulting syrupy amide ($[\alpha]_D^{12°}$ + 27° in water, *c* 0·9) (0·057 g.) gave a precipitate of hydrazodicarbonamide (0·01 g.), m. p. 254° (decomp.), when subjected to the " Weerman " test.

The *hydroxy-ester* " D " (0·4 g.) was twice methylated with Purdie's reagents to yield a product (0·32 g.), b. p. 110—115°/0·02 mm. (bath temp.), $n_D^{11°}$ 1·4437, $[\alpha]_D^{11°}$ + 0° in methyl alcohol (*c*, 1·5) (Found : OMe, 59·7. Calc. for $C_{10}H_{18}O_7$: OMe, 62·0%). This ester was proved to be *i*-xylotrimethoxyglutaric ester by conversion into the corresponding amide, m. p. 194°, unchanged by an authentic specimen, $[\alpha]_D^{16°}$ + 0° in water (*c*, 1·2) (Found : C, 44·1; H, 7·0; OMe, 40·9. Calc. for $C_8H_{16}O_5N_2$: C, 43·6; H, 7·3; OMe, 42·3%).

Isolation of l-*Dimethoxysuccinamide.*—The oxidation of fraction II with nitric acid was carried out by heating for 7 hours at 90°. After the usual treatment a mixture of esters was obtained which partly crystallised on addition to alcoholic ammonia. The crystals showed $[\alpha]_D^{17°}$ − 90° in water (*c*, 0·3), m. p. 270—280° (decomp.), mixed m. p. with *d*-dimethoxysuccinamide 254° (decomp.).

Examination of Fraction III.—Methylation and hydrolysis. Fraction III (0·5 g.) $n_D^{16°}$ 1·4635, $[\alpha]_D^{17°}$ + 75° in chloroform (*c*, 0·7), was methylated three times with methyl iodide and silver oxide, and the resulting syrup distilled to give a mobile syrup (0·6 g.), b. p. 100°/0·02 mm. (bath temp.), $n_D^{14°}$ 1·4450, $[\alpha]_D^{15°}$ + 64° in water (*c*, 0·9) (Found : OMe, 60·1%).

Hydrolysis with hydrochloric acid (20 c.c.; 7%) until the rotation became constant (+ 37°; 1·5 hours), followed by neutralisation with silver carbonate, etc., yielded a colourless syrup (0·45 g.), which deposited crystals (0·34 g.), m. p. 89°, not depressed by trimethyl xylopyranose, $[\alpha]_D^{15°}$ + 56° in water (*c*, 0·7); + 33° (equilibrium) (Found : OMe, 48·1. Calc. for $C_8H_{10}O_5$: OMe, 48·4%).

The residual syrup (0·1 g.) on treatment with aniline yielded a crystalline anilide (0·03 g.),

m. p. 196°, unchanged on admixture with tetramethyl galactopyranose anilide (Found : OMe, 38·9. Calc. for $C_{16}H_{25}O_5N$: OMe, 39·8%).

Hydrolysis of fraction III. Fraction III (0·54 g.) was hydrolysed at 95° with hydrochloric acid (50 c.c.; 7%) until the rotation became constant (6 hours, + 35°). After neutralisation with silver carbonate a syrup (0·4 g.) was obtained which showed $[\alpha]_D^{14°}$ + 23° in water (*c*, 0·6) (Found : OMe, 36·2. Calc. for $C_7H_{14}O_5$: OMe, 34·8%).

Isolation of 2 : 4 : 6-*Trimethyl Galactose Anilide* [with R. BURNETT].—The hydrolysis product from another experiment (0·3 g.) in alcohol was heated for 1 hour at 95°. On evaporation and standing, crystals (0·02 g.) were obtained, m. p. 169°, mixed m. p. with 2 : 3 : 4-trimethyl galactose anilide 157°, unchanged on admixture with 2 : 4 : 6-trimethyl galactose anilide.

Oxidation. The syrupy sugar (0·3 g.) was oxidised with bromine as previously described. A lactone (0·18 g.), b. p. 150°/0·04 mm. (bath temp.), $n_D^{14°}$ 1·4693, was obtained (Found : OMe, 37·2. Calc. for $C_7H_{12}O_5$: OMe, 36·1%), $[\alpha]_D^{16°}$ + 50° (5 minutes in water, *c* 3·8); + 49° (1 hour); + 45° (3 hours); + 40° (7 hours, constant value). The lactone (0·1132 g.) required 12·0 c.c. of N/20-sodium hydroxide for complete neutralisation in the cold. Calc. for $C_7H_{12}O_5$: 11·9 c.c. The amide obtained from this lactone gave a positive " Weerman " test.

Preliminary Study of Fraction IV.—Hydrolysis. The viscous syrup (0·5 g.), $[\alpha]_D^{18°}$ + 90° in chloroform (*c*, 0·8), was hydrolysed at 95° with hydrochloric acid (50 c.c., 7%) until the rotation became constant (+ 39°). The solution was neutralised with silver carbonate, and silver ions removed by hydrogen sulphide; then followed treatment with barium carbonate, filtration, evaporation, and extraction with ether, giving a soluble syrup " E " (0·3 g.), $[\alpha]_D^{15°}$ + 44° in water (*c*, 0·7) (Found : OMe, 27·7%), and a barium salt " F " (0·15 g.), $[\alpha]_D^{15°}$ + 43° in water (*c*, 0·4) (Found : OMe, 17·7%). Owing to the quantity available no crystalline derivatives were obtained from " F " by methylation, hydrolysis, and oxidation.

" E " on treatment with aniline in the usual way gave an anilide (0·3 g.), m. p. 170°, unchanged on admixture with 2 : 4 : 6-trimethyl galactose anilide; m. p. 149° on admixture with 2 : 3 : 4-trimethyl galactose anilide (Found : OMe, 29·5. Calc. for $C_{15}H_{23}O_5N$: OMe, 31·3%).

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