POLYSACCHARIDES OF TROPICAL GRASS SPECIES. I. GIANT STAR GRASS (*Cynodon plectostachyus*)

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ABSTRACT

An arabinoglucuronoxylan isolated[1] from the leaf and stem of the tropical-pasture species *Cynodon plectostachyus* contained neutral sugars in molar proportions: D-xylose, 17; L-arabinose, 2.2; and D-glucose, 1. 2-*O*-(4-*O*-Methyl-α-D-glucopyranosiduronic acid)-D-xylose was also present. Methylation analysis showed the presence of 2,3,4-tri-, 2,3-di-, and 2-*O*-methyl-D-xylose, together with 2,3,5-tri-*O*-methyl-L-arabinose. The polysaccharide has structural features similar to those isolated from temperate-grass species.

INTRODUCTION

The first study of hemicelluloses in a tropical pasture-grass was a preliminary investigation of Giant Star grass (*Cynodon plectostachyus*)[1]. Subsequently, the hemicelluloses of speargrass (*Heteropogon contortus*) were examined[2]. The present study completes the work on the *Cynodon* polysaccharide. The investigation was undertaken to compare the structures of hemicelluloses of tropical-grass species with those of temperate grasses already studied by other workers. A comparison of various methylation procedures for both *Cynodon* and *Setaria* hemicelluloses has already been reported[3].

RESULTS AND DISCUSSION

Isolation and purification of *Cynodon* hemicellulose has been described by McIlroy[1], who also showed, by partial hydrolysis with 0.01M oxalic acid, that virtually all of the L-arabinose residues were attached as side-chains to a backbone of (1→4)-β-D-xylose residues. Some 3–4% of uronic acid residues was also found.

Determination of neutral sugars. — The unmethylated polysaccharide was hydrolysed and the hydrolysate separated by ion-exchange chromatography into neutral and acidic fractions, essentially according to Blake and Richards[11]. Neutral sugars were determined as their alditol acetates by g.l.c.[11,12], and as the free sugars

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by an automated column-chromatographic procedure. Results are shown in Table I. The amount of xylose involved in aldobiouronic acids is not allowed for.

The aldobiouronic acid. — The aldobiouronic acid, isolated as the barium salt, was qualitatively shown to consist of D-xylose and 4-*O*-methyl-D-glucuronic acid, and was further characterised as the well known 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose by examination of the partially methylated derivative obtained upon hydrolysis of the fully methylated polysaccharide.

TABLE I

ANALYSIS OF SUGARS IN UNMETHYLATED *Cynodon* HEMICELLULOSE

Sugar	*Molar ratio by autoanalysis*	*Molar ratio by alditol acetates*
L-Arabinose	2.22	2.27
D-Glucose	1.0	1.0
D-Xylose	17.2	16.9

Partial acid hydrolysis. — The partial hydrolytic procedure adopted was essentially that of Aspinall *et al*[13]. The three oligosaccharides isolated were xylobiose, xylotriose, and xylotetraose. A plot of log $[R_X/(1-R_X)]$ *vs.* n, was linear [R_X is the distance moved on a paper chromatogram relative to xylose, and n the degree of polymerisation (xylose residues) assumed for the oligosaccharides]. This correlation indicates a homologous series of oligosaccharides.

The partial hydrolysis thus indicated that the main chain consisted of β-(1→4)-linked D-xylopyranose residues. The existence of small proportions of other types of linkage or side chains cannot be ruled out in view of the low recovery of oligosaccharides. Indeed, the amount of tri-*O*-methylxylose recovered from the hydrolysate of the methylated polysaccharide is concordant with a low degree of branching.

Examination of the methylated polysaccharide. — A sample of *Cynodon* hemicellulose methylated by the Haworth[7] and Purdie[8] procedures was fractionated by extraction with light petroleum–chloroform mixtures (Table II). Over 92% of the

TABLE II

FRACTIONATION OF METHYLATED *Cynodon* HEMICELLULOSE

Fraction	*Solvent*[a]	*Yield* (%)	*Methoxyl* (%)	$[\alpha]_D^{25}$, *degrees*
1	0:100	0.6		
2	10:90	1.4	27.4	−26.3
3	20:80	3.8	32.6	−48.4
4	30:70	82.2	38.2	−82
5	35:65	10.6	39.0	−84
6	40:60	0.8	33	
residue		0.6		

[a]Ratios are chloroform–light petroleum (b.p. 40–60°).

material was extracted over a fairly narrow concentration range (30–35% of chloroform, fractions 4 and 5) indicating that it was relatively homogeneous. The $\overline{M}_n$ before fractionation was 6,700, and that of combined fractions 4 and 5 (after one further Purdie methylation) was 6,900 as determined by vapour-phase osmometry.

Acid hydrolysis of material from fractions 4 and 5, and separation of the products on a cellulose column, yielded four fractions, the first of which contained 2,3,4-tri-*O*-methyl D-xylose and 2,3,5-tri-*O*-methyl-L-arabinose, and the second 2,3-di-*O*-methyl-D-xylose. The third fraction crystallized and was shown to be 2-*O*-methyl-D-xylose.

The aldobiouronic acid derivative, eluted as a fourth fraction by water, on hydrolysis gave 2,3,4-tri-*O*-methyl-D-glucuronic acid and 3-*O*-methyl-D-xylose. Permethylation of the reduced aldobiouronic acid yielded a disaccharide whose high positive optical rotation (+98°) indicated an α-D-anomeric linkage.

Quantitative examination of the neutral sugars from the methylated polysaccharide. — The hydrolytic procedure used was essentially that of Garegg and Lindberg[14]. The temperature for primary hydrolysis in 72% sulphuric acid was kept at 0° for 1 h, instead of at room temperature, so as to cause less degradation[15].

Extraction of the hydrolysate with dichloromethane removed the tri-*O*-methylpentoses completely, together with a proportion of the less-substituted sugars. The dichloromethane was allowed to evaporate at 40–45°, diminished pressure being avoided to minimise losses of tri-*O*-methylpentose. The dichloromethane fraction was then made up to 10 ml in a standard flask.

Neutral and acidic sugar derivatives left in the acid hydrolysate after extraction with dichloromethane were separated by ion-exchange, and the neutral sugars were converted into the acetylated nitrile derivatives[10] for g.l.c. examination.

Because not all of the various standard sugars were available, the type of calibration adopted for the alditol acetates could not be used. Peak areas per mole were taken to be the same for all sugar derivatives used, and so relative peak-areas were assumed to reflect relative molar amounts. Results are shown in Table III.

TABLE III

METHYLATED SUGAR COMPOSITION OF METHYLATED *Cynodon* HEMICELLULOSE

Sugar derivative	*Molar ratio*
2,3,4-Tri-*O*-methyl-D-xylose	2
2,3,5-Tri-*O*-methyl-L-arabinose	4
2,3-Di-*O*-methyl-D-xylose	38
2-*O*-Methyl-D-xylose	5

Deduction of some structural details of the polysaccharide. — The $\overline{M}_n$ of the methylated polysaccharide, 6,900 ±300, corresponds to a degree of polymerisation of 44 ±2 residues (arabinose and uronic acid included). The only estimate that can be made of the proportion of aldobiouronic acid present in the methylated material is

from the weight of aldobiouronic acid (0.376 g) relative to the total sugars (4.46 g) recovered from the cellulose column. Thus the aldobiouronic acid constitutes about 9% by weight of the methylated polysaccharide, or about 620 g per mole (taken as 6,900 g). The expected molecular weight for the free-acid form of the 3-*O*-methyl-2-*O*-(2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose, is 382. Thus at the maximum, only two aldobiouronic acid residues could be accommodated per molecule.

On the basis of the molecular weight, aldobiouronic acid, and methylated sugar determinations it is tempting to suggest a molecular composition of approximately 47 D-xylose, 4 L-arabinose and two 4-*O*-methyl-D-glucuronic acid residues. Factors that could affect the accuracy of the data must however be considered: (*a*) The methylation technique employed yielded less than 25% of the theoretical amount of a product that had $\overline{M}_n$ of 6,900. This material may not reflect the composition of the original polysaccharide; (*b*) the method of determining the aldobiouronic acid is probably not very accurate; and (*c*) the assumption that molar responses were equal for all of the acetylated nitrile derivatives could introduce errors (±5%) into the values for the methylated sugars.

The proportion of 2,3,4-tri-*O*-methyl-D-xylose indicates about one branch point per molecule.

The results of partial hydrolysis with 0.01M oxalic acid indicate that virtually all of the arabinose is present as side chains. These are probably attached as single residues, as in most arabinoglucuronoxylans from grasses[10].

Overall, the structural features found for the hemicellulose from *Cynodon* are similar to those detected in the hemicelluloses of temperate species of the *Gramineae*.

EXPERIMENTAL

General methods. — Paper chromatography was performed on Whatman No. 1 paper with the following solvent systems (*v/v*): (*A*) ethyl acetate–pyridine–water (10:4:3), (*B*) *n*-butanol–ethanol–water (4:1:1), (*C*) butanone–water (8:5:7), (*D*) benzene–ethanol–water (200:47:15), (*E*) *n*-butanol–benzene–pyridine–water (5:1:3:3, upper layer), (*F*) ethyl acetate–acetic acid–formic acid–water (18:3:1:4). Alkaline silver nitrate and *p*-anisidine hydrochloride were used as spray reagents.

G.l.c. was performed with a Varian-Aerograph model 1520 chromatograph fitted with flame-ionization detectors and with columns (5 ft × ⅛ in.) of stainless steel. The carrier gas was nitrogen. The following packings and liquid phases were used: (*i*) 3% ECNSS-M on Gas-Chrom Q (100–120 mesh), (*ii*) 5% LAC-4R-886 on Gas-Chrom Q (100–120 mesh), (*iii*) 15% butane-1,4-diol succinate on Chromosorb W (80–100 mesh), (*iv*) 14% ethylene glycol succinate on Chromosorb W (80–100 mesh). Columns (*iii*) and (*iv*) were used at a column temperature of 160°; injector, 220°; detector, 220°; and a nitrogen flow-rate of 35 ml.min^{-1}, unless otherwise stated. I.r. spectra were recorded with a Perkin–Elmer model 237 spectrophotometer. Melting points were determined on an Electrothermal melting-point apparatus and are

corrected. Optical rotations were determined by using a Perkin–Elmer model 141 polarimeter.

Paper electrophoresis of monosaccharides and partially methylated monosaccharides was conducted on strips (20 × 10 cm) of Whatman No. 1 paper in borate buffer (0.05M, pH, 9.6) at 500 V and 20–30 mA current. Vapour-phase osmometry (v.p.o.) procedures have been described previously[3]. Isolation and purification procedures have also been described[1].

Hydrolysis. — *Cynodon* polysaccharide (150 mg) was initially hydrolysed in 72% sulphuric acid (1 ml) for 1 h at 25°, after which time water (28 ml) was added. The tube was sealed and heated on a boiling water-bath for 3 h. The solution was titrated at 60–70° with barium hydroxide (0.25M) to an end-point with Congo Red to remove sulphate ions, The suspension resulting was filtered and further titrated at room temperature with 0.1M potassium hydroxide to a final pH of 8.5–9.0. The sample, after concentration was passed through a column that contained in the top half 10 ml of Amberlite IR-120(H^+), and in the bottom half 10 ml of Amberlite IRA-400 (Ac^-), by use of water (~100 ml). The upper portion of resin was removed, and the remaining anion-exchange resin was washed with water (50 ml) to remove all neutral sugars. The neutral effluents were concentrated to exactly 25 ml to give a concentration of approx. 5–6 $mg.ml^{-1}$.

Analysis of neutral sugars as alditol acetates. — An aliquot of the neutral sugar solution (5 ml) was reduced, and the resultant alditols converted into their acetates[11,12]. Samples were examined by g.l.c. [column (*i*)] under the following conditions: column temperature 185°, injector 245°, detector 240°, nitrogen flow-rate 32 $ml.min^{-1}$. After qualitative examination, mannitol hexaacetate was added as internal standard for quantitative determination. Peak areas were calculated by tracing the charts on to aluminium foil (in triplicate), excising, and weighing. Detector responses for the various alditol acetates (relative to mannitol hexaacetate) were determined to be: D-xylitol, 1.04; L-arabinitol, 1.03; D-glucitol, 0.97; and D-galactitol, 0.99. The calibration plots were nearly linear over the concentration range employed. Results for the *Cynodon* polysaccharide are summarised in Table I.

Autoanalysis of neutral sugars. — An aliquot (0.25 ml) of the neutral-sugar fraction of *Cynodon* polysaccharide was adsorbed on a Deacidite-FF column (0.6 × 66 cm) water-jacketed at 40°, equilibrated with 0.1M boric acid, pH 8.0. The sugars were analysed with an automated Technicon Sugar Chromatography System at a flowrate of 0.75 $ml.min.^{-1}$. A standard gradient (450 ml) of borate–sodium chloride was used for elution, the pH and ionic strength being raised during the run. Colour was developed with 1% orcinol solution and 70% sulphuric acid, and measured at 415 nm. Standard sugar solutions of D-xylose, L-arabinose, and D-glucose were applied to allow quantiation of results (Table I).

Partial hydrolysis with 0.01M oxalic acid has been described previously[1].

Examination of acidic oligosaccharides. — *Cynodon* polysaccharide (5 g) was heated with 0.5M sulphuric acid (200 ml) for 4 h at 100°. The hydrolysate was neutralised to a Congo red end-point at 60–70° with barium hydroxide solution, filtered, and

concentrated to ~25 ml. The pH was then raised carefully at ~25° to 8.0 by using barium hydroxide solution (25 mM) and a pH meter. The solution was kept for 5 h at ~25° and then concentrated to low volume. Addition of excess methanol precipitated the barium salt of the oligosaccharide (42 mg), which was washed with methanol, and then taken up in water and passed through a column of Amberlite IR-120 (H^+) resin to convert into it the freeacid form. The eluate end washings were concentrated to 2 ml. Ethylene oxide (1 ml) was added, the flask sealed, and stored for 6 days at ~25°, during which time the pH rose from 3.8 to 6.4. Ethylene oxide was removed by bubbling air through the solution, and the hydroxyethyl ester was reduced with potassium borohydride for 16 h at 10°. Potassium ions and borate were removed as already described. The reduced compound was hydrolysed with 0.5M sulphuric acid for 4 h at 100°. Chromatography on paper in solvents *A* and *E*, and paper electrophoresis, showed the presence of xylitol, 4-*O*-methylglucose, and glucose (faint). Analysis through the alditol acetates of the hydrolysate by g.l.c. on column (*i*) confirmed the presence of the acetates of xylitol, 4-*O*-methylglucitol, and a trace of glucitol.

Partial acid hydrolysis by sulphuric acid. — A suspension of *Cynodon* hemicellulose (6 g in 0.25M sulphuric acid, 1.5 l) was heated for 35 min on a boiling water-bath, and insoluble polysaccharide was separated by centrifugation. The cooled, supernatant liquid was neutralised (barium carbonate) and concentrated to about 50 ml in a rotary evaporator at 50°. Ethanol (3 vols.) was added to precipitate degraded polysaccharide. The combined precipitates were re-hydrolysed in a similar fashion. A total of seven such hydrolytic treatments were performed, the last three in 500 ml of acid because of the decreased quantity of material. The mother liquors remaining after the precipitation steps were combined, concentrated, and titrated with 0.05M potassium hydroxide to a final pH of 8.3. Metal ions and acidic sugars were removed by passage through columns of IR-120 (H^+) and IRA-400 (Ac^-) resins. Concentration of the eluate yielded a syrup (3.1 g) that by paper chromatography in solvent *A* showed the presence of xylose, arabinose, glucose, three oligosaccharides R_{Xylose} 0.62, 0.34, and 0.17, and traces of higher oligosaccharides. These oligosaccharides are hereafter referred to as disaccharide, trisaccharide, and tetrasaccharide respectively. The syrupy partial hydrolysate (3.1 g.) was placed on a column (40 × 4 cm) of charcoal–Celite (1:1). Solvent was pumped through the column at approx. 19 ml/h. Eluting solvents were, in order of use: water (1.6 l), 5% ethanol (2.1 l), 10% ethanol (2.0 l) and, 15% ethanol (1.0 l).

Seven major fractions were obtained, as shown by paper chromatography: *fraction* 1, monosaccharides only; *fraction* 2, monosaccharide plus disaccharide; *fraction* 3, disaccharide only; *fraction* 4, disaccharide plus trisaccharide; *fraction* 5, trisaccharide only; *fraction* 6, trisaccharide plus tetrasaccharide; *fraction* 7, tetrasaccharide.

Fractions 2, 4, and 6 were concentrated, and the oligosaccharides in each were separated on sheets of Whatman 3 MM paper in solvent *A*. They were eluted from the paper with warm water. The solutions were filtered and combined with their

respective fractions (3, 5, and 7) to yield, on concentration, chromatographically pure disaccharide (200 mg), trisaccharide (94 mg), and tetrasaccharide (50 mg). Celite in each case was removed as suggested by Whistler and BeMiller[5].

Examination of the disaccharide having R_X *0.62 in solvent* A. — Recrystallized from methanol (yield 160 mg) it had m.p. 184–186°, $[\alpha]_D^{26}$ −24.8° (*c* 0.98, water). Hydrolysis in 0.5M sulphuric acid followed by paper chromatography in solvents *A* and *B* showed only xylose to be present. Methylation of 10 mg with methyl iodide (1 ml) and silver oxide (2 g) in *N,N*-dimethylformamide (1 ml) for 5 days at room temperature in the dark, followed by isolation from the solvent, methanolysis in 3% methanolic HCl for 4 h at 65°, neutralisation (silver carbonate), and g.l.c. examination on column (*iv*) showed the presence of 2,3,4-tri-*O*-methylxylose and 2,3-di-*O*-methylxylose in approximately equimolar proportions. The disaccharide was thus 4-*O*-*β*-D-xylopyranosyl-D-xylose (xylobiose).

Examination of the trisaccharide having R_X *0.34 in solvent* A. — This sugar could not be crystallized. No xylo-oligosaccharides were available for comparison purposes. The optical rotation, $[\alpha]_D^{26}$ −42° (*c* 1.0, water) was of the correct order for xylotriose[6] (47°). Complete hydrolysis showed only xylose to be present. Methylation analysis as described for xylobiose showed 2,3,4-tri-*O*-methylxylose and 2,3-di-*O*-methylxylose to be present in approximately 1:2 molar proportions.

Examination of the tetrasaccharide having R_X *0.17 in solvent* A. — This sugar was not obtained crystalline; it had $[\alpha]_D^{26}$ −55° (*c* 0.55, water) (lit.[6] −60°). Acid hydrolysis yielded only xylose, and methylation analysis showed 2,3,4-tri-*O*-methylxylose and 2,3-di-*O*-methylxylose in the approximate molar ratios of 1:3. The sugar was considered to be xylotetraose.

Examination of the methylated polysaccharide. — *Cynodon* hemicellulose (20 g) was methylated with methyl sulphate–sodium hydroxide solution[7] (5 treatments) followed by methyl iodide–silver oxide[8] (4 treatments) to yield 8.4 g of material (OMe 38.6%), showing virtually no hydroxyl absorption in the infrared, $\overline{M}_n$ 6700 by v.p.o. The methylated polysaccharide (6.2 g) was extracted with mixtures of light petroleum (b.p. 40–60°)–chloroform (two 50-ml batches of each mixture) for 1 h periods under reflux. The solvent was removed in a rotary evaporator and the residues dried *in vacuo* for 6 h at 70°. The results are summarized in Table II.

Fractions 4 and 5 were combined and methylated once more with silver oxide–methyl iodide; yield 5.75 g, OMe 38.9%; $[\alpha]_D^{26}$ −82.3° (*c* 1.4, chloroform); M_n 6,900 ±300 by v.p.o. Methanolysis of 10 mg of the methylated polysaccharide in 3% methanolic hydrogen chloride for 18 h at 60°, and g.l.c. examination of the resulting glycosides on columns (*iii*) and (*iv*) indicated the presence of the methyl glycosides of the following sugars: 2,3,4-tri-*O*-methylxylose, 2,3,5-tri-*O*-methylarabinose, 2,3-di-*O*-methylxylose, and 2-*O*-methylxylose.

For hydrolysis, the methylated *Cynodon* hemicellulose (5.3 g) was kept overnight at ~25° in 4% methanolic HCl (500 ml), and then refluxed to constant optical rotation. Methanol was removed in a rotary evaporator (bath temp. 45°), the volume of liquid being kept constant by admitting water periodically. The solution was

finally made M in hydrochloric acid and heated at 100° to constant optical rotation (4 h). The acid was neutralised with silver carbonate, and silver ions were removed by passing hydrogen sulphide through the solution. The resultant suspension was then filtered. The filtrate was treated with barium carbonate to convert acids into their barium salts and then concentrated to a syrup (4.6 g).

Paper chromatography in solvents *B* and *C*, and visualization with *p*-anisidine hydrochloride spray, indicated the presence of tri-*O*-methylpentoses, 2,3-di-*O*-methylxylose, and 2-*O*-methylxylose, and a heart-shaped spot near the origin (aldobiouronic acid). The tri-*O*-methylpentoses corresponded by paper chromatography in solvent *D* to be 2,3,4-tri-*O*-methylxylose and 2,3,5-tri-*O*-methylarabinose. The syrupy mixture (4.51 g) of methylated sugars was absorbed on to a column of cellulose (Whatman C.F.11, 75 × 3.5 cm) that was eluted first with light petroleum (b.p. 100–120°)–butyl alcohol saturated with water, (7:3, 1.2 litres), next with butyl alcohol three-quarters saturated with water (700 ml), and finally with water (300 ml). Fractions (3 ml) were collected and examined by t.l.c. in solvent *C*. The contents of tubes containing the same sugars were combined to give four major fractions: fraction 1, tri-*O*-methylsugars (0.52 g); fraction 2, di-*O*-methylxylose (3.01 g); fraction 3, mono-*O*-methylxylose (0.54 g); and fraction 4, aldobiouronic acid (0.376 g).

Examination of the methylated sugars. — Fraction 1 had $[\alpha]_D^{26}$ −34.2° (*c* 0.9, water), which corresponded to about 90% of 2,3,5-tri-*O*-methyl-L-arabinose, ($[\alpha]_D^{26}$ −39.5°) and 10% of 2,3,4-tri-*O*-methyl-D-xylose, ($[\alpha]_D^{26}$ +18°). The fraction was separated into its two components by chromatography on Whatman 3 MM paper in solvent *D*, followed by elution from the paper. The supposed, 2,3,5-tri-*O*-methyl-L-arabinose (310 mg) on demethylation (48% aqueous hydrobromic acid[9]) showed only arabinose on paper chromatography. G.l.c. of the methyl glycosides on columns (*iii*) and (*iv*) also indicated the tri-*O*-methylarabinose. The suspected 2,3,4-tri-*O*-methyl-D-xylose (35 mg) was similarly examined by g.l.c. and by demethylation. Conversion into the aniline derivative (m.p. and mixed m.p. 100–101°) confirmed the identification. Fraction 2 was identical with 2,3-di-*O*-methylxylose by paper chromatography (solvents *B* and *C*) and by g.l.c. of the methyl glycosides. Demethylation yielded only D-xylose. The sugar was converted into the aniline derivative, m.p. 123–124°, $[\alpha]_D^{23}$ +188° (*c* 1.15, ethyl acetate).

Fraction 3 crystallized on concentration of the solvent (0.54 g). Recrystallization from abs. ethanol afforded 2-*O*-methyl-D-xylose, m.p. and mixed m.p. 135–136°, $[\alpha]_D^{24}$ +34° (*c* 0.4, water, equil.). The aniline derivative had m.p. and mixed m.p. 125–126°, $[\alpha]_D^{24}$ +223° (*c* 0.18, ethyl acetate). Fraction 4 was converted from the barium salt into the free-acid form by passage through a column of Amberlite IR-120 (H^+) resin. The effluent was evaporated and the residue converted into the methyl ester by refluxing with 4% methanolic HCl. The ester was reduced with potassium borohydride (200 mg) in water (10 ml) for 14 h at room temperature. The borohydride was neutralised with 25mM sulphuric acid, and the reduced, partly methylated disaccharide was extracted with chloroform (2 × 30 ml). The chloroform solution was washed with saturated sodium hydrogen carbonate solution, dried over magnesium sulphate, and

concentrated to a syrup (130 mg). A portion (5 mg) of the syrup was dried *in vacuo* over phosphorus pentaoxide, and then heated with 4% methanolic HCl, neutralised (Ag_2CO_3), and subjected to g.l.c. on columns (*iii*) and (*iv*). The glycosides of 2,3,4-tri-*O*-methylglucose and 3-*O*-methylxylose were detected. A further portion (20 mg) of the syrupy disaccharide was hydrolysed (0.5M sulphuric acid, 4 h at 100°). Paper chromatography (solvents *B* and *C*) showed the hydrolysate to consist of 2,3,4-tri-*O*-methylglucose and 3-*O*-methylxylose.

A third fraction (100 mg) was methylated (twice) with silver oxide and methyl iodide to yield the fully methylated disaccharide, $[\alpha]_D^{25}$ +98° (*c* 1.17, chloroform). Methanolysis of a portion of this product, followed by g.l.c., showed the presence of the methyl glycosides of 2,3,4,6-tetra-*O*-methylglucose and those presumed to be of 3,4-di-*O*-methylxylose, although a reference sample was not available for the latter.

Quantitative examination of the methylated Cynodon *hemicellulose.* — Methylated *Cynodon* hemicellulose (130 mg) was dissolved in 72% sulphuric acid (1 ml) at 0°, and kept for 1 h at that temperature. Water (8 ml) was added, and the tube was sealed and heated for 4 h at 100°. The hydrolysate was filtered and continuously extracted with dichloromethane (50 ml) for 36 h. The extract was shaken with saturated sodium hydrogen carbonate solution, and then water, and dried over anhydrous magnesium sulphate. Most of the dichloromethane was allowed to evaporate at 40–45° at atmospheric pressure. The solution was then transferred quantitatively to a 10-ml standard flask, which was filled to the mark with dry dichloromethane (fraction *a*). The water-soluble fraction was neutralized with 0.25M barium hydroxide solution and finally barium carbonate to pH 7–8, and centrifuged. The precipitate was agitated with water, and then hot 80% aqueous ethanol, and centrifuged. The washings and extracts were concentrated and passed through a column filled with Amberlite IR-120 (H^+) (10 ml, top half) and Amberlite IRA-400 (Ac^-) (10 ml, bottom half). Water (60 ml) eluted neutral sugars.

The solution was concentrated and made up in a standard flask to 10 ml with water (fraction *b*).

An aliquot of fraction *b* (2 ml) was pipetted into a 5-ml vial, evaporated to dryness *in vacuo* at 40–45° and kept overnight in a vacuum desiccator at 10 torr with concentrated sulphuric acid as desiccant. Fraction *a* (2 ml) was then added, and the dichloromethane evaporated at 40° and atmospheric pressure, by passing warm, dry nitrogen over the vial in a desiccator containing phosphorus pentaoxide. Final drying was achieved by keeping the vial for 45 min at 45° and 300 torr over phosphorus pentaoxide; yield of methylated neutral sugars, 16.2 mg.

The neutral sugars were treated with pyridine (1.5 ml) and hydroxylamine hydrochloride (15 mg) for 1 h at 90°. Acetic anhydride (1.5 ml) was added, and the tubes were resealed and heated for a further h at 90°. The resultant, acetylated nitriles[10] were examined by g.l.c. on column (*ii*) under the following conditions: column, 185°; injector 245°; detector, 240°; nitrogen flow-rate, 35 ml.min^{-1}. Qualitative analysis was followed by quantitation. Peak areas per mole were assumed to be

the same for all sugar derivatives, and so relative peak areas were assumed to reflect relative molar amounts. Peak areas were determined as described for the analysis of alditol acetates (see Table III).

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