Phytochemistry, 1975, Vol. 14, pp. 1793–1798. Pergamon Press. Printed in England.

AN ACIDIC OLIGOSACCHARIDE FROM MAIZE SLIME

K. WRIGHT and D. H. NORTHCOTE

Department of Biochemistry, University of Cambridge, Cambridge, CB2 1QW, England

(*Received* 15 *November* 1974)

Key Word Index—*Zea mays*; Gramineae; maize; root-cap; slime; polysaccharide; fucose; differentiation; electrophoresis; aldobiouronic acid; uronic acid.

Abstract—The main uronic acid product of acid hydrolysis of maize root-cap polysaccharide ran marginally behind galacturonic acid on paper electrophoresis at pH 3·5. Material from both radioactive and non-radioactive slime preparations was used to provide evidence that this was an oligomeric partial hydrolysis product containing both galacturonic acid and glucose. The consequences of this upon the structure of the slime are discussed.

INTRODUCTION

The maize root-cap synthesizes a polysaccharide which is extremely hydrophilic and serves to protect the growing tip from physical damage and dehydration. The polymer contains a high proportion of fucose [1] and since this is not found elsewhere in the maize seedling [2] its formation represents the end-product of a differentiation process specific to the maize outer root-cap cell. The polymer is made of neutral and acidic components which can be separated by glass-fibre paper electrophoresis [2]. We have previously analysed the distribution of radioactivity between the monomers of such fractions prepared from slime isolated from roots fed with radioactive glucose [2]. During the analysis of acid hydrolysates of the fractions it was observed that the main peak of radioactivity in the uronic acid fraction did not coincide with galacturonic acid (GalA) during a paper electrophoretic separation and it was probably due to an aldobiouronic acid. Aldobiouronic acids have been obtained from the acid hydrolysates of a large number of plant gums and mucilages [3,4]. We have examined material from both radioactive and non-radioactive maize slime and conclude that each material was an oligomeric partial hydrolysis product which contained both GalA and glucose.

Maize slime also contains protein [5,6], the function of which is unknown. The amino-acid content of the purified slime preparations was determined to aid the interpretation of titration data.

RESULTS AND DISCUSSION

Electrophoresis of oligosaccharides from oak xylan and pectin

A xylan preparation known to contain 4-*O*-methylglucuronic acid[7] was made from oak shavings and hydrolysed with 3% H_2SO_4. The neutralized hydrolysate was subjected to PC (overnight, Solvent 1) to remove neutral sugars, and the immobile components were eluted and applied to paper for electrophoresis at pH 3·5. This was run at 4 kV for 45 min and the samples were stained with both aniline phthalate and $AgNO_3$. A number of spots were visualized between the single position of all the neutral sugar markers and authentic GalA. The material in each spot was eluted and hydrolysed again under identical conditions. The fastest-running component was unchanged and ran with the same mobility as authentic 4-*O*-methylglucuronic acid which occurs as single unit branch at the 2-position of the xylose in the main chain. The

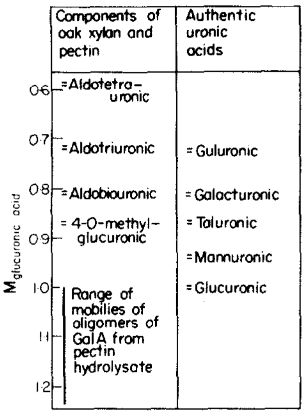

Fig. 1.

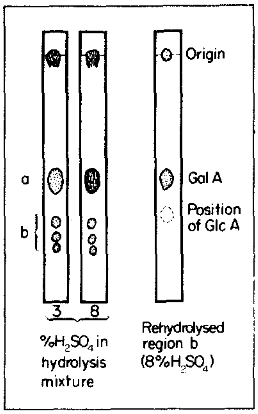

Fig. 2.

Fig. 1. Electrophoretic mobilities of uronic acids. On the left are the $M_{glucuronic\ acid}$ values of components obtained from separate hydrolyses of oak xylan and apple pectin; authentic uronic acids are on the right.

Fig. 2. Electrophoretic behaviour of incomplete hydrolysis products of apple pectin. The degree of shading indicates the relative staining intensities. Acid strengths were 3·1 (3) and 8·3 (8) % H_2SO_4. Region *a* was GalA and region *b* comprised oligomers of GalA which moved more quickly at pH 3·5.

other materials were aldobiouronic, aldotriouronic and aldotetrauronic acids since on repeated hydrolysis they yielded, progressively, materials with higher mobilities and eventually 4-*O*-methylglucuronic acid and xylose. The mobilities of these components are shown in Fig. 1, together with mobilities of standard uronic acids.

An approx 6% (w/v) solution of apple pectin (B.D.H.) in H_2O was mixed with equal volumes of H_2SO_4 to give two solutions with acid concentrations of 3·1 and 8·3% (w/w). The solutions were autoclaved, neutralized with *N,N*-dioctylmethylamine and electrophoresed at pH 3·5. Samples of these hydrolysates were stained with $AgNO_3$. The visible spots other than the neutral material were in two regions (Fig. 2). The spot in region *b*, which was electrophoretically indistinguishable from glucuronic acid (GlcA), gave a different coloration on treatment with aniline phthalate. At the lower acid concentration relatively more material was found in region *b* compared with region *a*. The material from an area of untreated paper corresponding to region *a* was eluted and rerun; material isolated in the same way from region *b* was rehydrolysed with 8·3% H_2SO_4 before further electrophoresis. The results are shown in Fig. 2. Region *a* was GalA. After rehydrolysis all the spots of region *b* disappeared to yield a spot with the mobility of GalA and a small amount of neutral material.

While electrophoresis has been used for some time as a method for separating uronic acids [1,8,9] the behaviour of aldobiouronic acids in this system has not been critically examined. The results obtained by partial hydrolysis of pectin showed that oligomers of GalA ran more quickly than GalA, and some had the same mobility as GlcA. It is therefore necessary to ensure that complete hydrolysis has taken place before a radioactive peak in this position can be unambiguously designated GlcA. Some substances ran faster than GlcA and these may have been tri- or tetragalacturonic acids which have a slightly larger charge density than the monomer. Radioactive material with a faster mobility than GlcA on paper electrophoresis at pH 3·5 has been observed [2,10] and this therefore probably represented incompletely hydrolysed portions of polygalacturonic acid [11]. It was confirmed that faster-running material from radioactive maize slime could be made to run more slowly by more vigorous hydrolysis conditions (8·3%, H_2SO_4). From these results it can be seen that oligouronic acids have increased mobility on paper electrophoresis at pH 3·5, while aldo-oligouronic acids have decreased mobility compared with the uronic acid.

Identity of uronic acid in maize-root slime

Because of the difficulties of achieving complete acid hydrolysis of polyuronic acids and of subsequently identifying the monomers in the presence of mixtures of oligouronic acids and aldo-oligouronic acids it is not possible to rely solely upon a simple chromatographic or electrophoretic analysis [1,2,6,9]. It was therefore necessary to carry out additional investigations on the materials in order to identify the uronic acid.

Hydrolysis with 3% H_2SO_4 of maize slime isolated from roots supplied with D-glucose-[U-^{14}C] for 2·5 hr yielded a peak of radioactivity located 10–20 mm behind a GalA marker after paper electrophoresis at pH 3·5. This was repeatedly observed in all the analyses reported previously [2] and was confirmed by mixing the material with an authentic sample of GalA, submitting the mixture to electrophoresis and subsequently staining the strips which had already been

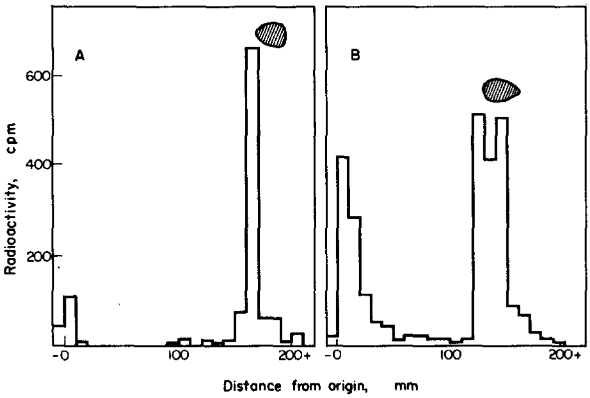

Fig. 3. Electrophoresis of the uronic acid fraction from radioactive slime hydrolysates. (A) Material which did not move in Solvent 1 after acid hydrolysis. The large peak is the acidic oligosaccharide. (B) Rehydrolysis of acidic oligosaccharide obtained as in (A). The hatched areas indicate the position of GalA.

counted for radioactivity (Fig. 3A). The mobility of this material (an acidic oligosaccharide) was not inconsistent with its being an aldobiouronic acid. When the retarded radioactive material was eluted from the electrophoretogram and rehydrolysed the profile of radioactivity showed either a broadening of the peak into the GalA region or the production of a discrete peak with the mobility of GalA (Fig. 3B). In both cases a neutral component was produced, but this remained at the origin on a chromotogram (Solvent 1) and was therefore not a monosaccharide. Neither was it mobile on electrophoresis at pH 2 and it was therefore not an amino-acid; we consider it to be a degradation product. No radioactive glucuronic or mannuronic acid was ever found.

Radioactive slime was prepared and the strongly-acidic components isolated by glass-fibre paper electrophoresis at pH 6·5 [2,12]. The hydroxy-ethyl ester was synthesized by treatment with ethylene oxide and samples were withdrawn at intervals and electrophoresed to show to what extent the polymer had been neutralized by esterification. After 178 hr the esterified polymer was reduced with $NaBH_4$ and dialysed to remove excess reagents. Glass-fibre paper electrophoresis at pH 6·5 showed the presence of a neutral peak and one which was slightly acidic; the latter is thought to have been caused by alkaline hydrolysis of the ester link before reduction. The neutral material was hydrolysed with 3% H_2SO_4 and neutralized with *N*,*N*-dioctylmethylamine. The component sugars of the neutralized hydrolysate were separated by chromatography in Solvent 1 and their radioactivities compared with those of sugars in hydrolysates of untreated material. The percentage distribution of the label between the neutral sugars and uronic acid are shown in Table 1. There was a decrease in the proportion of radioactivity in the uronic acid material of the hydrolysate and a compensating increase in the proportions in galactose and glucose after reduction. There was a greater increase in label in glucose than galactose after reduction. However this does not necessarily indicate any stoichiometry of the material whose resistance to acid hydrolysis was lowered, since the GalA and glucose need not have been labelled to the same degree during the course of the incubation with radioactive glucose. Since no glucuronic acid was ever detected after hydrolytic breakdown of the slime the results indicated that GalA was the only uronic acid present and this was attached to glucose by a uronosidic linkage.

In another experiment a sample (approx 40 mg) of the 80% EtOH-insoluble component of the slime was acetylated using a mixture of acetic anhydride and pyridine [13]. The product was precipitated by H_2O, extracted into methylene chloride and divided into two fractions. One was rotary evaporated to remove the methylene chloride, taken up in dimethoxyethanol and reduced with diborane generated *in situ* [14]. The other was maintained as a control. Both samples were again precipitated by H_2O and extracted with methylene chloride. They were hydrolysed (3% H_2SO_4) and the alditol acetate derivatives prepared and analysed by GLC. The galactose content rose from 20 to 25% as a result of the reduction whereas the glucose content changed from

Table 1. Distribution of radioactivity between constituents of the strongly-acidic fraction of maize slime before and after esterification and reduction

	Radioactivity (%)		
Constituent	Untreated	Esterified and reduced	Change
Acidic oligosaccharide	33	16	−17
Galactose	20	28	+8
Glucose	19	31	+12
Fucose	28	25	−3

18 to 11%. These results confirmed that galacturonosyl linkages were present. However, the glucose content did not rise after this direct reduction; this may have been because the GalA units which were successfully reduced were present in a region of the molecule which contained little neutral sugar.

Identity of neutral sugar linked to the galacturonic acid

Material isolated from a peak of radioactivity located 10–20 mm behind GalA was isolated from a hydrolysate (3% H_2SO_4) of the slime and rehydrolysed by refluxing for 2 hr with 2 N HCl. Subsequent chromatography (Solvent 1) gave, in addition to the peak which ran at the origin, a smaller peak in the position of glucose. Hence glucose must have been present in the oligomer.

Slime and other soluble polymers were isolated from approximately 500 maize seedlings. The solution was rotary evaporated almost to dryness and the residue redissolved in 3% H_2SO_4, hydrolysed, neutralized and electrophoresed at pH 3·5. The entire uronic acid region (that enclosed by marker GalA and GlcA and a region 50 mm nearer the origin than GalA) was eluted and re-electrophoresed to remove contaminating neutral sugars. A small sample of the material was run separately and stained with silver nitrate. In the uronic acid region three spots were revealed, one corresponding to GalA and the others to slightly retarded components. All three components were eluted together. One quarter of the material was retained while the bulk of it was acetylated, directly reduced with diborane generated *in situ* and deacetylated [14]. It was hydrolysed, neutralized with *N,N*-dioctylmethylamine and chromatographed in Solvent 1 in conjunction with untreated control material and sugar markers. Origin material, a substance with the R_f of a disaccharide, and materials chromatographically identical with galactose and glucose were produced in the approximate ratio of 1:2:2:10 (measured by a densitometer-trace of material treated with alkaline $AgNO_3$). All the material in the non-reduced hydrolysate remained at the origin. Hence reduction had facilitated hydrolysis even though material with the R_f of a disaccharide was one of the products. The material at the origin was presumably unreduced uronic acid since acidic substances are not mobile in this solvent. Galactose was also produced, but the major product was chromatographically identical with glucose.

Amino-acid and carboxyl group content

The protein content of the slime was investigated in order to aid interpretation of titration data which were obtained to verify the uronic acid content of the slime. The 80% EtOH-insoluble portion of the slime was prepared, and the protein content of a solution was measured. The sample contained 30% (w/w) protein of which an unusually high proportion of amino-acids were basic (Composition, moles %: Lys, 11·4; His, 3·8; Arg, 10·5; Trp, 1·9; Asp, 9·2; Thr, 4·9; Ser, 5·4; Glu, 8·2; Pro, 0·3; Gly, 11·6; Ala, 6·2; Val, 7·3; Ileu, 4·3; Leu, 7·1; Tyr, 1·5; Phe, 3·2). Whether this large amount of protein has a function within the slime or whether it simply represents some of the contents of dying cells is not known.

The carboxyl group content was measured by back titration of a solution of slime in 0·01 N NaOH. A sample (9·75 mg) of slime contained 9·5 μmol carboxyl group. Amino-acids also contributed carboxyl groups, and aspartic acid and glutamic acid together constituted about 18% (w/w) of the protein, which itself comprised 30% (w/w) of the slime. Thus 5·4% of the slime was aspartate and glutamate and the content of GalA equivalents was 12% (w/w). This was in good agreement with a measurement of 14% (w/w) obtained from a replicate sample using the carbazole procedure, and one of 12% obtained by Jones and Morré [15] also using the carbazole reaction.

Structure of the slime

A radioactive sample of slime was prepared [2]. It was dialysed against water but not fractionated into its components. Samples containing approx 40000 cpm were then hydrolysed with acid strengths of 2·1, 3·1, 4·1 and 8·3% (w/w). Since each radioactive slime preparation contained less than 1 mg material and the volume of acid in which it was hydrolysed was 2 ml, the concentration of polysaccharide was constant at a value of less than 0·05% (w/w). The samples were neutralized with *N,N*-dioctylmethylamine and the neutral sugars and uronic acids separated. The distribution of label between uronic acid, galac-

Table 2. Effect of hydrolysis with different acid strengths on the distribution of label between components of the uronic acid fraction of maize slime

	Radioactivity (%)		
Acid strength (% w/w)	Origin material	Material with approx mobility of GalA	Material with approx mobility of GlcA
2·1	5·1	49·6	45·2
3·1	0·8	68·9	30·3
4·1	0	78·1	21·9
8·3	0	87·4	12·6

tose–glucose, mannose, arabinose and xylose–fucose was the same in each case. However, when the uronic acid material was eluted and separated by paper electrophoresis at pH 3·5 the distributions shown in Table 2 were obtained. Material which, at lower acid concentrations, had run with a mobility identical to that of marker GlcA moved to occupy the region near GalA at higher acid strengths. These results demonstrate that at least short sequences of GalA do occur within the slime.

Although hydrolysis of both radioactive and non-radioactive preparations of slime frequently gave rise to material which ran behind authentic GalA this was not invariably the case, and the peak of radioactivity in this region was occasionally coincident with GalA and sometimes marginally ahead of it. We attribute these differences to variations in the hydrolytic procedure resulting from an inability to standardize the concentration of slime in the hydrolysis mixture owing to the small quantities of material which were available. Our interpretation of the results is that the acidic polymers of the slime consist of sequences of polygalacturonic acid which may be interspersed with regions of glucose residues, resulting in the possibility of formation of oligomers containing GalA and glucose in variable amounts after acid hydrolysis. The amount of glucose attached in this way might depend on the extent of formation of glucose-polymer, a factor known to be variable [12].

Since in our initial hydrolysis experiments it was shown that little if any free GalA was formed [2] the slime probably does not consist predominantly of a continuous galacturonic acid chain. The glucose residues could be integrated at fairly regular intervals along the chain or they might be present as blocks, either within the chain or at the reducing end. It is possible that the slime consists of a glucose chain carrying short branches of GalA oligomers. The evidence will not distinguish between these possibilities. To do so, more selective techniques of degradation, in particular enzymic hydrolysis at specific residues will be required.

EXPERIMENTAL

Preparation of xylan. Oak shavings were boiled under reflux for 2 hr with sodium hexametaphosphate (2% w/w, pH 3·7) to remove pectin. The residue, after washing, was treated with 2 × 100 ml KOH (5% w/v) for 2 hr at room temp. and the supernatant neutralized with glacial HOAc. The neutral soln was dialysed overnight against H_2O, digested with a few drops of salivary amylase [16] and dialysed once more. Material inside the dialysis bag was freeze-dried.

Preparation of radioactive slime and its components. Seeds of *Zea mays* (var. Caldera 535) were germinated under sterile conditions [12]. The roots on the intact seedlings were incubated with D-glucose-[U-^{14}C] for 2·5 hr and the slime isolated [2,12]. When the final soln was conc by rotary evaporation it was not taken to dryness but either stored frozen or freeze-dried. Slime was separated into its component polymers by electrophoresis [2,12].

Preparation of non-radioactive slime. The acidic oligosaccharide was prepared from approx 500 sterile seedlings [12]. The slime was isolated in the same way as the radioactive slime except that a correspondingly larger volume of water was used to wash the slime from the roots.

For the studies on the whole slime 2000–3000 sterile roots per preparation were used. The soln of slime in H_2O was conc and the slime precipitated by addition of 4 vol. of EtOH. This 80% (v/v) EtOH-insoluble material was removed by centrifugation and washed in 80% EtOH. Finally it was dissolved in a few ml H_2O and freeze-dried (yield 40–60 mg).

Titration of slime. Slime (39 mg) was dissolved in 0·01 N NaOH and made up to 20 ml. Samples (5 ml) were back titrated with 0·01 N HCl using a Radiometer PHM26 pH meter fitted with a combination glass/reference electrode and the titration curve compared with that of 0·01 N NaOH in which the slime was dissolved. The difference in the volumes of acid required was due to the presence of acidic groups in the slime. The 0·01 N HCl was standardized against Na_2CO_3 and the number of moles of carboxyl group calculated.

Preparation of uronic acid esters of radioactive material. A sample of the radioactive strongly-acidic component of the slime was isolated by glass-fibre paper electrophoresis and dissolved in 0·5 ml H_2O [2]. Ethylene oxide (10 ml) was added and the mixture was allowed to stand for a number of days in solid CO_2. Ethylene oxide was replenished occasionally and samples were withdrawn at intervals and electrophoresed on glass-fibre paper to determine progress of the esterification.

General methods of hydrolysis and analysis. Polysaccharides were hydrolysed in an autoclave at 120°, 103·4 kN/m^2 (15 psi) for 1 hr with 4·14% (w/w) H_2SO_4 unless otherwise stated. This strength of acid was obtained by mixing 28 ml H_2O with 1 ml 72·9% (w/v) H_2SO_4 (41 ml conc H_2SO_4 plus 26 ml H_2O). Other strengths of acid were obtained by appropriate dilutions of 72·9% (w/v) H_2SO_4.

Except when a sample was being prepared for GLC analysis, the hydrolysate was neutralized by repeated washing with a soln of 10% (v/v) *N,N*-dioctylmethylamine in $CHCl_3$ until the aq. layer was neutral [1]. When the amine was used the aq. layer was always washed 3 × with $CHCl_3$, rotary evaporated to dryness, taken up in H_2O and electrophoresed for 30 min, 5 kV, pH 2 (80 ml HOAc, 20 ml HCO_2H made up to 1 litre with H_2O) to remove amino-acids and residual amine salts. Neutral material was eluted from the dried electrophoretogram. Material to be analysed by GLC was neutralized with $BaCO_3$, filtered and conc by rotary evaporation.

On paper, neutral sugars could be separated by descending chromatography in EtOAc–C_6H_5N–H_2O (8:2:1) (Solvent 1) for 40 hr. Charged uronic acid material remained at the origin and was eluted and applied to paper for electrophoresis for 45 min, 4 kV, pH 3·5 (5 ml C_6H_5N, 50 ml HOAc made up to 1 l. with H_2O [1]. Components were identified by reference to marker sugars which were visualized either by aniline phthalate [17] or alkaline $AgNO_3$ [18]. To determine radioactivity profiles papers were cut into strips and counted in a liquid scintillation counter [1]. To locate non-radioactive material on strips which had already been counted, they were washed in toluene and benzene to remove scintillant [2], dried, and stained with aniline phthalate.

Neutral sugars of non-radioactive material were also determined by GLC [2]. The acetylation was accomplished in a mixture of 1 ml Ac_2O and 0·1 ml conc H_2SO_4 at 60° for 1 hr [19].

Diborane reduction of carboxyl groups of non-radioactive material. The acidic material from the GalA region of the pH 3·5 electrophoretogram was eluted, rotary evaporated to dryness and acetylated in a mixture of 1 ml Ac_2O and 0·1 ml H_2SO_4. The acetylated material was precipitated in approx 10 ml H_2O and extracted with 4 × 5 ml additions of CH_2Cl_2. This was removed by rotary evaporation and the product dissolved in 1 ml dimethoxyethane. $LiBH_4$ (10 mg) was added followed by 1 ml portions of a mixture of 0·88 ml BF_3 etherate and 4 ml dimethoxyethane over a period of 1 hr [14]. The mixture was shaken and then allowed to stand for 18 hr. A ppt formed on the addition of cold H_2O and it was extracted in CH_2Cl_2. The CH_2Cl_2 was removed by rotary evaporation and the solid dissolved in MeOH. The methanolic soln was cooled and dry NH_3 was bubbled through the soln to bring about deacetylation [20]. MeOH and NH_3 were removed under red pres.

A similar method was used to reduce the uronic acid residues of the 80% EtOH-insoluble fraction of the slime, except that initial acetylation was carried out by stirring the moist slime (approx 40 mg dry wt) in 2 ml C_6H_5N and adding 5 ml Ac_2O over a period of 3 days; the mixture was maintained at 70° [13]. Insoluble material was removed by centrifugation and the supernatant added to cold H_2O to ppt. the acetylated polymer. The ppt. was extracted with CH_2Cl_2 and thereafter treated as above. In this case the final deacetylation was effected by the acid hydrolysis.

Protein and amino-acid estimation. Protein was measured by the method of Lowry [21] with bovine serum albumin as a standard. For amino-acid analysis a soln of slime was hydrolysed with HCl (6 M) in a sealed tube for 18 hr at 100°. It was passed down a column of Deacidite FF-IP to remove "humin" before analysis with a Beckmann Amino Acid analyser [22].

Acknowledgements—K. W. thanks Tate and Lyle Ltd for a Fellowship, during the tenure of which this work was carried out. The 4-*O*-methylglucuronic acid was a gift from Dr. D. M. W. Anderson of Edinburgh University and taluronic acid was kindly given by Dr. K. Larsson, Chalmers University of Technology, Göteborg, Sweden.

REFERENCES

1. Harris, P. J. and Northcote, D. H. (1970) *Biochem. J.* **120,** 479.
2. Wright, K. and Northcote, D. H. (1974) *Biochem. J.* **139,** 525.
3. Staněk, J., Černý, M. and Pacák. J. (1965) *The Oligosaccharides*, p. 476, Academic Press, New York.
4. Smith, F. and Montgomery, R. (1959) *Am. Chem. Soc. Monogr. no. 141: The Chemistry of Plant Gums and Mucilages,* Reinhold, New York.
5. Juniper, B. E. and Roberts, R. M. (1966) *J. Roy. Micros. Soc.* **85,** 63.
6. Jones, D. D. and Morré, D. J. (1967) *Z. Pflanzenphysiol.* **56,** 166.
7. Northcote, D. H. (1958) *Biol. Rev.* **33,** 53.
8. Hanke, D. E. and Northcote, D. H. (1974) *J. Cell Sci.* **14,** 29.
9. Bowles, D. J. and Northcote, D. H. (1972) *Biochem. J.* **130,** 1133.
10. Wright, K. and Bowles, D. J. (1974) *J. Cell Sci.* **16,** 433.
11. Hamilton, J. K. and Thompson, N. S. (1957) *J. Am. Chem. Soc.* **79,** 6464.
12. Wright, K. (1975) *Phytochemistry* **14,** 759.
13. BeMiller, J. N. (1965) *Methods in Carbohydrate Chemistry* (Whistler, R. L., ed.), Vol. V, pp. 65–69, Academic Press, New York.
14. Aspinall, G. O. (1965) *Methods in Carbohydrate Chemistry* (Whistler, R. L., ed.), Vol. V, pp. 397–400, Academic Press, New York.
15. Jones, D. D. and Morré, D. J. (1973) *Physiol Plantarum* **29,** 68.
16. Olaitan, S. A. and Northcote, D. H. (1962) *Biochem. J.* **82,** 509.
17. Wilson, C. M. (1959) *Analyt. Chem.* **31,** 1199.
18. Trevelyan, W. E., Procter, D. P. and Harrison, J. S. (1950) *Nature* **166,** 444.
19. Borchardt, L. G. and Piper, C. V. (1970) *Tappi* **53,** 257.
20. Thompson, A. and Wolfrom, M. L. (1963) *Methods in Carbohydrate Chemistry* (Whistler, R. L. and Wolfrom, M. L., eds.), Vol. II, pp. 215–220, Academic Press, New York.
21. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265.
22. Larsen, I. and Mǿrtensen, J. V. (1967) *Analyt. Biochem.* **21,** 466.

Present address: Dr. K. Wright, Chesterford Park Research Station, Fisons Ltd., Nr. Saffron Walden, Essex, CB10 1XL, England.