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HYDROLYSIS OF PLANT CELL WALLS WITH TRIFLUOROACETIC ACID

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Abstract—A method is presented for the determination of the neutral monosaccharide units present in plant cell walls. The cell wall sample is dissolved by stirring for 18 hr at 37° with trifluoroacetic acid. The cellulosic component is hydrolysed by the addition of small amounts of water during heating at 100° so that the cellulose, during depolymerisation, stays in solution. The hemicellulosic component is hydrolysed under more dilute conditions at 100° such that the hemicellulosic pentose units undergo minimum degradation. The stability of the monosaccharide units under the different hydrolytic conditions is presented. The reproducibility of the method is demonstrated by comparing results of the new method with those from more established methods using samples of Italian ryegrass leaf and stem, lucerne stem, sainfoin leaf, birch sawdust and barley straw.

INTRODUCTION

The cell walls of all organisms have a degree of robustness since their primary function is to contain the contents of the cell. In plants, there is a division between primary cell walls which contain the cell when it is actively extending before reaching its full size and the secondary cell wall which is laid down when the cell size is reached and creates a tougher and more rigid structure around the cell [1] Both the primary and the secondary cell wall contain the same types of polysaccharides, namely cellulose, hemicelluloses and pectin [2], but the proportion of each type of polysaccharide is very different between primary and secondary walls In addition, the composition of each type of polysaccharide is different while secondary walls also contain the non-carbohydrate polymer, lignin The composition of each type of polysaccharide depends on the species, variety and a number of other factors including the environmental conditions prevailing during the life of the plant [3] Although celluloses from primary and secondary cell walls are different in respect of such parameters as average chain length and degree of crystallinity, this point is irrelevant if a hydrolytic method, based on the release of the constituent monosaccharide residue D-glucose, is used for their determination.

To determine the composition of plant cell walls, a number of procedures have been proposed. Some of the earlier methods relied on the isolation of individual polysaccharides The method of Crampton and Maynard [4] claimed to remove all the non-cellulosic constituents (including lignin) from the plant sample and the cellulose could be estimated gravimetrically, but it has been observed that monosaccharide units other than D-glucose are found in hydrolysates of these residues [5]. Van Soest [6] proposed a scheme involving extractions with detergents at pH 7 0 (neutral detergent residue, NDR) and pH 1 0 (acid detergent residue, ADR). If the lignin content (L) of the sample is known, the cellulose and hemicellulose contents could be determined from the equations

$$\text{Hemicellulose} = \text{NDR} - \text{ADR}$$

$$\text{Cellulose} = \text{ADR} - \text{L}.$$

In addition to the requirement for a number of correction steps, the procedure also suffers from the fact that not all the hemicellulosic polysaccharides are fully hydrolysed under the acidic conditions present during the extraction with the detergent at pH 1.0 [5].

To gain the maximum amount of information about the composition of the polysaccharides of plant cell walls, it is considered essential to depolymerise the material into its component monosaccharide units and, although enzymes may be useful, at present this needs to be carried out under acidic conditions Most hydrolyses are carried out in sulphuric acid as it appears to cause less destruction of the released monosaccharides than either hydrochloric or nitric acids If the composition of the hemicelluloses alone is required, they can be hydrolysed almost completely with 1 M sulphuric acid at 100° for 16 hr but to hydrolyse the complete cell wall (including the cellulose) the sample requires a prehydrolysis in 72% sulphuric acid at 37° for *ca* 1 hr followed by dilution to 1 M concentration and further heating at 100° for *ca* 16 hr [7]. A major disadvantage of using sulphuric acid is the problem of removing the acid after the hydrolysis This is usually done by neutralizing with barium carbonate and filtering off the precipitate of barium sulphate It is a lengthy and messy procedure.

Trifluoroacetic acid (TFA) was first used by Albersheim *et al* [8] as a 2 M solution to hydrolyse hemicellulosic polysaccharides and it has been adopted by many other workers in the field. It has the major advantage that it is volatile (bp 72°) and therefore easily removed To hydrolyse the cellulosic component, more concentrated solutions of TFA are required [9, 10] but these can

cause extensive degradation of some of the sugar residues, particularly D-xylose A single hydrolysis also has the disadvantage that it is impossible to differentiate between D-glucose units arising from cellulose and those from hemicelluloses such as mixed linkage β-glucans or glucomannans

This paper presents a method for estimating the neutral sugar residues present in plant cell walls, including lignified cell walls The method allows the hemicelluloses and cellulose to be determined simultaneously in a single sample

RESULTS AND DISCUSSION

The major monosaccharide unit present in cell walls is D-glucose which arises from the cellulose component. The major pentose unit present in lignified cell walls is D-xylose From preliminary experiments, it was noted that D-xylose was less stable in TFA solutions than the other pentose commonly found, L-arabinose D-Glucose, methyl-β-D-glucopyranoside, methyl-α-D-glucopyranoside, D-xylose and methyl-β-D-xylopyranoside were dissolved in TFA overnight at 37° then diluted to 80% TFA and heated at 100° Samples were removed at regular intervals and analysed for monosaccharide content by GC as aldononitrile acetates The results are shown in Fig. 1 In the samples with methyl glycosides, the amount of residual glycoside could also be determined The results show that D-glucose was fully recovered after dissolution in TFA and it was only slowly degraded in hot 80% TFA On the other hand, three to four % of D-xylose was lost overnight and the rest was rapidly degraded in 80% TFA, 25% being degraded after only 1 hr Methyl-β-D-xyloside was extensively hydrolysed overnight (>50%) and thereafter rapidly hydrolysed and degraded The two methyl glucosides were hydrolysed more slowly overnight (<20%) but were then hydrolysed rapidly at 100° The α-glucoside was more stable than the β-anomer

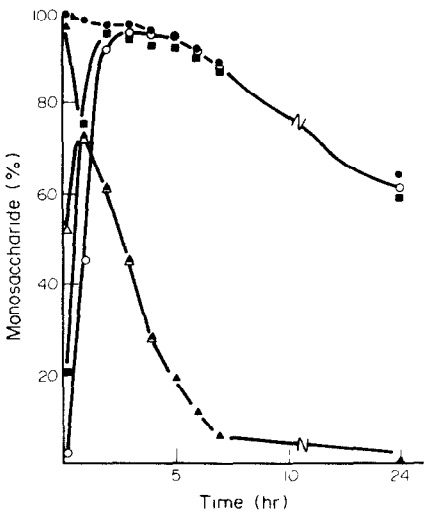

Fig 1 Stability of sugars in 80% TFA at 100° after dissolution in 100% TFA at 37° for 18 hr ● D-glucose, ○ Methyl-α-D-glucopyranoside, ■ Methyl-β-D-glucopyranoside, ▲ D-xylose, △ Methyl-β-D-xylopyranoside

These investigations were repeated under the conditions for cellulose and hemicellulose hydrolysis given in the Experimental and the results are shown in Figs 2 and 3, respectively They show that D-glucose is stable (<5% loss) under both the cellulose and hemicellulose conditions and that the glucosides are fully hydrolysed The xyloside is completely hydrolysed under both conditions but although xylose is still degraded under the cellulose condition, it is recovered quantitatively (<5% loss) under the hemicellulose conditions

Cellulose powder (Whatman No 1) was dissolved in TFA overnight at 37 and then subjected to both the cellulose and hemicellulose conditions After the overnight dissolution, no free glucose was detected in the solution Under the conditions for hemicellulose hydrolysis, the cellulose powder gave 1 8% D-xylose and 2 4% D-glucose showing that negligible hydrolysis had occur-

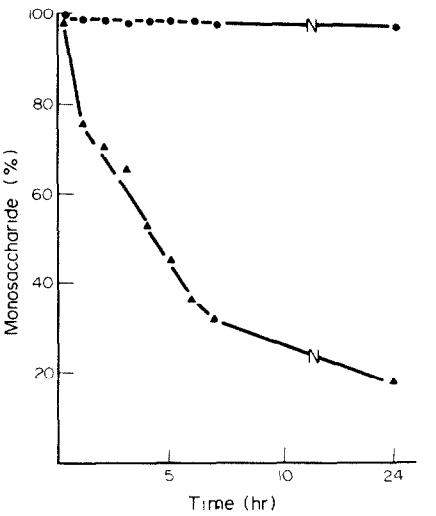

Fig 2 Stability of sugars under conditions for cellulose hydrolysis ● D-glucose, ▲ D-xylose

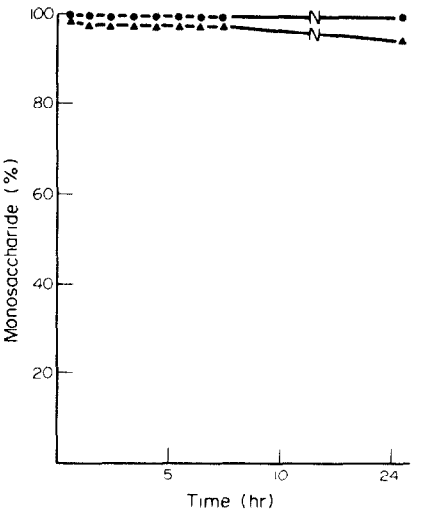

Fig 3 Stability of sugars under conditions for hemicellulose hydrolysis ● D-glucose, ▲ D-xylose

red. Indeed the D-glucose produced may have arisen from some of the native cellulose being partially degraded to shorter chain length forms during the preparation of the cellulose powder. When hydrolysed under the conditions for cellulose hydrolysis, a yield of >95% D-glucose was obtained showing that the cellulose was fully hydrolysed with little, if any, degradation.

When preliminary experiments were being carried out, it was observed that the total sample, particulary with lignified samples, was not completely soluble in TFA (as compared to cellulose powder) The insoluble material was isolated by centrifugation and washed with TFA, ethanol and diethyl ether. It was analysed for monosaccharide composition by the 72% sulphuric acid method [7] and for lignin by the acetyl bromide method [11] The only monosaccharide residue detected was D-glucose which accounted for <2% of the sample dry matter. The rest of the sample appeared to be lignin (>80%) It is possible that some protein could be present in this material but no analyses were performed either for nitrogen content or amino-acid composition

To test the reproducibility of the proposed method, the monosaccharide composition of Italian ryegrass leaf and stem, lucerne stem, sainfoin leaf, birch sawdust and barley straw were determined by the hemicellulose and cellulose methods and the results are shown in Table 1. Also included are the results for a 2 M TFA hydrolysis [8] and a 72% sulphuric acid hydrolysis [7] of the same samples It can be seen that very similar results are obtained by the two methods. Indeed, higher total recoveries were obtained by the new method than by a combination of 2 M TFA and 72% sulphuric acid for all but the birch sawdust

The hemicellulose results are very similar to those obtained with 2 M TFA and the cellulose values are very similar to those obtained with 72% sulphuric acid. The major difference is in the reduced recovery of other monosaccharide residues with the proposed cellulose method. Thus, a single hydrolysis cannot be used with 100% TFA, while meaningful results are obtained with 72% sulphuric acid alone However, a single hydrolysis with 72% sulphuric acid will overestimate the cellulose content of a sample since any hemicellulosic D-glucose will be assumed to come from cellulose As there are a number of sources of hemicellulosic D-glucose residues (mixed linkage β-glucans, xyloglucans and glucoman-

Table 1 Neutral monosaccharide residues produced by different hydrolytic conditions (% sample dry matter)

Plant material		Monosaccharide				
		Ara	Xyl	Man	Glc	Gal
Italian ryegrass stem	A	5 48	22 50	0 10	4 16	1 33
	B	3 94	6 72	0 36	35 39	1 39
	C	5 50	21 46	0 16	3 92	1 21
	D	4 96	20 24	0 10	34 96	1 42
Italian ryegrass leaf	A	9 30	23 00	—	5 20	1 16
	B	2 48	4 61	—	48 23	1 84
	C	8 44	21 96	—	2 29	1 14
	D	7 33	20 66	—	40.25	1 31
Lucerne stem	A	2 73	14 21	1 96	8 28	1 74
	B	2 25	4 76	2 42	40 09	1 64
	C	2 61	14 12	1 87	3 34	1 89
	D	2 68	12 62	2 20	38 64	2 14
Sainfoin leaf	A	7 50	8 59	1 79	7 94	3 65
	B	5 19	2 81	2 23	28 98	3.76
	C	7 95	7 24	1 16	6 34	2 85
	D	8 14	6 92	1 64	27 43	3 95
Birch sawdust	A	2 06	23 35	1 84	2 23	1 42
	B	1 34	9 65	3 96	36 85	1 02
	C	2 68	23 94	2 13	4 28	1.13
	D	2 24	25 63	4 24	37 92	1 61
Barley straw	A	3 24	24 53	—	3 61	1 01
	B	2 13	7 36	—	45 96	1 34
	C	3 04	21 98	—	4 41	1 83
	D	3 08	24 38	—	45 90	1 65

A 100% TFA + hemicellulose hydrolysis
B 100% TFA + cellulose hydrolysis
C 2 M TFA
D 72% H_2SO_4
All hydrolysates were analysed by GC as aldononitrile acetates The values quoted for the monosaccharides are for anhydro sugar residues

nans), a two-stage hydrolysis is essential to obtain full information about the composition of the cell wall The proposed method provides this information from a single sample and without the presence of sulphuric acid which is difficult to remove The proposed method has the advantage over that of Fengel *et al* [9] in that correction factors to compensate for the degradation of monosaccharide residues in hot strong TFA are not required

In conclusion, the proposed method is a reliable alternative to the 72% sulphuric acid method and is suitable for use with highly lignified plant cell wall materials

EXPERIMENTAL

Plant material Samples of Italian ryegrass (*Lolium multiflorum*) leaf and stem, lucerne (*Medicago sativa*) stem, sainfoin (*Onobrychis viciifolia*) leaf, birchwood (*Betula alba*) and barley (*Hordeum vulgare*) straw were freeze-dried and ground in a hammer-mill to pass a 1 mm screen Crude cell wall prepns were prepd by extracting the material with H_2O at 70 , filtering and washing with water (70), EtOH Me_2CO and Et_2O before air-drying

Hydrolysis procedures Standard hydrolysis procedures with 72% H_2SO_4 [7] and 2 M TFA [8] were used The following procedure was used with TFA (99% + purity) The sample (20 mg) is weighed into a glass vial (7 ml) and TFA (2 ml) added along with a small teflon-coated magnetic follower The vial is capped using a butyl rubber/teflon (not silicone rubber/teflon) disc and stirred overnight at 37 An aliquot (0 5 ml) is transferred to a glass vial (3 5 ml) H_2O (1 ml) and *meso*-inositol (2 mg/ml, 0 5 ml) are added and the vial capped as above This sample, for hemicellulose hydrolysis, is heated at 100 for 30 min and then evapd to dryness A second aliquot (0 5 ml) is transferred to a similar vial and H_2O (0 1 ml) is added The capped vial is heated at 100 for 30 min The addition of H_2O (0 1 ml) is repeated at 30 min intervals until total vol of 0 5 ml is added *Meso*-inositol soln (0 5 ml) is added as above and heating continued for a further 2 hr before evapn to dryness

Analysis of monosaccharides Monosaccharides released by the hydrolysis procedures were determined by GC as aldononitrile acetates as previously described [12]

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