Phytochemistry, 1971, Vol. 10, pp. 1065 to 1073. Pergamon Press. Printed in England.

POLYSACCHARIDE MANNOSE IN NEW ZEALAND FERNS

R. W. BAILEY and VALERIE PAIN

Applied Biochemistry Division, D.S.I.R., Palmerston North, New Zealand

(*Received* 10 *August* 1970)

Abstract—Mannose has been measured in hydrolysates of cell wall carbohydrates from fronds of 109 species of ferns from 40 genera. Sixty-four species from 24 genera contained high (10–20%) or medium-high (5–10%) levels of mannose in the cell wall carbohydrates of their green leaf (laminar) tissue. Forty-five species in 16 genera however (principally *Thelypteris*, *Asplenium*, *Blechnum*, *Doodia* and *Polystichum*) contained little or no mannose (<2%) in the cell wall carbohydrates from laminar tissues. The few species examined from fern orders other than the Filicales also had low levels of cell-wall mannose. Only one genus, *Phymatodes*, gave species with high and low levels respectively of polymer mannose in the lamina. Cell-wall mannose levels were also measured in midribs from the low mannose ferns and found to be low only in the *Asplenium* spp. Holocellulose (11·5 g) from one high mannose species, *Pyrrosia serpens*, was fractionated with alkali into hemicellulose (1·2 g), possible galactoglucomannan (4·5 g) and cellulose (2·7 g).

INTRODUCTION

THE MONOSACCHARIDE compositions of the structural carbohydrates of higher plant cell walls have been extensively investigated but only limited studies[1,2] appear to have been made on these polysaccharides in ferns (Filicopsida). Preliminary studies in this laboratory indicated that while hydrolysates of various fern tissue cell-walls always contained xylose, arabinose, glucose and galactose this was not so with mannose. In frond laminar (green leaf) tissue in particular some species were rich in cell-wall mannose while others were virtually mannose-free. Some 150 species of ferns, many endemic, from a wide range of families and genera occur in New Zealand. As plant cell-wall mannans have been suggested to be of some taxonomic or evolutionary significance[1,3] the levels of this polysaccharide mannose in the New Zealand ferns have been measured.

RESULTS

Cell-wall Mannose in Fern Laminar Tissue

Mannose levels in the cell-wall carbohydrates of the frond laminar tissues are given in Table 1 with the species listed in the order given in Allan[4] and the mannose values assigned to low, medium or high level groups. From 0–3% of mannose in the total cell-wall reducing sugars was considered to be a low level and over 10% a high level.

The levels of total cell-wall carbohydrates, as anhydro-reducing sugars liberated in two sequential acid hydrolyses, are also listed in Tables 1 and 2. For most plant tissues the second, 72%, acid hydrolysate is generally recorded as cellulose. In the high mannose fern samples, however, much of the reducing sugar in these stronger acid hydrolysates will have

[1] T. E. TIMELL, *Svensk. Papperstidn.* **65**, 266 (1962).
[2] G. BERTI and F. BOTTARI, *Progress in Phytochemistry* (edited by L. REINHOLD and Y. LEWSCHITZ), Vol. 1, p. 589, Interscience, London (1968).
[3] C. M. STEWART, The Chemistry of Secondary Growth in Trees, *Div. For. Prods Tech. Paper* 43, C.S.I.R.O., Melbourne (1966).
[4] H. H. ALLAN, *Flora of New Zealand*, Vol. 1, N.Z. Govt. Printer, Wellington (1961).

Table 1. Mannose and cell-wall carbohydrate levels in fern laminae

Family	Species (herbarium No.)	Mannose (% of total anhydro-reducing sugars) High	Medium	Low	Anhydro-reducing sugars liberated sequentially by N-acid (% of oven dried laminae)	72% acid (% of oven dried laminae)
Ophioglossales						
	Botrychium australe var. *australe* R. Br. (CHR155880)			2·4	5·4	5·4
	B. australe var. *millefolium* Prantl (CHR155882)			2·1	5·3	4·3
	*Ophioglossum pedunculosum** Desv.			1·7	8·5	12·5
Marattiales						
	Marattia salicina Smith (CHR195024)			0	4·5	6·4
Osmundaceae						
	Todea barbara (L.) Moore (CHR155884)		9·2 (7·4)†		13·4 (13·0)	12·5 (32·5)
	T. superba Col. (CHR155885)		6·6		8·2	16·0
	T. hymenophylloides A. Rich. (CHR155886)	10·0			8·6	18·2
Schizaeaceae						
	Lygodium articulatum A. Rich. (CHR155887)		6·0		16·7	16·5
	Schizaea dichotoma (L.) Smith (CHR155888)	13·1			9·6	23·5
Gleicheniaceae						
	Gleichenia microphylla R. Br. (CHR155889)	11·4			11·1	14·6
	G. circinata Swartz. (CHR155890)	17·8			11·2	15·6
	G. cunninghamii Heward ex Hook. (CHR 195020)	19·3			10·2	9·5
	G. linearis (Burman) C. B. Clarke (CHR155892)	18·6			9·2	10·8
Loxomaceae						
	Loxoma cunninghamii R.Br. ex A. Cunn. (CHR155893)	13·2			15·4	22·1
Hymenophyllaceae						
	Hymenophyllum flabellatum Labill. (CHR155894)‡	21·9			13·0	13·0
	H. dilatatum (Forst. f.) Swartz (CHR155895)	14·7 (11·2)			10·5 (14·5)	10·3 (28·3)
	H. rarum R. Br. (CHR155896)§		7·5		12·7	11·7
	H. sanguinolentum (Forst. f.) Swartz (CHR155897)‡		8·6		13·7	12·9
	H. scabrum A. Rich. (CHR155898)‡	10·2			15·7	10·7
	H. demissum (Forst. f.) Swartz (CHR155899)‡	26·0			8·9	8·0
	H. pulcherrimum Col. (CHR155900)‡	15·8			15·3	18·3
	H. lyallii Hook. (CHR155901)‡		5·6		9·6	15·4
	H. ferrugineum Colla (CHR155902)‡	15·6			15·5	28·6
	H. malingii (Hook) Mett. (CHR155903)‡	12·5			10·3	16·0
	H. revolutum Col. (CHR155904)‡		7·2		10·7	12·0
	H. peltatum (Poir.) Desv. (CHR155905)‡	10·1			10·1	12·8
	H. multifidum (Forst. f.) Swartz (CHR155907)‡	11·8			7·8	9·6

H. bivalve (Forst. f.) Swartz (CHR155908)‡	12·3			7·3	9·3
Trichomanes venosum R. Br. (CHR155909)§	11·1			13·3	19·1
T. endlicherianum Presl (CHR155910)‡	11·3			11·8	18·6
T. elongatum A. Cunn. (CHR155911)‡		7·8		12·9	15·3
T. reniforme (Forst. f.) (CHR155912)	20·0			8·2	12·5
Dicksoniaceae					
Dicksonia squarrosa (Forst. f.) Swartz (CHR195023)		8·8 (12·8)		7·4 (9·7)	13·7 (24)
D. fibrosa Col. (CHR155913)		8·0 (11·7)		6·6 (12·8)	16·9 (28·5)
D. lanata Col. (CHR155914)	10·3			5·9	18·8
Cyatheaceae					
Cyathea dealbata (Forst. f.) Swartz (CHR155915)		8·6 (8·4		7·2 (11·5)	14·1 (32·3)
C. medullaris (Forst. f.) Swartz (CHR195022)	21·2			13·8	12·2
C. cunninghamii Hook. f. in Hook. (CHR155916)		7·3		12·8	21·6
C. smithii Hook. f. (CHR155917)	15·1			7·6	9·6
Polypodiaceae					
Pyrrosia serpens (Forst. f.) Ching (CHR195029)	25·1			11·0	12·3
Anarthropteris lanceolata (J. Smith) L. B. Moore (CHR155918)		6·5		20·4	17·3
Phymatodes scandens (Forst. f.) Presl (CHR195028)			1·1	8·5	13·2
P. diversifolium (Willd.) Pic. Ser. (CHR195027)	13·8 (8·4)			10·6 (10·1)	8·8 (30·1)
P. novae-zelandiae (Baker) Pic. Ser. (CHR195021)	10·5 (12·1)			16·7 (16·3)	16·4 (24·3)
Grammitidaceae					
Grammitis heterophylla Labill. (CHR155919)	12·9			17·0	18·0
C. billardieri Willd. (CHR155920)	10·7			17·8	14·7
Thelypteridaceae					
Thelypteris pennigera (Forst. f.) Allan (CHR155921)			0	4·4	9·3
T. gongylodes (Schkuhr) Allan (CHR155922)			1·2	6·5	17·5
T. dentata (Forsk.) Allan (CHR155923)			0	8·4	9·6
Dennstaediaceae					
Leptolepia novae-zelandiae (Col.) Kuhn (CHR155924)		6·1		7·2	20·8
Hypolepis rugosula (Labill.) J. Smith (CHR155925)		7·8		14·7	32·6
H. distans Hook. (CHR155926)		7·7		11·2	17·3
H. millefolium Hook. (CHR155927)		8·0		7·1	15·0
H. tenuifolia (Forst. f.) Bernh. in Schrad. (CHR155928)		6·3		7·3	19·7
H. puncata (Thunb.) Mett. in Kuhn (CHR155929)		9·6		6·9	11·8
Lindsaeaceae					
Lindsaea linearis Swartz (CHR155930)	10·7			5·4	6·1
L. trichomanoides Dryand. (CHR155931)	12·3			10·5	15·7
Davalliaceae					
Davallia tasmanii H. C. Field (CHR155932)	16·2			7·8	12·5
Arthropteris tenella (Forst. f.) Smith in Hook (CHR155933)		3·2		8·9	13·9
Nephrolepis cordifolia (L.) Presl (CHR155934)			2·8	6·6	10·5
N. exaltata (L.) Schott (CHR155935)		3·5		8·8	9·4

TABLE 1.—*cont.*

Family	Species (herbarium No.)	Mannose (% of total anhydro-reducing sugars) High	Medium	Low	Anhydro-reducing sugars liberated sequentially by *N*-acid (% of oven dried laminae)	72% acid (% of oven dried laminae)
Pteridaceae						
	Paesia scaberula (A. Rich.) Kuhn (CHR155937)	17·2			7·0	12·7
	Histiopteris incisa (Thunb.) J. Smith (CHR155938)	10·7			9·0	16·7
	Pteridium aquilinum var. *esculentum* (Forst. f.) Kuhn (CHR155939)		7·4		9·7	21·6
	Pteris comans Forst. f. (CHR155941)		8·8 (8·7)		8·9 (9·9)	15·7 (41·2)
	P. macilenta A. Rich. CHR155942)	10·8			9·0	17·4
Aspleniaceae						
	Asplenium flabellifolium Cav. (CHR190313)			2·8	8·6	12·7
	A. lucidum var. *lucidum* Forst. f. (CHR195026)			0	7·1	12·8
	A. lucidum var. *aucklandicum* (Hook. f.) Allan (CHR69409)			1·9	6·3	20·8
	A. obtusatum Forst. f. (CHR69322)			0·5	7·7	20·9
	A. falcatum Lam. (CHR195025)			0	12·3	15·7
	A. lamprophyllum Carse (CHR155945)			1·5	7·7	13·2
	A. bulbiferum Forst. f. (CHR155946)			0	6·9	18·2
	A. hookerianum Col. (CHR155949)			0·9	8·9	12·5
	A. flaccidum Forst. f. (CHR155950)			1·2	7·1	21·7
	Pleurosaurus rutaefolius Fee (CHR195061)*		4·4		5·3	12·8
Blechnaceae						
	Doodia media R. Br. (CHR155952)			0	4·8	13·5
	D. caudata (Cav.) R. Br. (CHR155953)			0·5	6·2	13·8
	Blechnum fraseri (A. Cunn.) Luerss. (CHR155954)			0·5	10·4	13·5
	B. filiforme (A. Cunn.) Ettingshausen (CHR155955)			0	6·2	14·5
	B. patersonii (R. Br.) Mett. (CHR155956)			0·1	5·4	11·8
	B. capense (L.) Schlecht. (CHR155957)			0·5	6·8	10·4
	B. minus (R. Br.) Allan (CHR155958)			2·0	6·7	22·0
	B. penna-marina (Poir.) Kuhn (CHR155960)			0·7	5·5	16·7
	B. nigrum (Col.) Mett (CHR155961)			1·1	7·3	17·6
	B. banksii (Hook, f.) Mett. ex Diels (CHR69321)			1·2	6·9	14·9
	B. discolor (Forst. f.) Keys (CHR155962)			0	7·4	15·6
	B. vulcanicum (Blume) Kuhn (CHR155943)			0	4·1	9·1
	B. lanceolatum (R. Br.) Sturm (CHR155964)			0	9·1	21·6
	B. membranaceum (Col.) Mett. (CHR155965)			0·9	8·1	22·5
	B. fluviatile (R. Br.) Salom (CHR155966)			0	6·6	15·5

Dryopteridaceae					
Polystichum vestitum (Forst. f.) Presl (CHR155967)			2·7	7·5	24·9
P. silvaticum (Col.) Diels (CHR155968)			1·8	6·8	24·0
P. richardii (Hook.) J. Smith (CHR1559670)		4·0		8·5	23·7
Ctenitis decomposita (R. Br.) Cop. (CHR155971)			2·4	5·8	12·7
C. glabella (A. Cunn.) Cop. (CHR155972)			2·6	7·0	16·7
C. velutina (A. Rich.) Cop. (CHR155973)		5·3		7·3	13·8
Rumohra adiantiformis (Forst. f.) Ching (CHR155974)		4·3		14·5	25·1
R. hispida (Swartz) Cop. (CHR155975)		5·6		8·8	7·4
Athyriaceae					
Athyrium australe (R. Br.) Presl (CHR155976)		3·3		4·2	10·6
A. japonicum (Thunb.) Cop. (CHR155977)			0·7	7·1	8·4
Adiantaceae					
Adiantum aethiopicum L. (CHR155978)	13·1			6·3	13·4
A. diaphanum Blume (CHR155979)	12·1			9·6	18·2
A. hispidulum Swartz (CHR155980)	11·6			13·6	33·0
A. formosum R. Br. (CHR155981)	10·0			6·7	15·6
A. cunninghamii Hook. (CHR155982)	19·4			6·6	13·8
Cheilanthes sieberi Kunze (CHR155983)‡		7·5		12·1	12·5
Pellaea rotundifolia (Forst. f.) Hook (CHR195030)	14·3			16·2	11·0
P. falcata (R. Br.) Fee (CHR155984)		6·6		13·6	10·2
Azollaceae					
Azolla rubra R. Br. (CHR155985)		3·8		6·1	8·1

* Whole frond taken.
† Values in brackets from midribs.
‡ Lamina separated from midrib only.
§ Whole frond above stipe taken.

come from the gluco- or galactogluco-mannan; for this reason the term cellulose has been avoided.

As far as possible the laminar tissue was dissected from the fronds to be free from midrib or secondary vein tissue. In a few cases as indicated in the Table the whole frond was taken. With some species, particularly from the genera *Hymenophyllum* and *Trichomanes* genera, it was almost impossible to free the membranous laminae from the wiry midribs and in these cases the whole frond above the main stem or stipe was taken. As a check that the high mannose levels recorded for these species was not merely due to the presence of midrib tissue the sample of *H. dilatatum* laminae used to obtain the result in Table 1 was carefully dissected from fronds to be entirely free of midrib and veinal tissue. Similarly the

TABLE 2. MANNOSE AND CELL-WALL CARBOHYDRATE CONTENTS OF FERN MIDRIBS

Species	Mannose (% of total anhydro-reducing sugars)	Anhydro-reducing sugars liberated by (% of oven-dried laminae)	
		N-acid	72% acid
Botrychium australe var. *australe*	5·7	5·9	9·1
B. australe var. *millefolium*	3·1	6·0	12·9
Marattia salicina	8·0	6·0	25·5
Phymatodes scandens	7·0	8·4	23·0
Thelypteris pennigera	4·7	6·3	22·1
T. dentata	3·1	13·7	24·5
Arthropteris tenella	3·2	10·2	27·1
Nephrolepis cordifolia	6·9	9·4	33·0
N. exaltata	9·5	11·0	30·9
Asplenium flabellifolium	2·5	10·4	21·3
A. lucidum var. *lucidum*	4·6	6·3	26·0
A. lucidum var. *aucklandicum*	4·2	5·8	32·0
A. obtusatum	1·3	9·3	27·0
A. falcatum	9·2	11·0	26·3
A. lamprophyllum	3·5	17·8	46·2
A. bulbiferum	1·1	5·8	27·5
A. hookerianum	1·4	21·8	31·7
A. flaccidum	2·4	8·1	28·9
Doodia media	3·4	7·7	37·1
Blechnum fraseri	4·9	17·2	53·3
B. patersonii	8·2	12·5	30·7
B. capense	9·3	18·0	58·7
B. minus	3·9	20·4	53·0
B. penna-marina	3·6	15·0	44·8
B. nigrum	1·6	10·8	29·4
B. banksii	10·0	9·5	20·9
B. discolor	5·5	10·8	32·2
B. vulcanicum	4·9	13·0	51·1
B. lanceolatum	3·8	25·0	48·7
B. fluviatile	5·2	9·8	34·5
Polystichum vestitum	3·8	10·0	25·6
P. silvaticum	7·6	9·3	25·0
P. richardii	6·1	11·1	29·1
Ctenitis decomposita	5·1	7·7	40·3
C. glabella	3·8	16·2	27·2
Rumohra adiantiformis	9·8	11·8	28·6
Athyrium australe	3·9	5·7	33·8
A. japonicum	2·8	8·2	30·6

sample of *Pyrrosia serpens* laminae was carefully separated from midribs while the *T. reniforme* laminae were easily separated from their stipes. These three samples of laminae all gave high mannose levels (Table 1).

Cell-wall Mannose in Fern Frond Midribs

Qualitative analyses and a few quantitative analyses (Table 1 in brackets) indicated that species with high mannose levels in their laminae cell walls also had high mannose levels in their frond midribs. Mannose levels found in cell-wall hydrolysates from the midribs of species with low mannose levels in their laminar tissue are given in Table 2.

Extraction of Mannan Polysaccharide from Pyrrosia serpens *Fronds*

The results given in Table 1 indicate that in many cases if the mannose is present in a gluco- or galactogluco-mannan then this mannan would be a major component of the cell-wall carbohydrates. In some species, e.g. *Hymenophyllum demissum, Trichomanes reniforme* and *P. serpens* this polysaccharide could exceed the true cellulose.

Freeze-dried *P. serpens* laminae were therefore fractionated into hemicellulose A + B, possible mannan and cellulose. The yields and monosaccharide compositions of the fractions given in Table 3 confirm that a mannan which is probably a galactoglucomannan is the major cell-wall polysaccharide in *P. serpens* laminar tissue. Extraction of the mannan into strong alkali was difficult and required seven extractions.

TABLE 3. CELL-WALL POLYSACCHARIDES FROM *Pyrrosia serpens* LAMINAE

Fraction	Wt.* (g, ash and moisture-free)	Monosaccharide composition
Soluble in dilute alkali	1·2	Xylose, arabinose, glucose, galactose.
Soluble in strong alkali–boric acid	4·5	Mannose glucose, galactose, xylose. 3 : 1 : 0·24 : 0·14
Insoluble in strong alkali–boric acid	2·7	Glucose, mannose. 10 : 1

* Yields from 11·5 g of holocellulose from 21 g of freeze-dried laminae (all ash and moisture free values).

Other Monosaccharides in Fern Cell-wall Hydrolysates

In addition to mannose the hemicellulose hydrolysates always showed on chromatograms xylose, arabinose, glucose, galactose and occasionally rhamnose while the cellulose hydrolysates contained glucose and some xylose. These individual monosaccharides were not measured quantitatively. From visual assessments of spot intensities it was concluded that frond laminae cell walls generally contained either about equal amounts of galactose, arabinose and xylose or a little less xylose than the other two sugars. In contrast chromatograms of midrib hydrolysates indicated the presence of more xylose than arabinose or galactose in these cell-walls.

DISCUSSION

In a study of 14 fern species Timell[1] recorded mannose levels ranging from 5 to 18% of the stem or midrib cell-wall carbohydrates. The present results suggest that the division of ferns into two classes with high and low levels of cell-wall mannose respectively is more obvious if laminar (green frond) tissue is examined. None of the 14 species examined by

Timell[1] occurs in this country but within the genera that we have examined he recorded low (5–6%) levels of midrib mannose in *Thelypteris* (3) and *Athyrium* (1) spp. and high levels (12–18) in *Adiantum* (1) and *Pteridium* (1) spp. These values are substantially in agreement with the present results.

The division of ferns into groups with high or low levels of mannose in the laminar cell-wall polysaccharides appears to be of taxonomic interest for the following reasons. The few Filicopsida examined from orders other than Filicales, i.e. *Botrychium*, *Ophioglossum* (Ophioglossales), *Marattia* (Marattiales) and *Azolla* (Salvinales) species, were all low in cell-wall mannose. Within the order Filicales species from the first seven (paleobotanically 'old')[5] families (Osmundaceae, Schizaceae, Gleichenaceae, Loxomaceae, Hymenophyllaceae, Dicksoniaceae and Cyatheaceae) consistently contained high or medium-high mannose levels. In the remaining 12 Filicales families of Allan[4] (grouped in the geologically younger Polypodiaceae by Smith)[5] low mannose levels occur consistently in species from five families, namely the Thelypteridaceae, Aspleniaceae, Blechnaceae, Athyriaceae and possibly the Dryopteridaceae. In the Davalliaceae there are possibly high level (*Davallia* sp. and low level (*Arthropteris*, *Nephrolepis* spp.) mannose genera in the one family. Only one genus examined in the Filicales, *Phymatodes* contained species with high (*P. diversifolium* and *P. novae zelandiae*) and low (*P. scandens*) levels of mannose in the laminae cell-walls. It is perhaps relevant that all of the low mannose species occur among the families and sub-families of the Filicales where recent taxonomic studies have shown considerable variation.[4–7]

Plant cell wall mannose is generally present in gymnosperms at high levels as galactoglucomannan but only in traces in angiosperms as glucomannan.[2] The mannan from *Osmunda cinnamomea* L. has been shown to be a galactoglucomannan[8] and the results for *Pyrrosia serpens* are also in agreement with the mannan of this species being a galactoglucomannan. Assuming that the mannose in the high mannose ferns arises from the same polymer then these ferns are chemically nearer to the gymnosperms.[1] Nevertheless so far as laminae are concerned some fern genera are closer in mannose levels to the angiosperms in their mannose levels.

The *N*-acid, total hemicellulose, hydrolysate monosaccharides consist of a mixture of xylose, arabinose, galactose, glucose and mannose derived from various polysaccharides based on xylan as well as the galactoglucomannan. The 72% acid, cellulosic, hydrolysate monosaccharides consist principally of glucose and mannose derived from cellulose and galactoglucomannan. The results in Table 1 suggest a higher ratio of cellulosic monosaccharides to hemicellulose monosaccharides in the low mannose species compared with the high mannose species. After making allowance for the contribution to these total hydrolysates from the galactoglucomannan the results are also in agreement with the general conclusion that as the mannan levels decline the cellulose levels increase in the laminae.

EXPERIMENTAL

Fern Samples

Whole ferns or large fronds were collected from natural habitats. In a few cases cultivated plants, which had come originally from natural sites, were sampled; i.e. *Todea barbara*, *Davallia tasmanii*, *Pellae falcata*, *Marattia salicina*, *Thelypteris gongylodes* and *T. dentata*. Fronds were divided into two samples; (a) the laminae or green frond tissue free as far as possible from midribs (stipes and rachis) and sideribs, (b) midrib

[5] G. M. SMITH, *Cryptogamic Botany*, Vol. 2, p. 266, 2nd edition, McGraw-Hill, New York (1955).
[6] R. E. HOLTTUM, *Biol. Rev.* **24**, 267 (1949).
[7] E. B. COPELAND, *Genera Filicum*, Chronica Botanica, Co., Waltham Mass, U.S.A. (1947).
[8] T. E. TIMELL, *Svensk. Papperstidn.* **65**, 173 (1962).

and stalk. Only one sample of each species was collected, except in the case of *Phymatodes scandens.* As many species as possible from each genus were collected.

Samples (10–100 g green wt.) were freeze-dried and ground in a Casella mill to pass a 1-mm sieve. Herbarium specimens were lodged with the herbarium at Botany Division, D.S.I.R., Lincoln, New Zealand and herbarium numbers are given in Table 1. Identifications were confirmed by reference to one or more taxonomists. The nomenclature and classification systems used by Allan,[4] based on that of Holttum,[6] have been followed in the present paper.

Cell-wall Hydrolysates

Crude cell walls were prepared from freeze-dried samples (1 g) by treatment with ethanol and ammonium oxalate. The cell-wall residues were sequentially hydrolysed by treatment with *N* H_2SO_4 (200 ml refluxed 2 hr) followed by 72% H_2SO_4 (20 ml at room temperature 4 hr, diluted to 400 ml, refluxed 2 hr).[9,10]

Carbohydrate Analyses

Total reducing sugars in the two hydrolysates were measured by a micro cuprimetric method[11] using a glucose standard and recorded as anhydro-sugar.

Mannose was measured in the two hydrolysates by quantitative paper chromatography[12] using acid-washed paper and aniline hydrogen phosphate spray reagent. Papers were developed with (1) butyl acetate–water–ethanol–pyridine (8:1:2:2), (2) ethyl acetate–water–pyridine (2:2:1) cellulose hydrolysates only, or (3) ethyl acetate–acetic acid–formic acid–water (9:1·5:0·2:2·0). While solvent (1) gave a good separation of mannose from other sugars it was very slow (3 days). As mannose and arabinose moved at the same rates in solvent 2 this solvent was only suitable for 72% acid hydrolysates. Solvent (3) separated mannose from arabinose in *N*-acid, hemicellulose, hydrolysates but desalting to remove interfering uronic acids was necessary. Hydrolysates (25 ml) were neutralised with $BaCO_3$, filtered, freeze-dried and taken up in a little (1 ml) water. Aliquots of 50 and 100 μl were applied to the chromatograms and conditions were such that as little as 0·2% of monosaccharide (in the total cell-wall carbohydrates) could be measured. Mannose was often liberated in both acid hydrolysates. The combined mannose present in both hydrolysates was, therefore, calculated as a per cent of the total cell wall carbohydrates, i.e. the combined reducing anhydro-sugars measured in the *N*-acid and 72% acid hydrolysates.

Extraction of Mannan

Freeze-dried laminae were freed from soluble substances, including pectin and protein, by treatment with neutral detergent[13] then delignified with sodium chlorite to give a holocellulose.[14] Hemicellulose (A + B) was extracted with dilute alkali (10%, w/v)[9] after which possible mannan was dissolved by repeated extraction with strong sodium hydroxide (24%, w/v)–boric acid (4%, w/v).[15] Polysaccharides in the various extracts were precipitated with ethanol (3 vol.) after acidification with acetic acid, centrifuged, dialysed and finally freeze-dried.

Acknowledgements—Thanks are due to Dr D. R. Given, Mr H. E. Connor and Mr A. E. Esler, Botany Division, D.S.I.R. and Mr R. M. Greenwood, Applied Biochemistry Division, D.S.I.R. for advice on the taxonomy and identification of ferns. Also to the above-mentioned and Dr R. Fergusson, Mr J. Braggins, Miss B. Paris, Mrs Bedford and Mrs Hynes, who kindly collected or arranged for the collection of fern material

[9] R. W. Bailey and B. D. E. Gaillard, *Phytochem.* **7**, 2037 (1968).
[10] R. W. Bailey, *N.Z. J. Agri. Res.* **10**, 15 (1967).
[11] N. Nelson, *J. Biol. Chem.* **153**, 375 (1944).
[12] C. M. Wilson, *Anal. Chem.* **31**, 1199 (1959).
[13] P. J. Van Soest, *J. Ass. Off. Agri. Chem.* **46**, 825 (1963).
[14] R. L. Whistler and J. Bemiller, *Methods in Carbohydrate Chemistry* (edited by R. L. Whistler), Vol. 3, p. 21, Academic Press, New York (1965).
[15] T. E. Timell, *Methods in Carbohyrdate Chemistry* (edited by R. L. Whistler), Vol. 5, p. 134, Academic Press, New York (1965).