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# COMPOSITION OF *ACTINIDIA* MUCILAGE

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**Key Word Index**—*Actinidia chinensis*; Actinidiaceae; mucilage; polysaccharide; glucuronomannan.

**Abstract**—Purified mucilages extracted from several plant parts of *Actinidia chinensis* and from the leaves of nine *Actinidia* species, were shown to be acidic polysaccharides, containing galactose, arabinose, mannose and glucuronic acid. Fucose and xylose were also present in the mucilages from *A. chinensis* and in the leaf mucilage of four other species. Partial hydrolysis studies suggested that all the mucilages may belong to the glucuronomannan family of polysaccharides, with a repeating disaccharide core of glucuronosylmannose. Division of the *Actinidia* genus into subgenera may be possible on the basis of properties and monosaccharide compositions of the mucilages.

## INTRODUCTION

The kiwi fruit (*Actinidia chinensis* Planch. var. *hispida* C.F. Liang.) has enjoyed remarkable success as a commercial crop in New Zealand in recent years. The area occupied by the plant in 1980 exceeded 5000 hectares and predicted plantings indicate that by 1990 fruit production will have increased 10-fold.

For centuries the Chinese have known that the plant contains a mucilage which can be extracted and used as a constituent in road paving, a protective covering for walls, for the sizing of paper and in the manufacture of inks and dyes [1]. Despite this long use and the recent attention focussed on *Actinidia chinensis* there have been only two reports on the composition of the mucilage and little else on its properties, chemistry or structure. Thus, Kihara 1938 [2] demonstrated the presence of galactose and arabinose in the mucilage from the bark of *A. callosa*, and Takei 1958 [3] found, in addition, glucuronic acid and rhamnose in the mucilage from the bark of *A. rufa*.

Classification within the genus *Actinidia* has proved difficult with many of the species being poorly defined and the nomenclature confused [4, 5]. Knowledge of the compositions and chemical structures of the mucilages from *Actinidia* species could contribute to better definition of the species within the genus.

This study presents analytical data for the mucilages extracted from various plant parts of *A. chinensis* and also from the leaves of nine *Actinidia* species. On the basis of this knowledge, groupings within the genus are proposed.

## RESULTS AND DISCUSSION

### *Homogeneity and properties of purified mucilage*

The purified mucilages (see Tables 1 and 2) all dissolved readily in water to give clear viscous solutions. The mucilage in these solutions was retained on columns of DEAE-Sephadex, and in all cases no neutral carbohydrates were eluted from the columns with water. This retention of the mucilage on the anion exchanger indicated that each was an acidic polysaccharide, and they were recovered from the column with unaltered composition by elution with potassium bicarbonate. Cation analysis showed them to exist as mixed salts (Ca, Mg, K). A solution of the free acid form of each mucilage was prepared using the cation exchanger SP Sephadex in the hydrogen form. After solutions of deionized mucilage were freeze dried the dried mucilage was no longer water soluble.

Examination of the mucilages for homogeneity by zone

Table 1. Chemical composition and specific rotations of purified mucilages from *Actinidia chinensis*

| Source | Yield of crude mucilage (mg/g dry wt) | Specific rotation ($[\alpha]_D^{25}$) | Composition (w/w) of mucilage (%) | | | | | | Total accounted for (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | | Neutral carbohydrate* | Uronic acid | Protein | Ca | Mg | K | |
| Root | 96 | −34.5° | 72.9 | 11.3 | 1.3 | 0.39 | 0.40 | 0.16 | 86.5 |
| Pith | 19 | −27.2° | 75.4 | 11.8 | 0.6 | 0.92 | 0.11 | 0.03 | 88.9 |
| Bark | 51 | −20.1° | 73.4 | 12.8 | 1.0 | 1.2 | 0.14 | 0.14 | 88.7 |
| Bulk pith | 9 | −33.6° | 79.0 | 11.3 | 0.3 | 0.65 | 0.14 | 0.12 | 91.5 |
| Leaf | 21 | − 5.9° | 66.9 | 18.0 | 1.1 | 1.9 | 0.11 | 0.04 | 88.1 |
| Leaf stalk | 47 | − 5.6° | 52.3 | 14.9 | 0.7 | 7.1 | 0.08 | 0.20 | 75.3 |
| Fruit stalk | 54 | −12.7° | 77.8 | 15.9 | 1.6 | 0.67 | 0.16 | 1.47 | 97.6 |
| Fruit | 9 | +70.8° | 41.9 | 27.9 | 2.6 | 1.2 | 0.60 | 0.28 | 74.5 |

*Sugars and uronic acid determined as anhydro units.

Table 2. Chemical composition and specific rotations of purified mucilages from leaves of nine *Actinidia* species

| Species | Yield of crude mucilage (mg/g dry wt) | Specific rotation ($[\alpha]_D^{25}$) | Composition (w/w) of mucilage (%) | | | | | | Total accounted for (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | | Neutral carbohydrate* | Uronic acid | Protein | Ca | Mg | K | |
| *A. arguta* | 21 | +31.2° | 54.5 | 24.6 | 2.1 | 3.7 | 0.06 | 0.05 | 85.0 |
| *A. giraldii* | 23 | +24.1° | 51.8 | 22.2 | 1.8 | 4.1 | 0.05 | 0.11 | 80.1 |
| *A. melanandra* | 15 | +30.3° | 57.9 | 23.1 | 0.63 | 2.8 | 0.11 | 0.04 | 84.6 |
| *A. purpurea* | 19 | +18.1° | 57.3 | 21.0 | 5.0 | 2.8 | 0.21 | 0.10 | 86.4 |
| *A. chinensis* | 21 | − 5.9° | 66.9 | 18.0 | 1.10 | 1.9 | 0.11 | 0.04 | 88.1 |
| *A. eriantha* | 14 | − 6.2° | 66.7 | 17.6 | 1.7 | 1.9 | 0.21 | 0.04 | 88.2 |
| *A. rufa* | 33 | − 3.0° | 65.9 | 14.3 | 0.70 | 1.7 | 0.20 | 0.01 | 82.8 |
| *A. callosa* | 39 | −25.6° | 73.1 | 14.0 | 0.20 | 1.2 | 0.01 | — | 88.5 |
| *A. polygama* | 14 | +11.3° | 64.7 | 17.7 | 2.5 | 2.0 | 0.22 | 0.02 | 87.1 |

*Sugars and uronic acid determined as anhydro units.

electrophoresis on cellulose acetate showed a single component in 12 of the 16 mucilages in two buffer systems. Of the four heterogenous polysaccharides those from the leaves of *A. chinensis*, *A. eriantha* and *A. rufa* all contained a minor secondary component, while the fruit mucilage from *A. chinensis* consisted of equal amounts of two components.

The sedimentation behaviour of *A. chinensis* bulk pith (BP) mucilage showed a single component which sedimented slowly, giving a broad boundary indicative of polydispersity.

*Time-course of hydrolysis of* $^{14}$C*-labelled, mucilage from* A. chinensis *leaves*

The level of $^{14}$C incorporation into the monosaccharides of the polysaccharide, approximated the percentage composition of the same monosaccharides determined by hydrolysis-GC and uronic acid analysis (Table 3). This result indicated a non-specific incorporation into the monosaccharides, and allowed valid conclusions to be made from experiments which used the $^{14}$C-labelled mucilage as a tool for monitoring the depolymerization of the molecule and for assessing degradative losses during hydrolysis.

Hydrolysis of the $^{14}$C-labelled mucilage in 2 M TFA (100°) was 97.5% complete after 6 hr, and radioactivity was distributed among the compounds listed (Table 3). The aldobiuronic acid, identified by hydrolysis-GC, was shown to contain equimolar proportions of mannose and glucuronic acid. Hydrolysis-GC analysis of the borohydride reduced disaccharide indicated that the reducing group was on the mannose. After hydrolysis for 2 hr, fucose, arabinose and galactose were released from the mucilage. Thus, the final 4 hr of hydrolysis depolymerized the remaining material to the aldobiuronic acid and some further hydrolysis of the latter led to some mannose and glucuronic acid. These results implied that after 2 hr hydrolysis, the unhydrolysed material was an oligo- or polysaccharide composed solely of glucuronosylmannose units.

Losses due to decomposition during hydrolysis were

Table 3. Time course hydrolysis of $^{14}$C-labelled leaf mucilage from *Actinidia chinensis*

| Component released | Yield at hydrolysis time (cpm) | | | | | | Composition after 6 hr hydrolysis (%) | |
|---|---|---|---|---|---|---|---|---|
| | 15 min | 30 min | 1 hr | 2 hr | 4 hr | 6 hr | cpm | w/w* |
| Fucose | 2175 | 2200 | 2261 | 2290 | 2311 | 2314 | 12.1 | 11.6 |
| Arabinose | 3563 | 3848 | 4158 | 4160 | 4257 | 4210 | 22.0 | 22.7 |
| Galactose | 2025 | 2893 | 5391 | 6024 | 6106 | 6159 | 32.2 | 30.1 |
| Glucuronylmannose | 398 | 737 | 996 | 2133 | 3573 | 3710 | — | — |
| Mannose | 59 | 98 | 160 | 376 | 784 | 1221 | 16.8† | 16.2 |
| Glucuronic acid‡ | 33 | 70 | 95 | 298 | 754 | 998 | 16.8 | 15.1 |
| Origin | 10806 | 8940 | 5936 | 3576 | 1326 | 500 | — | — |
| Total cpm | 19059 | 18786 | 18996 | 18857 | 19111 | 19112 | — | — |
| Theoretical total§ | 19035 | — | — | — | — | — | — | — |

5 mg $^{14}$C-labelled mucilage was hydrolysed in 1.0 ml 2 M TFA at 100°. Aliquots were removed at the above times and 15 μl applied as a 2.5 cm band to a TLC plate. Sugars were separated by two runs in solvent A. Following autoradiography, the layer was coated with cellulose acetate and the radioactive bands cut out and counted.

*Composition determined by GC of alditol acetates and the *m*-hydroxydiphenyl method for uronic acids.

†The total cpm for mannose and glucuronic acid were obtained by summing the cpm for glucuronosylmannose, mannose, glucuronic acid and the origin in the 6 hr sample and dividing by two.

‡The cpm for glucuronic acid are the sum of the glucuronic acid and glucuronolactone bands.

§The cpm contained in equivalent amount of original $^{14}$C-labelled mucilage.

negligible because the radioactivity contained in the mucilage was quantitatively recovered at each of the hydrolysis times. Also, early products of hydrolysis (fucose, arabinose) did not show a decrease in activity at later sampling times.

*Monosaccharide composition of the mucilages*

The time-course hydrolysis of the $^{14}C$-labelled mucilage showed that optimum conditions for the release of monosaccharides from the mucilages were 2 M TFA for 3 hr at 100° (Table 3). The product mixture was fractionated into neutral and acidic fractions by ion-exchange chromatography on QAE-Sephadex. The monosaccharides in the neutral fractions were identified by TLC and quantitated by GC as their alditol acetate derivatives (Tables 4 and 5). A portion of the acidic fraction when hydrolysed further with 50% TFA (6 hr, 100°) and examined by TLC revealed mannose and glucuronolactone as the major components from all mucilage samples. A result consistent with this finding was obtained when carboxyl reduced derivatives were prepared from the remainder of the acidic fraction and examined by hydrolysis-GC analysis. Thus, the acid fraction from all but the fruit mucilage contained only mannose and glucose in equimolar proportions, confirming that mannose and glucuronic acid were the components of the acidic fractions. With the fruit mucilage hydrolysate, a large quantity of galactose was found in the carboxyl-reduced acid fraction in addition to the equimolar amounts of mannose and glucose. However, the 50% TFA hydrolysate of the acidic fraction of the fruit mucilage revealed only a trace of galactose. Thus the galactose in the carboxyl-reduced acidic fraction is likely to have originated from galacturonic acid. It is almost certainly a contaminant as the fruit is known to contain pectin (0.6% dry wt) [Lodge N., personal communication] and no steps were taken to remove this during isolation of the fruit mucilage.

The mol % compositions and specific rotations of the pith, root and bark mucilages (Table 1) showed only small differences. However, since the BP and pith mucilages are in effect duplicate samples, and since they gave almost identical mol % compositions, the greater variation found among the compositions of the pith, root and bark mucilages is likely to be significant.

The same six monosaccharides were identified as components of all the mucilages extracted from the various plant parts of *A. chinensis* (Table 4). There were clear differences in the proportions of the component monosaccharides in the leaf, leaf stalk, fruit and fruit stalk mucilages. The mol ratios of fucose and arabinose varied between 1:2.7 (leaf) and 1:0.87 (fruit stalk). The glucuronic acid contents of these mucilages were all significantly higher than those found for the pith, root and bark mucilages; the maximum difference was between the leaf stalk and pith samples (20.1% and 11.0%, respectively).

Relatively low carbohydrate contents were found for the leaf stalk and fruit mucilages. That for the leaf stalk mucilage, confirmed by the phenol–sulphuric method [6],

Table 4. Monosaccharide composition of purified mucilages from *Actinidia chinensis*

| | Composition after hydrolysis (mol %) | | | | | | |
|---|---|---|---|---|---|---|---|
| Source | Fucose | Arabinose | Galactose | Xylose | Mannose | Glucuronic acid | Galacturonic acid |
| Root | 11.6 | 22.3 | 38.8 | 3.7 | 11.8 | 11.8 | — |
| Bark | 15.3 | 16.1 | 41.3 | 0.9 | 13.2 | 13.2 | — |
| Pith | 15.6 | 20.4 | 39.6 | 0.58 | 11.9 | 11.9 | — |
| Bulk pith | 15.8 | 20.3 | 41.5 | 0.34 | 11.0 | 11.0 | — |
| Leaf | 9.2 | 24.8 | 27.6 | 0.92 | 18.8 | 18.8 | — |
| Leaf stalk | 8.5 | 16.4 | 34.3 | 0.65 | 20.1 | 20.1 | — |
| Fruit stalk | 15.7 | 13.7 | 39.2 | 1.1 | 15.2 | 15.2 | — |
| Fruit | 4.9 | 10.0 | 31.3 | 2.3 | 14.3 | 14.3 | 22.8 |

Table 5. Monosaccharide composition of purified mucilages from leaves of nine *Actinidia* species

| | Composition after hydrolysis (mol %) | | | | | |
|---|---|---|---|---|---|---|
| Species | Fucose | Arabinose | Galactose | Xylose | Mannose | Glucuronic acid |
| *A. arguta* | — | 23.6 | 20.1 | — | 28.2 | 28.2 |
| *A. giraldii* | — | 28.3 | 15.4 | — | 28.1 | 28.1 |
| *A. melanandra* | — | 26.8 | 22.1 | — | 25.6 | 25.6 |
| *A. purpurea* | 1.3 | 26.8 | 21.2 | — | 25.4 | 25.4 |
| *A. chinensis* | 9.2 | 24.8 | 27.6 | 0.92 | 18.8 | 18.8 |
| *A. eriantha* | 10.7 | 20.2 | 25.9 | 5.5 | 18.8 | 18.8 |
| *A. rufa* | 10.6 | 20.9 | 34.9 | 2.0 | 15.8 | 15.8 |
| *A. callosa* | 13.7 | 24.1 | 31.2 | 4.7 | 13.2 | 13.2 |
| *A. polygama* | 12.5 | 2.7 | 32.0 | 13.7 | 19.5 | 19.5 |

implied the presence of an undetected constituent but this possibility was not investigated further. During hydrolysis of the fruit mucilage in 2 M TFA, a white precipitate was formed, a property not demonstrated by any of the other mucilages. This precipitate was separated from the hydrolysate. The phenol–sulphuric acid method showed it to contain carbohydrate but no further studies were made of this material.

*Grouping of* Actinidia *species*

The nine leaf mucilages separated clearly into three groups. Those from *A. arguta*, *A. giraldii*, *A. melanandra* and *A. purpurea* all exhibited positive specific rotations, had identical electrophoretic behaviour and contained arabinose, galactose, mannose and glucuronic acid as their primary constituents. The mol ratio of these four sugars was similar throughout the group. Their glucuronic acid contents were *ca* twice that of the *A. chinensis* mucilages.

The second group included the mucilages from *A. chinensis*, *A. eriantha*, *A. rufa* and *A. callosa*. The similarities within this group were not as marked but they all had negative specific rotations, three of them contained secondary components when electrophoresed, and all contained the same sugars as the *A. chinensis* polysaccharides.

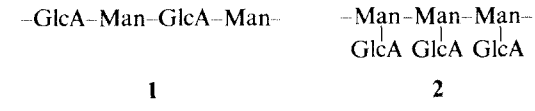
The third group contained only *A. polygama* which possessed the positive specific rotation of the first group and the sugar components of the second. However, it differed markedly from the second group by its unusually low arabinose (2.7%) and high xylose (13.7%) content.

The above groupings of the nine *Actinidia* species were compared with those of C. F. Liang's 1979 classification [Liang, C. F., personal communication]. *A. arguta*, *A. melanandra*, *A. purpurea*, *A. giraldii* and *A. polygama* were grouped in the Leiocarpae by Liang, although *A. purpurea* and *A. giraldii* were not considered as separate species, but as varieties of *A. arguta*. Therefore, with the exception of *A. polygama* the Leiocarpae correspond with group one of this study. Of the group 2 species, Liang lists *A. chinensis* and *A. eriantha* in the group Stellatae, *A. callosa* in the Maculatae, while *A. rufa* does not appear to be recognized as a valid entity in his classification. On the basis of its mucilage composition *A. rufa* would be placed in the Stellatae. Thus within the limited scope of this survey, the subdivision of the genus *Actinidia* in terms of mucilage composition seems a distinct possibility.

*Partial hydrolysis and mucilage structures*

Two degraded polysaccharides, A and B, were prepared from the BP mucilage (Table 6) by partial hydrolysis and their compositions determined.

Polysaccharide A was prepared under hydrolysis conditions that released most of the fucose, arabinose, galactose and xylose from the parent molecule and was separated from these products by dialysis in a membrane with a MW cut off of 50000. Polysaccharide A was subjected to carboxyl reduction by borohydride treatment of the carbodi-imide derivative, and then by hydrolysis-GC analysis was shown to contain equimolar proportions of mannose and glucose and 2.5% galactose (Table 6). These results, when considered in conjunction with the earlier findings from the $^{14}C$-labelled mucilage hydrolysis that: (a) glucuronosylmannose was a major component of the completely hydrolysed mucilage; and (b) the liberation of mannose from the mucilage was concomitant with the release of an equimolar amount of glucuronic acid, suggested that the mucilage contained an acid-resistant glucuronomannan core which consisted of a repeating disaccharide unit of glucuronosylmannose. Two tentative structures can be proposed for the core:




**1** **2**

Structure **1** has been reported as a backbone in several gums [7–10]. However, based on the results of this limited study, structure **2** is a possible alternative. It is hoped to resolve the question by methylation analysis of the core polymer.

The 2.5% galactose in polysaccharide A was believed to be a remnant of galactose side chains rather than an internal constituent of the glucuronomannan chain. This was based on the observation that the galactose concentration of polysaccharide A was further reduced to > 0.5% by prolonging the hydrolysis (2 M TFA, 2 hr), without disrupting the integrity of the high MW polymer.

All 16 mucilages were subjected to the same hydrolysis–dialysis procedure used to obtain polysaccharide A. All gave amounts of high MW glucuronomannan.

Polysaccharide B was prepared under conditions that removed nearly all the fucose, arabinose and 26% of the galactose from the original mucilage. It was separated from these products by dialysis. The resultant polysaccharide contained glucuronomannan and galactose in *ca* 1:1 ratio, and a small amount of xylose. Hence, 74% of the galactose content of the mucilage is linked directly to the glucuronomannan backbone. However, the remainder of the galactose was susceptible to oxalic acid hydrolysis,

Table 6. Monosaccharide components of polysaccharides obtained from partial hydrolyses of bulk pith mucilage

| | | | Composition after hydrolysis (mol %) | | | | | |
|---|---|---|---|---|---|---|---|---|
| Polysaccharide | Optical rotation ($[\alpha]_D^{25}$) | Wt (mg) | Fucose | Arabinose | Galactose | Xylose | Mannose | Glucuronic acid |
| Original, bulk pith | −33.6° | 200 | 15.8 | 20.3 | 41.5 | 0.34 | 11.0 | 11.0 |
| A* | − 1.9° | 22 | — | — | 2.5 | — | 48.7 | 48.7 |
| B† | +54° | 96 | 0.58 | 0.47 | 50.1 | 0.60 | 24.1 | 24.1 |

*Polysaccharide A after hydrolysis in 2 M TFA, 100°, 1.5 hr.

†Polysaccharide B after hydrolysis in 0.5% oxalic acid, 100°, 3 hr.

which suggested that it occurred in the outer regions of the side chains separated from the core linked galactose, or the core itself, by more labile arabinose or fucose units.

The time-course release of components during hydrolysis of the mucilage in 0.5% oxalic acid (100°) was closely monitored by TLC. The BP and $^{14}C$-labelled mucilages gave identical results. After 5 min, traces of fucose, arabinose and an unknown compound were detected. After 1 hr, the first trace of galactose appeared but the concentration of the unknown compound had decreased. This implied that it was undergoing hydrolysis. The immediate and simultaneous release of fucose and arabinose point to at least a proportion of these sugars occupying terminal positions on side chains of the molecule.

The unknown compound was isolated by prep. TLC after partial hydrolysis of the $^{14}C$-labelled mucilage. When subjected to mild hydrolysis it yielded arabinose only. When the glycitol was prepared and hydrolysed, similar amounts of radioactivity were obtained in arabitol and arabinose. These data indicated that the unknown was an arabinosylaraboside.

## EXPERIMENTAL

*General procedures.* Specific rotations were recorded at 589 nm with a Perkin-Elmer model 241 spectropolarimeter. All evaporations were conducted at 40° under red. pres. TLC used 20 × 20 cm plates coated with MN300 cellulose using the solvent systems: (A) PrOAc–$HCO_2H$–$H_2O$ (11:5:3); (B) *n*-BuOH–pyridine–$H_2O$ (2:2:1). Sugars were detected by *p*-anisidine–$KIO_4$ [11]. Radioactive compounds were detected by autoradiography and measured for radioactivity by the procedure of Redgwell *et al.* [12].

Analytical GC (FID) used a 2.0 m × 2 mm i.d. stainless steel column packed with SP 2330 on 100/120 Supelcoport. The temp. was maintained at 210°, carrier gas $N_2$, 30 ml/min. Sugars were separated as their alditol acetates prepared as described in the Supelco Bulletin 774 [13] with inositol as int. standard. $Ac_2O$ was removed azeotropically with toluene and $Me_2CO$ solns of the acetates injected into the gas chromatograph.

Ca, Mg and K were determined by atomic absorption after $HClO_4$ digestion. Protein N was determined by the Kjeldahl method.

Uronic acid contents of the native mucilages were determined using the *m*-hydroxydiphenyl reagent [14] with glucuronic acid as a standard. The difference in *A* for equimolar amounts of glucuronic acid and galacturonic acid was very slight and ignored when determining the uronic acid content of the fruit mucilage. The amount of mannose in each mucilage was assumed to be the same as its glucuronic acid content, since the carboxyl-reduced acidic fractions for all mucilages gave a molar ratio of 1:1 for glucose and mannose.

*Plant material.* Leaves, leaf stalks, fruit, fruit stalks, roots and stems were harvested during March 1981 from a single *A. chinensis* plant (cv Hayward) growing at the DSIR Research Orchard, Kumeu. The leaves of eight other *Actinidia* species were collected during May 1981 from the DSIR Research Orchard, Te Puke. Plant parts were immersed in liquid $N_2$ on the day of harvesting, freeze-dried and ground in a ring grinder. Bark (consisting of the bark and phloem tissues) could be easily peeled from the wood after freeze-drying the stems. Bark and wood (the latter henceforth referred to as the pith sample) were then ground separately.

*Extraction of plant part mucilages.* Plant powder (*ca* 12 g) was immersed in boiling 80% EtOH (200 ml) for 5 min, then centrifuged. The supernatant was discarded, and the residue was then sequentially extracted with 50 ml aliquots of the following solvents: 80% EtOH (× 2), MeOH–$CHCl_3$–$H_2O$ (12:5:3) (× 6) [15] and again with 80% EtOH (× 2). The residue was suspended in $H_2O$ (200 ml), stirred for 2 hr at ambient temp. and centrifuged at 13 000 *g* for 20 min. The supernatant containing the mucilage was decanted and the residue re-extracted in $H_2O$ (100 ml) for 2 hr. The combined supernatants were dialysed, firstly for 16 hr at ambient temp. with α-amylase (0.5 ml of a 1 mg/ml soln in $H_2O$ of Rohalase (M. Rohm Products)) and toluene as a biostat, then for 72 hr at 4°, with frequent changes of $H_2O$. The resultant mucilage solns, which all gave a negative $I_2$–KI reaction for starch, were concd then freeze-dried. The crude mucilages were stored desiccated at 4° (see Tables 1 and 2 for yields).

*Extraction of BP mucilage.* The BP mucilage was isolated from 1-year-old canes of *A. chinensis* (4 kg wet wt), harvested in Sept. 1980 from the DSIR Research Orchard, Te Puke. Fresh stems were split longitudinally then the mucilage and septa scraped from the pith cavity into cold $H_2O$, stirred overnight at 4° and then filtered through Miracloth to remove the septa. The viscous filtrate (negative $I_2$–KI test) was concd and freeze-dried, yielding crude BP mucilage (12 g).

*Purification of plant part mucilages.* Although EtOH was an effective pptant for the BP mucilage it gave poor yields for the smaller quantities of the plant part mucilages. Of the solvents tested, *t*-BuOH was the most efficient for this purpose. Crude mucilage (0.2 g) was dissolved in $H_2O$ (40 ml) and ppted with 2 vols. *t*-BuOH. The supernatant was concd to 33% vol. and a second crop of ppt obtained by the addition of a further 2 vols. *t*-BuOH. The ppts were combined, washed in 50% *t*-BuOH, dissolved in 20 ml $H_2O$ and the total procedure repeated. The resulting ppt was dissolved in $H_2O$, filtered through a pad of prewashed Polyclar AT on glass fibre paper, and clarified by millipore filtration (3 μm followed by 1 μm pore size). The filtrates were freeze dried to give white to cream coloured polysaccharides. It was important to freeze-dry the mucilage filtrates without concentrating them, otherwise mucilages were obtained which dissolved to give cloudy solns. Yields ranged between 70 mg (*A. giraldii*) and 120 mg (*A. chinensis*) pith.

*Purification of BP mucilage.* The crude mucilage (4 g) in $H_2O$ (600 ml) was dialysed against $H_2O$ for 72 hr at 4°, then centrifuged (20 000 *g* 1 hr), and the supernatant decanted into 4 vols. EtOH at 4°. The ppted mucilage was washed with 50% EtOH, dissolved in $H_2O$ and the pptn repeated. The final ppt was dissolved in $H_2O$ and clarified by millipore filtration. The filtrate was frozen in liquid $N_2$, ground in a mortar and pestle and freeze-dried to give a snow-white, fluffy powder (1.4 g).

*Preparation of $^{14}C$-labelled mucilage.* Labelling was carried out *in vivo* using an *A. chinensis* plant (cv Hayward) with branchlets of young developing leaves. Two branchlets were each enclosed in a plastic bag which carried a septum-sealed vial, containing 2.5 mCi $NaH^{14}CO_3$, to which 1.0 ml 30% lactic acid had been added. The bag was sealed around the stem with tape and the $^{14}CO_2$ released by unscrewing the cap through the bag. 1 hr after $^{14}CO_2$ release the bag was removed. A week later a second pulse of $^{14}CO_2$ was administered to the same branchlets in an identical manner, then after 5 days all leaves were stripped from the stem (52 g fr. wt), killed in boiling 80% EtOH, macerated in a Virtis homogenizer and the $^{14}C$-labelled mucilage extracted and purified as described for the plant part mucilages, yielding 0.13 g, with activity 210 000 dpm/mg.

*Ion exchange chromatography.* Columns (7 × 1.5 cm) of DEAE-Sephadex were prepared in the $HCO_3^-$ form. A soln of each mucilage (10 mg/50 ml) was loaded on to the column and the column washed with $H_2O$. The effluent was tested for carbohydrate by the PhOH–$H_2SO_4$ method. The mucilages were ex-

changed off the Sephadex by elution with 50 ml 0.5 M $KHCO_3$ and were then dialysed and freeze-dried.

*Zone electrophoresis.* This used cellulose acetate membranes in the Beckman R-101 microzone electrophoresis cell. Mucilages (0.25 $\mu$l of a 1 mg/ml soln in 0.1 M NaCl) were electrophoresed for 1 hr (1 mA/cm) in either 0.05 M Pi buffer pH 7.2, or 0.2 M $ZnSO_4$, pH 5:1. The membrane was stained for 15 min in 1% Alcian blue-8-GX in EtOH–acetate (1:1) buffer, pH 5.8. Destaining was carried out in an aq. soln of 5% HOAc and 10% EtOH, until the background was white [16].

*Ultracentrifugation.* This was carried out on a Beckman model E ultracentrifuge at 44 000 rpm. A soln of BP mucilage in 0.1 M NaCl (0.5 mg/ml) was used.

*Hydrolysis.* Purified mucilage (*ca* 5 mg), dissolved in a vial overnight in 0.5 ml $H_2O$. TFA (0.5 ml, 4 M) was added, the vials sealed with Teflon lined screw caps, and hydrolyses carried out in a temp. block at 100° for 3 hr. The TFA was removed by co-distillation with $H_2O$ and the hydrolysate added to a column of QAE-Sephadex (5 ml bed vol. $HCOO^-$ form). The neutral sugars were recovered by washing with $H_2O$ and the acidic fraction subsequently eluted with 30 ml 5% $HCO_2H$. The neutral fraction was concd to dryness, dissolved in 1.0 ml 10% *iso*-PrOH and analysed by TLC and GC.

The dried acidic fraction was dissolved in 1.0 ml $H_2O$ and 0.5 ml aliquots dispensed into separate vials. To one vial was added 0.5 ml conc. TFA which was heated for 6 hr at 100° and the hydrolysate examined by TLC. The contents of the other vial were freeze-dried and methylated with methanolic-HCl (1.0 ml of 2.5%) at 40° for 16 hr. The MeOH was evaporated and the HCl removed under red. pres. over KOH pellets. The methyl esters were reduced with $NaBH_4$, hydrolysed in 2 M TFA (4 hr 100°) and the products analysed by GC.

*Isolation of polysaccharide A.* BP mucilage (200 mg), dissolved overnight in $H_2O$ (20 ml), was maintained under reflux with TFA (20 ml, 4 M) for 90 min in a boiling water bath. The hydrolysate was cooled to 4° and dialysed for 72 hr in a Spectrapor membrane with a MW cut-off of 50 000 (Spectrum Medical Industries). The retentate was concd, filtered (0.45 $\mu$millipore) and freeze-dried, yielding 22 mg.

Polysaccharide A was reduced using the carbodi-imide technique of Taylor *et al.* [17]. After a second reduction the polysaccharide gave a negative response to the *m*-hydroxydiphenyl test for uronic acids. The reduced polysaccharide was hydrolysed in 2 M TFA (4 hr 100°) and analysed by GC.

*Isolation of polysaccharide B.* BP mucilage (200 mg), was dissolved overnight in $H_2O$ (20 ml) then maintained under reflux with oxalic acid (20 ml 1%) for 3 hr in a boiling water bath. The hydrolysate was cooled to 4° and dialysed for 72 hr in a membrane with a MW cut off of 12 000–14 000 (Union Carbide). The retentate was concd, filtered and freeze-dried, yielding 98 mg.

Polysaccharide B was hydrolysed in 2 M TFA (2 hr, 100°) and the neutral sugars quantitated by GC. The glucuronic acid (and by inference the mannose) content of polymer B was determined using the *m*-hydroxydiphenyl reagent.

The dialysate from the preparation of B was concd and adjusted to pH 5.5 with $Ba(OH)_2$ soln. The Ba oxalate ppt was removed by centrifugation and washed twice with $H_2O$. Supernatants were passed through a column of SP Sephadex ($H^+$) to remove $Ba^{2+}$ and freeze-dried to yield *ca* 120 mg of a deliquescent mixture of sugars. An amount (5 mg) was hydrolysed in 2 M TFA (1 hr, 100°) and the monosaccharides of the hydrolysate and the original dialysate quantitated by GC.

*Identification of glucuronosylmannose.* $^{14}C$-labelled mucilage (5 mg) was hydrolysed in 1 ml 2 M TFA (6 hr, 100°). The hydrolysate was spotted across two TLC plates and run twice in solvent A. The aldobiuronic acid was located by autoradiography ($R_{fuc}$ 0.45), scraped from the plate and suspended in 5 ml $H_2O$ for 30 min with vigorous shaking. The suspension was filtered (0.45 millipore) and the filtrate eluted on to a QAE-Sephadex column (1 × 5 cm $HCOO^-$ form) which was washed with 50 ml $H_2O$. The aldobiuronic acid was recovered in 40 ml 5% $HCO_2H$, concd to dryness and dissolved in 1 ml $H_2O$. A portion of this soln was subjected to a sequence of $NaBH_4$ reduction; esterification (2.5% methanolic HCl) and $NaBH_4$ reduction; the latter two steps were repeated and then the product was hydrolysed in 2 M TFA (4 hr, 100°). The hydrolysate was examined by GC of the acetylated sugars and the acetylated alditols.

*Identification of arabinosylaraboside.* $^{14}C$-labelled mucilage (5 mg) was hydrolysed in 0.5% oxalic acid (1 hr, 100°). The hydrolysate was spotted across two TLC plates and run once in solvent B. The unknown was located by autoradiography ($R_{fuc}$ 0.82) and travelled in partial contact with arabinose but clear of galactose. It was extracted from the layer as described for the aldobiuronic acid isolation. The filtrate was concd and re-spotted across a single TLC plate and run in solvent A (twice) to remove the contaminating arabinose. In this solvent the unknown had the same mobility as galactose ($R_{fuc}$ 0.82). It was extracted from the layer and the chromatographic process repeated in solvent B to ensure complete removal of galactose. An aliquot of the unknown was subjected to $NaBH_4$ reduction, and both the original unknown and the reduced form were hydrolysed in 0.5 M TFA (2 hr, 100°) and examined by TLC in solvent A. Autoradiography and *p*-anisidine–$KIO_4$ spray revealed that the unknown contained only arabinose and the reduced compound gave equal radioactivity in arabinose and arabitol, demonstrating that it was a disaccharide.

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