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PARTIAL STRUCTURE OF A POLYSACCHARIDE FROM LEAVES OF *WELWITSCHIA MIRABILIS*

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Abstract—Gum-tears from the leaves of *Welwitschia mirabilis* contain a polysaccharide composed of arabinose, galactose and glucuronic acid as main constituents with xylose, fucose and rhamnose in smaller quantities. Periodate oxidation and permethylation studies indicated that the gum could consist of a framework of glucuronic acid residues linked 1 → 4 and galactose residues linked 1 → 6 and of short chains of arabinose, xylose, fucose and rhamnose linked 1 → 3 to both residues. All rhamnose and fucose and part of arabinose were found as non-reducing terminal units.

INTRODUCTION

Welwitschia mirabilis, a desert-plant with naked seeds, seems to be a surviving relic from the mesozoic period. The taxonomic relationship of this isolated plant within the plant kingdom is not clear, although there have been many interpretations. At the present time this plant is placed taxonomically as a gymnosperm within the Cycadophytina although several characteristics of this curious plant point to phylogenetic connections with the angiosperms. As a gymnosperm *Welwitschia* is normally placed with *Gnetum* and *Ephedra* in the class Gnetatae within the Cycadophytina. However Markgraf [1] supposed a relationship with the Cycadaceae since plants of this family have similar slime channels to those of *W. mirabilis*. Stephen [2, 3] too suggested a connection between the polysaccharide exudate found on the stems of *W. mirabilis* and that from *Eucephalartos longifolius* [4].

RESULTS AND DISCUSSION

The ethanol precipitated gum *W. mirabilis* having $[\alpha]_D^{20} = -39.5^0$ (H_2O, *c* 3.9) was analyzed for uronic acids (32%). Following total acid hydrolysis, the only uronic acid detected by TLC was glucuronic acid [R_{ara} 0.46 solvent (a)]. The sugars of the hydrolyzed gum were analyzed by GLC as alditol acetates (column 1 and 2) and accounted (as anhydro sugars) for 92% of the gum (Table 1). The main constituents were arabinose, glucuronic acid and galactose, with lesser quantities of rhamnose, fucose and and xylose. Essentially the same results were obtained when the gum was further purified by precipitation with cetyltrimethylammonium bromide [5]. Partial acid hydrolysis of the purified polysaccharide was followed by GLC (column 2). After 1 hr only arabinose and a trace of xylose were released. After 2 hr the other neutral sugars appeared gradually, indicating that arabinose and possibly xylose were linked in the furanose form.

The gum was degraded by periodate oxidation. Following reduction by $NaBH_4$ and acid hydrolysis of the polyol, the neutral sugars were identified by GLC (column 2) in the following molar proportion: glycerol (1), arabinose (0.3), galactose (0.3). Traces of erythritol, threitol and mannose could be detected while rhamnose, fucose and xylose were absent.

Attempts to methylate the gum were unsuccessful and it was therefore reduced with borohydride after esterification. The reduced gum was analyzed for residual uronic acid (6%) but the uronic acid content could not be further decreased by repeating the procedure. The reduced gum was methylated twice and *O*-Me sugars were identified by GC–MS as alditol acetates (Table 1). The results were similar when the gum was remethylated. When the esterified polysaccharide was reduced with sodium borodeuteride and methylated twice, only *O*-Me glucoses were deuterated thus confirming the presence of glucuronic acid in the gum. All rhamnose and fucose and part of arabinose were found as non-reducing terminal end groups (30 mole). The presence of 2,3-,2,5-di-*O*-Me-arabinose, 2,3- (or 3,4-) and 3,5-di-*O*-Me-xylose (24 mole) suggested that short chains of arabinose and xylose are involved. Since 3,5-di-*O*-Me-xylose was detected (1 mole), it is possible that all xylose is in the furanose form as arabinose thus ruling out the presence of 3,4-di-*O*-Me-xylose in favour of 2,3-di-*O*-Me-xylose (8 mole). Xylose residues were not found in the terminal position as part of arabinose. Therefore it would be expected that xylofuranosyl units would be hydrolyzed more slowly than arabinose as found in the partial acid hydrolysis studies.

The presence of 2,3,4-tri-*O*-Me-galactose, 2,3,6-tri-*O*-Me-glucose and of the branched points 2,4-di-*O*-Me-galactose and 2-*O*-Me-glucose indicated that the polysaccharide had a framework of galactose residues linked 1 → 6 and glucuronic acid residues linked 1 → 4 with occasional side chains attached 1 → 3 on both sugar units. The lack of correlation between the proportion of terminal (30 mole) and branched residues (19 mole) is not

Table 1. Molar proportion of sugars of *W. mirabilis* polysaccharide

	Non-methylated	Methylated
Rhamnose	5	4
Fucose	6	8
Arabinose	44	35
Xylose	6	9
Mannose	- -	5
Galactose	14	16
Glucose	—	23
Glucuronic acid	25	—

unusual (see for example [6]) and could be partly explained by the presence of uronic acid residues in the reduced polysaccharide (6%) which could be branched but do not appear in the methylation data.

The proportion of individual sugars obtained by methylation analysis is given in Table 2. This confirmed the results obtained by acid hydrolysis with the exception of arabinose whose proportion is lower. The appearance of a small amount of mannose linked 1 → 6 is attributed to the fact that it could be adjacent to uronic acid residues and thus resistant to acid hydrolysis. The methylation data confirmed the results obtained by periodate oxidation of the polysaccharide: rhamnose, fucose and most of xylose should be oxidized while part of the arabinose and galactose should be resistant to periodate. The molar proportion of glycerol, galactose and arabinose was calculated from the methylation data to be 1, 0.3 and 0.2, respectively (found 1, 0.3, 0.3). Again the calculated proportion of arabinose was lower, which was attributed to a loss of terminal arabinofuranose units during the methylation procedure.

Up to now an unsufficient number of gum exudates have been examined carefully enough [7, 8] to enable any chemotaxononic implications to be made especially among higher taxa. Aspinall [9] bases his chemical classification of gums on the type of basic chain of the polysaccharide, so that he can relate them to other groups of plant polysaccharides, e.g. pectins (the galacturorhamnan group of gums), hemicelluloses (the xylan group of gums) and with the galactans and arabinogalactans of the coniferous woods (the galactan group of gums). Following this principle of classification it is evident, that the galactan group of gums, in which the gum exudate from the leaves of *W. mirabilis* can be placed, occurs only in families belonging to the subclass Rosidae of the Magnoliatae (= Dicotyledoneae) in the subdivision Magnoliophytina (= Angiospermae) and those of the Mimosaceae, Apiaceae, Meliaceae and Rutaceae. There is one exception, however, where this group occurs in one *Araucaria* species, that is in the subclass Pinidae (= Coniferae) of the class Pinatae in the subdivision Coniferophytina (= gymnospermae). In addition arabinogalactans do not occur in the wood of this species (*A. bidwilli*). A similar situation is found in the glucuronomannan group of gums where they occur in families in the subclass Rosidae, and those of the Rosaceae and Combretaceae but again with one exception; *Eucephalartos* of the class Cycadatae in the subdivision Cycadophytina (= Gymnospermae) [2].

Genealogical phylogeny is more difficult than characteristic phylogeny but the present results can best be interpreted in terms of the latter, that is there is a progression from the more simply constructed galactans and arabinogalactans of the conifers to the more complicated galactans of the angiosperm gum exudates. The investigations on *Araucaria bidwillii* show a vicarious appearance of both classes, and from this finding arises the question of whether other taxonomic characters also indicate that this is a comparatively derived taxon. In the case of *Welwitschia* the question can be answered in the affirmative. It would be worth checking to ascertain whether simple galactans or arabinogalactans are also present in the xylem of this species. The merely sporadic occurrence of this galactan type of natural product appears improbable with substances of such complicated structure. Bearing in mind the chemical relationship of gums and slimes to pectins and hemicelluloses, the phenomenon might be explained more plausibly by assuming a high degree of constancy in chemical constitution over broad phytogenetic spans.

This would then be analogousto what is found to a much more marked extent, in the histones which exhibit an astonishing similarity in widely separated taxa.

EXPERIMENTAL

Gum-tears from leaves of *W. mirabilis* (Botanical Garden Berlin-Dahlem, June 4. 1974 leg. Th. ECKARDT) were used.

Table 2. Methylation data and relative proportions of *O*-methyl sugars present in methylated reduced polysaccharide of *W. mirabilis*

O-Me sugars identified	Molar proportion	Relative retention time*			
		Column 1		Column 2	
		found	lit. [5]	found	lit. [5]
2,3,4-tri-*O*-Me-rhamnose	4†	0.55	0.46	0.41	0.41
2,3,4-tri-*O*-Me-fucose	8	0.7	0.65	0.53	0.58
2,3,5-tri-*O*-Me-arabinose	18†	0.55	0.48	0.41	—
2,3-di-*O*-Me-arabinose	7	1.5	—	1	1.07
2,5-di-*O*-Me-arabinose	8	1.15	1.1	0.8	—
2,3- or 3,4-di-*O*-Me-xylose	8	1.65	1.54	1.1	1.19
3,5-di-*O*-Me-xylose	1	1	1.08	0.71	—
2,3,4-tri-*O*-Me-mannose	5	2.25	2.49	2.05	2.19
2,4-di-*O*-Me-galactose	12	6.1	6.35	5.1	5.1
2,3,4-tri-*O*-Me-galactose	5	3.3	3.41	2.89	2.9
2-*O*-Me-glucose	7	7.6	7.9	6.1	6.6
2,3,6-tri-*O*-Me-glucose	17	2.5	2.5	2.32	2.32

* Relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-Me-glucitol.

† Estimated from the molar proportion of rhamnose in the polysaccharide.

TLC was carried out on Si gel in (a) *n*-BuOH–Me_2CO–HOAc–C_5H_5N–H_2O (2:3:2:2:1). Sugars were analyzed by GLC as alditol acetates on columns of (1) 3% ECNSS-M and (2) 3% OV-255 on Gas Chrom Q [10]. The identity of all *O*-Me sugars was confirmed by GC–MS (column 2) [11]. Uronic acids were measured by the carbazole tests [12] using glucuronolactone as a standard.

Purification of gum. Gum-tears were dissolved in H_2O and precipitated twice with EtOH containing 1% HCl. The ppt. was washed with EtOH, dissolved in H_2O and freeze-dried. The polysaccharide was further purified by precipitation with cetyltrimethylammonium bromide [5] with a yield of 50%.

Periodate oxidation of the polysaccharide (10 mg) was carried out in 0.05 M $NaIO_4$ (5 ml) at 4° in the dark and the periodate consumption was measured by spectrophotometry [13]. The oxidation was complete after 48 hr.

Hydrolysis. The optimum conditions for acid hydrolysis of the polysaccharide were with 0.5 M H_2SO_4 at 100° for 7 hr. The internal standard was *meso*-inositol. Partial acid hydrolysis of the polysaccharide (30 mg) was performed in 3 ml H_2O and 3 ml Dowex 50 (H^+) at 100°.

Methylation. Prior to methylation, the polysaccharide was deionized with Dowex 50 (H^+) and freeze-dried. The material (30 mg) was esterified with CH_2N_2 and reduced with $NaBH_4$ [14]. Methylation was carried out by the procedure of Hakamori as described in ref. [15]. The methylated polysaccharide was hydrolyzed by the HCO_2H–H_2SO_4 procedure [16].

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