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The gum exudate of *Encephalartos longifolius* Lehm. (female): further hydrolytic studies

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ABSTRACT

Sequential, acid hydrolysis of the gum exudate from *Encephalartos longifolius* cones gave the neutral disaccharides β-Gal-(1 → 3)-Ara (**1**), β-Gal-(1 → 3)-Gal, and β-Gal-(1 → 6)-Gal; the triouronic acid β-GlcA-(1 → 6)-β-Gal-(1 → 3)-Ara (**2**); and the biouronic acids described earlier, namely, β-GlcA-(1 → 6)-Gal (**3**), β-4-*O*-MeGlcA-(1 → 6)-Gal, and β-GlcA-(1 → 2)-Man (**4**). Oligomers up to the tetramer of β-GlcA-(1 → 2)-Man α-linked through O-4 of GlcA characterised the inner, core region of the complex acidic polysaccharide. Alternating GlcA and Man residues were indicated by FABMS of methylated, acid-degraded gum. The presence of the constituent sugar units was confirmed by methylation of the gum and partition chromatography of the products of acid hydrolysis. Partial hydrolysis gave fractions containing terminal and in-chain GlcA attached to Gal and Man. Base-catalysed degradation of the methylated products showed Rha to be exterior to GlcA in the periphery.

INTRODUCTION

Of the highly complex Cycad gums, the exudate from bark and cones of *Encephalartos longifolius* has hitherto been studied in greatest detail[1,2]. Determination of the constituent sugars and uronic acid, assay by GLC of the methanolysis products from the methylated gum, and analysis of the acidic products of partial hydrolysis of the polysaccharides showed that units of β-GlcA-(1 → 2)-Man (**4**) constituted the core, and that ramified assemblies of galactose, rhamnose, 3-*O*-methylrhamnose, arabinose and glucuronic acid were attached thereto. Further investigation of the gum has established detail concerning peripheral sugar and uronic acid residues, and verified the postulate that alternating sequences of GlcA and Man are the framework upon which the acidic, substituted arabinogalactan moieties are built.

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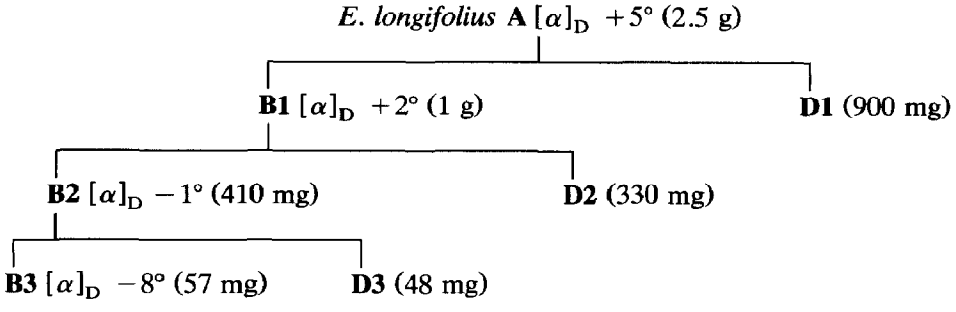
EXPERIMENTAL

General methods.—Solvent systems used in paper chromatography were *A*, 2:1:1 1-butanol–acetic acid–water; *B*, 18:3:1:4 EtOAc–acetic acid–formic acid–water; *C*, 4:1:5 upper-phase 1-butanol–EtOH–water; *D*, 8:2:1 EtOAc–pyridine–water; *E*, 10:4:3 EtOAc–pyridine–water; *F*, butanone–water azeotrope; *G*, 169:47:15 upper-phase benzene–EtOH–water. Sugars and their methyl ethers were detected in the usual way[3]. Conditions for assaying sugars and uronic acids, molecular weight measurements, methylation analysis procedures, and NMR determinations have been described[3,4].

Partial acid hydrolysis.—The gum exudate[1] from *E. longifolius* (16 g) was heated in a solution (500 mL), brought to pH 2 by addition of dil H_2SO_4, on a boiling water bath for 13 h, and the hydrolysate was neutralised ($BaCO_3$) and centrifuged. The supernatant solution was concentrated and poured into EtOH (4 vol), and the precipitated, autohydrolysed gum **A** (12 g) was washed with EtOH and Me_2CO, and dried in air. This product was treated further to obtain three degraded, polymeric products **B1–B3** by heating in 0.25 M H_2SO_4 on a boiling water bath for 1, 0.75, and 2.2 h as outlined in Scheme 1. Spectra/Por dialysis tubing with a nominal cut-off of ~ 3500 was used to remove the low molecular weight products of hydrolysis. Molecular weight profiles for **B2** and **B3** were obtained on Bio-Gel P-10, and sugar and uronic acid compositions were found for **B1–B3**. Methylation analysis furnished modes of linkage of the units in **B1–B3**, and dimsyl-catalysed base-degradation[5] indicated the sugars contiguous to GlcA in **B2** and **B3**. Further, partial acid hydrolysis (M CF_3CO_2H, 100°C, 1.5 h) of **B3** and paper chromatography (solvents *A* and *B*) gave products which were compared with authentic standards containing alternating GlcA and Man units. For FABMS analysis, methylated **B3** was separated into fractions 1 (24 mg) and 2 (11 mg), on Merckogel 2000 (2:1 EtOH–$CHCl_3$ as eluent). These fractions were analysed as the methyl ester methyl glycosides[6] by GLC. Dialysates **D1–D3** were examined by PC (solvents *A*, *B*, and *D*); two-dimensional use of solvents *A* and *D* differentiated the neutral and acidic components. Comparisons were made with authentic standard mono-



Scheme 1. Stages of depolymerisation of **A**, indicating yields of polysaccharides **B1–B3** and of dialysates **D1–D3**.

and oligo-saccharides, and with partial acid hydrolysates (0.25 M H_2SO_4, 100°C, for 2, 4, and 8 h) of an acidic oligosaccharide mixture, comprising two-thirds of the *E. longifolius* exudate, reported earlier[1]. Dialysate **D1**, as Ba^{2+} salts, was separated into EtOH-soluble (neutral sugars) and EtOH-insoluble (Ba^{2+} salts) fractions. The neutral components were eluted from a charcoal–Celite column with increasing concentrations of EtOH in water, and the acidic components from a cellulose column with decreasing concentrations of BuOH in BuOH–HOAc–H_2O mixtures (commencing at 3:1:1)[7].

Methylation analysis.—Fully methylated *E. longifolius* gum was analysed by GLC of the derived alditol acetates obtained after hydrolysis, and by methanolysis and GLC[8]. Base-catalysed degradation using potassium dimsyl (potassium methylsulphinylmethanide) in Me_2SO[5] was applied to the methylated gum.

Methylated *E. longifolius* gum (3.8 g) was solubilised in hot HCO_2H during 2 h, water was added, and the bulk of the volatile acid was removed in vacuo. The residual syrup was heated with 0.5 M H_2SO_4 on a boiling-water bath for 12 h, and the neutralised ($BaCO_3$) hydrolysate was concentrated to a syrup. Sugar methyl ethers were fractionated on a cellulose column at 30°C, using a gradient of light petroleum (bp 120°C) and 1-butanol saturated with water in the proportions 7:3 to 1:3, followed by half-saturated aq 1-butanol, 1-butanol–EtOH–water, and EtOH–water. Samples of fractions were monitored by PC (solvents *C*, *F*, and *G*) and combined appropriately[9]. The acidic products, which followed 4-*O*-methylgalactose on elution, were analysed as Ba salts by methanolysis–GLC; a mannose-containing oligomer, eluted late from the cellulose column, was submitted to detailed structural examination.

RESULTS AND DISCUSSION

The sample of *E. longifolius* gum used for partial acid hydrolysis was essentially the same in composition as that used in earlier studies[1,2], namely, for the neutral sugars (in mol), 3-Rha, 2; Rha, 16; Fuc (trace); Ara, 10; Xyl, 2; Man, 8; Gal, 31; uronic acid, mainly GlcA, 30%. The purpose was to cleave peripheral sugar units in stages, to identify the mono- and oligo-saccharides released, and to isolate the acidic mannose-containing core with minimum loss of Man and the highest practicable removal of the complex array of sugar and uronic acid units attached to the core. The successive dialysates (Scheme 1) indicated that, after preliminary removal of the bulk of Ara*f* in the production of the "autohydrolysis" product **A**, the remaining Ara*f*, Rha*p*, Xyl*p*, 3-Rha*p*, and Gal*p* were present in **D1** and **D2**, while, among the monosaccharides, Gal*p* and 3-Rha*p* alone constituted the bulk of **D3**; (1 → 3)- and (1 → 6)-linked galactobioses were present, the latter in traces, together with diminishing amounts of β-D-Gal*p*-(1 → 3)-L-Ara (**1**; identification described below). β-D-Glc*p*A-(1 → 6)-D-Gal (**3**) was preponderant among the acidic components from **D1** to **D3**, while β-D-Glc*p*A-(1 → 6)-β-D-Gal*p*-(1 → 3)-L-Ara (**2**; identification below) was prominent in **D1** but less so in **D3**. The dialysates

each contained a series of other acidic components chromatographically similar to those of hydrolysates reported earlier[1].

Fractionation of the neutral components of **D1** on charcoal–Celite yielded, after elution of Gal, Ara, Xyl, and Rha with water, 3-*O*-methyl-L-rhamnose (262 mg), mp 110–114°C, $[\alpha]_D$ +34° (*c* 3.7, H_2O); the sugar crystallised spontaneously on evaporation of the eluate obtained by using 3% ethanol in water. Assignment of the ^1H and ^{13}C chemical shifts for the NMR spectra of a solution in D_2O established the position of the MeO substituent.

Eluted from charcoal–Celite with 5% ethanol in water, a disaccharide (40 mg), $[\alpha]_D$ +42° (H_2O), that stained pink with *p*-anisidine hydrochloride after PC and had mobility in solvents *A*, *C*, and *D* slightly different from that of authentic α-Gal-(1 → 3)-Ara, was shown by acid hydrolysis to consist of 1:1 Gal and Ara (GLC of alditol acetates). Reduction of the disaccharide followed by hydrolysis gave Gal only. Assignments made for the chemical shifts of ^1H and ^{13}C resonances for a solution in D_2O established the β-D-Gal and 3-*O*-glycosylated L-Ara (α and β pyranosyl) moieties. A detailed interpretation of the NMR parameters and a comparison with those of α-Gal*p*-(1 → 3)-Ara[10] and of β-Gal*p*-(1 → 3)-Gal, which co-eluted from the charcoal–Celite column in an earlier fraction, have been published elsewhere[11].

Of the acidic oligosaccharide components of **D1–D3**, one, which was not apparent in earlier hydrolysis experiments[1], was eluted from the cellulose column immediately following the major biouronic acid β-Glc*p*A-(1 → 6)-Gal (R_{Gal} 0.44, solvent *A*). The oligosaccharide (R_{Gal} 0.33) on partial acid hydrolysis and PC (solvents *A* and *D*) showed unchanged material, staining pink with *p*-anisidine hydrochloride (+ + + +), **3** (+ + +), **1** (+), Gal (+), and Ara (+ + + +). On further hydrolysis (2 M CF_3CO_2H, 4 h, 100°C) some **3** remained and the only other components visible were GlcA, Gal, and Ara. With borohydride treatment prior to hydrolysis under similar conditions, the result was the same but with a lesser response of Ara. Analysis of the anomeric region of the ^1H NMR spectrum[3] for the oligosaccharide indicated the presence of terminal (T) β-linked GlcA (δ 4.52, $J_{1,2}$ 7.6 Hz), in-chain β-linked Gal (δ 4.60, $J_{1,2}$ 7.6), and α,β-Ara*p* (δ 4.56, $J_{1,2}$ 7.4; δ 5.24, $J_{1,2}$ 2.6). Reduction ($NaBD_4$), methylation, hydrolysis, and PC (solvent *C*) indicated the presence of T-GlcA, → 6)-Gal, and → 3)-Ara*p*. GLC and GLC–MS of the derived partially methylated alditol acetates of borodeuteride-reduced oligosaccharide confirmed the presence of T-GlcA (+ + +), → 6)-Gal (+ + +), → 3)-Ara-ol (+ +), and some → 3)-Ara (+). From these results, it is apparent that the ratios of Ara (total) to Gal to GlcA are 1:1:1, and that the structure is β-Glc*p*A-(1 → 6)-β-Gal*p*-(1 → 3)-Ara (**2**).

Five further acidic oligosaccharides, each homogeneous in PC (solvent *A*), were eluted from the cellulose column, and the first three of these (R_{Gal} 0.25, 0.19, and 0.1), on partial acid hydrolysis, each afforded **3**, Gal, GlcA, and Ara. The fourth (R_{Gal} < 0.05) yielded mainly **3** and Gal, and the last (eluted with aqueous ethanol; immobile in PC) a complex mixture including **3**, Gal, **4**, and Xyl. At this point, the

TABLE I

Analytical data for partial-hydrolysis products **B1** to **B3** from *E. longifolius* gum

	B1	**B2**	**B3**
M_w [a] $\times 10^3$		6	2.5
Uronic acid (mol%)	42	44	47
Neutral sugars (mol%) [b]			
3-Rha	4	2	
Ara	2	1	
Xyl	< 1		
Gal	21	8	4
Man	31	44	49
Modes of linkage [c] *(mol%)*			
T-Rha*p*	4	2	
T-Ara*p* [d]	2	1	
T-Gal*p*	2	tr	
→ 3)-Gal*p*	3	tr	
→ 6)-Gal*p*	9	4	
→ 3,6)-Gal*p*	5	4	
→ 2)-Man*p*	10	19	30
→ 2,3)-Man*p*	9	3	1
→ 2,4)-Man	8	13	12
→ 2,3,4)-Man	4	9	tr
T-Glc*p*A [e]	19	15	21
→ 4)-GlcA [e]	22	29	35

[a] From SEC (Bio-Gel P-10). [b] By GLC of alditol acetates. [c] By GLC–MS of partially methylated alditol acetates. [d] Traces also of → 3)-Ara in **B1** and **B2**. [e] Determined after $LiAlD_4$ reduction of methylated **B1**–**B3**.

molecular weight of the acidic oligosaccharide approaches that of the material retained in the dialysis bag. The retention of Ara residues suggests the proximity of this unit, as well as of Gal, to the core.

Non-dialysable polysaccharide fractions B1–B3.—Table I shows the compositions of the non-dialysable, partial-hydrolysis products from autohydrolysed *E. longifolius* gum. Preponderance of the GlcA and Man constituents is maintained throughout, with only **B1** containing a substantial (21 mol%) proportion of Gal residues. The mol wt distributions of **B2** and **B3** (Fig. 1) showed the trend towards a 14-unit product, comprising → 2)-Man and → 2,4)-Man and terminal and → 4)-linked GlcA, there being good agreement of the results of methylation analysis using GLC of alditol acetates and of methyl ester methyl glycosides. The ^1H NMR spectrum of **B3** showed anomeric signals in agreement with the units found by methylation, for units of: → 2)-α-Man-(1 → (δ 5.40, 4 H), → 2)-α-Man-OH (5.27, 0.6), → 2)-β-Man-OH (4.95, 0.4), β-GlcA-(1 → (4.51, –), and → 4)-β-GlcA-(1 → (4.47, 5).

Methylated **B2** and **B3** were subjected to base-catalysed β-elimination experiments in order to determine the sugars adjacent to the → 4)-linked GlcA. Dimsyl-catalysed reactions[5] confirmed that GlcA was exterior to Gal and Man at

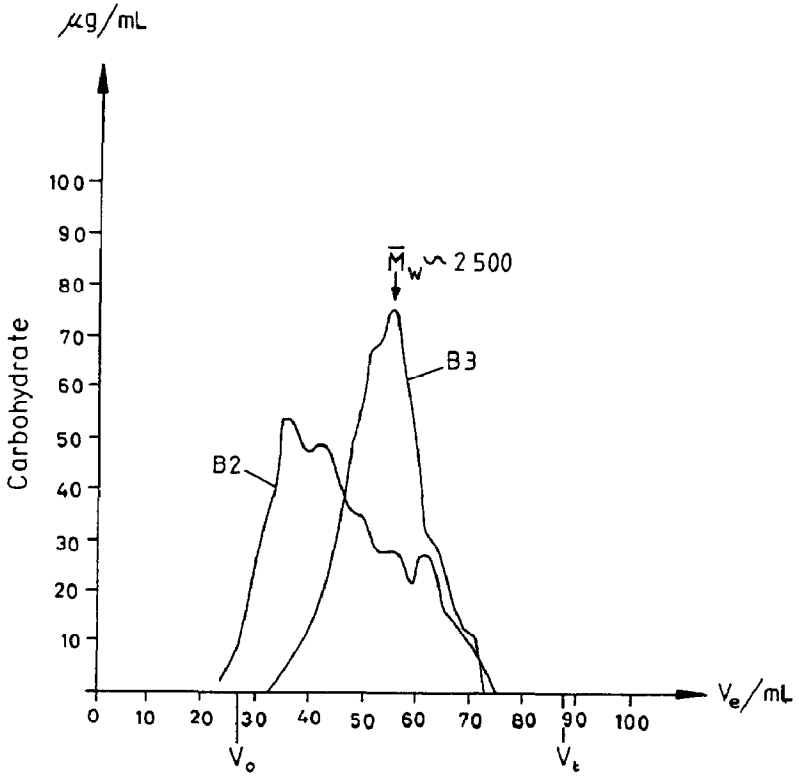


Fig. 1. Products **B2** and **B3** from *E. longifolius* gum: molecular weight profiles on steric-exclusion chromatography using Bio-Gel P-10. V_e, Volume eluted; V_o, void volume; V_t, total volume.

O-6 and O-2, as both became deuteriomethylated. Thus, referring to methylated **B2** as shown in Table I, → 6)-Gal (4) became T-Gal (4) with OCD_3 on C-6, and → 3,6)-Gal (4) similarly became → 3)-Gal (4), while → 2)-Man (19) became T-Man (4) with OCD_3 on C-2. During the treatment with base, newly released → 2)-Man was severely degraded, indicating its attachment to O-4 of GlcA, in both **B2** and **B3**. Furthermore, there was no evidence for → 4)-Man that would have been formed on base-catalysed degradation and deuteriomethylation from → 2,4)-Man (13), and this result is also accountable on the grounds that such Man is exterior to GlcA in methylated **B2**.

FABMS of fractions 1 and 2 (see Experimental) of methylated **B3** showed a multiplicity of cationised (NH_4^+ or Na^+) species corresponding to $GlcA_nMan_n$ ($n = 2$–4) together with ions corresponding to glycosidic cleavages[12] with subsequent loss of MeOH. Further ions could be rationalised on the basis of mannoglucuronoglycan sequences; these and other FAB-mass spectra will be discussed in full elsewhere. The spectra obtained may be compared with those of related methylated polysaccharides containing contiguous GlcA and Man units[13].

Small-scale partial hydrolysis of **B3** gave products that included β-GlcA-(1 → 2)-Man and its dimer [α-(1 → 4)-linked to GlcA], trimer, and tetramer; this was

shown by PC (solvents *A* and *B*) with authentic markers derived from *Hakea sericea* gum[14] and *Ornithogalum thyrsoides* mucilage[15]. The components gave a linear plot of log $[(1/R_{\text{Monomer}}) - 1]$ vs. the number of biouronic acid units. Therefore, a minimum of four alternating pairs of GlcA and Man units must be present in the linear core of **B3**, and, accordingly, of *E. longifolius* gum. Methylation analysis (Table I) requires that a second terminal GlcA and a branch at O-4 of Man should also be present in **B3**.

Methylation analysis of the intact gum.—A comparison of methylation analyses of *E. longifolius* gum by GLC–MS of derived alditol acetates and by GLC of the acetylated aldononitriles with the yields of methylated sugars from large-scale fractionation of the methyl ethers on cellulose showed good agreement; the largest discrepancies were for T-Rha, recovery from the column being low, and for →2,3)-Man and T-Ara*f* where GLC responses were low (5 and 10%, respectively, being recovered from the cellulose column). The proportions (mol%) of sugar units and their modes of linkage in *E. longifolius* gum found by GLC methods were as follows: T-Rha*p* (16), →)-Rha (5), T-Ara*f* (4), T-Ara*p*/T-Xyl*p* (5), →3)-Ara (7), T-Gal*p* (3), →3)-Gal (2), →6)-Gal (9), →3,6)-Gal (19), →3.4,6)-Gal (2), →2,3)-Man (trace), and →2,3,4)-Man (8). GLC traces for partially methylated alditol acetates and of the methylated glycosides derived from methylated *E. longifolius* gum have been published[8], the latter analyses indicating the presence in comparable amounts of T-Glc*p*A and →4)-GlcA. Additionally, 3% of →3,4)-GlcA was revealed by GLC analysis as the partially methylated alditol acetate derived from the reduced methylated gum.

Methyl ethers from the cellulose column were identified variously by methods (*a*)–(*g*) as follows: 2,3,4-Me_3Rha (*a–d*), 2,3,5-Me_3Ara (*a–d*), 2,3,4-Me_3Xyl (*a–d*), 2,3,4,6-Me_4Gal (*a–d*), 2,3-Me_2Rha (*a–c*), 2,3,4-Me_3Ara (*a–c*), 2,4,6-Me_3Gal (*a–c* and *f*), 2,3,4-Me_3Gal (*a–c* and *f*), 2,4-Me_2Ara (*a–d* and *g*), 4,6-Me_2Man (*a–d* and *g*), 3-MeRha (*a–d* and *g*), 2,4-Me_2Gal (*a–c*, *e*, and *f*), 6-MeMan (*a–c* and *f*), 2-MeGal (*a–c*), and 4-MeGal (*a–c*). The methods used were (*a*) PC (solvents *C*, *F*, and *G*), (*b*) GLC–MS of the alditol acetates, (*c*) GLC of the methyl glycosides, (*d*) *O*-demethylation and PC, (*e*) crystallisation on seeding, (*f*) NMR spectroscopy, and (*g*) probe MS. Traces of 2,3,4-Me_3Fuc, 3,4,6-Me_3Man, 2,4-Me_2Rha, 3,4-Me_2Rha, 2,3,6-Me_3Gal, and 2,3-Me_2Xyl were detected. The components in the later eluates were hydrolysed and methanolysed, and the products were analysed by PC and by GLC as the alditol acetates and as the methyl ester methyl glycosides. The ^1H and ^{13}C NMR spectral assignments (method *f*) for the sample of 6-*O*-methylmannose proved the site of attachment of the MeO to be at C-6 (identified by APT), a shift of 9.55 ppm being experienced relative to D-mannose[16]. The NMR spectra of 2,4,6-Me_3Gal, 2,3,4-Me_3Gal, and 2,4-Me_2Gal have been analysed and discussed elsewhere[17].

Five sub-fractions containing uronic acid, totalling ~28% by weight of the hydrolysate of methylated *E. longifolius* gum, were eluted last from the cellulose column. Analysis by GLC of the methyl ester methyl glycosides produced on

TABLE II

Methylation analysis employing GLC–MS of alditol acetates derived from $NaBD_4$-reduced acidic fraction and its $LiAlD_4$-reduced product

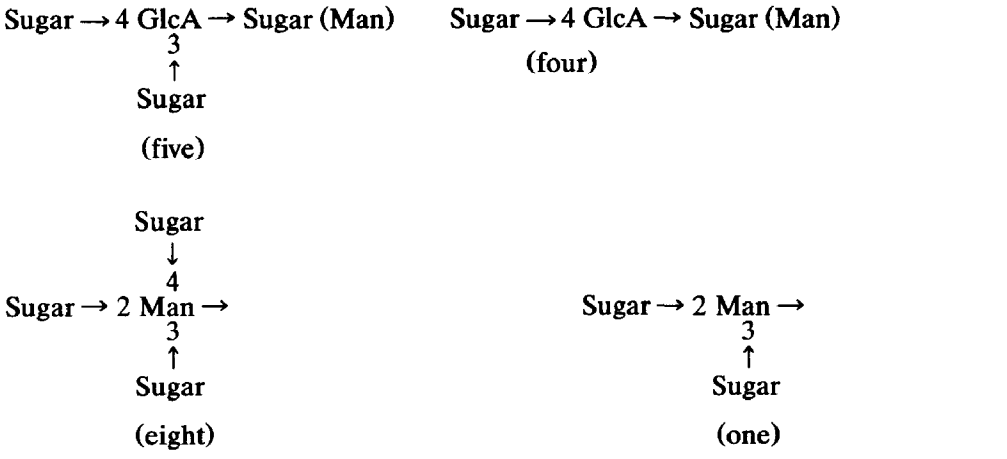
Modes of linkage	t_R [a]	Methylated $NaBD_4$-reduced compound	$LiAlD_4$-reduced product	CD_3O on	CH_3O on	Ratio CD_3O to CH_3O on
→ 2)-Mannitol	0.33	18	17	1,3,4,5	4,6	C-4 2:1
→ 2)-Man	1.51	29	27	3,4	4,6	C-4 8:1
Glucitol [b]	0.40		5	1,3,4,5	2	
T-Glc [b]	1.77		34	3,4	2,3	C-3 2:1
→ 4)-Glc [b]	3.10		17	3	2,3	C-3 1:2

[a] Retention relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol on a column of 3% OV-225 on Chromosorb W-HP, isothermal at 175°C. [b] Deuterium-labelled at C-6, and therefore derived from GlcA units.

methanolysis of the sub-fractions showed that the bulk of the uronic acid in the final eluates was in the form of 2,3,4-Me_3GlcA and 2,3-Me_2GlcA (mass ratio 4:1) with some 2-MeGlcA. Methylated neutral sugars were found as follows (ratios by weight): 2,3,4-Me_3Gal (6), 2,4-Me_2Gal (5), 4,6-Me_2Man (2), and 6-MeMan (4), the Man derivatives being confined to the last sub-fraction and the Gal to the third and fourth. The first two sub-fractions were minor and multi-component.

Although relatively small in proportion (2.5%) to the total hydrolysate from methylated gum, the final sub-fraction (eluted with 4:12:7 1-butanol–EtOH–water) was examined in detail to derive information regarding the pattern of substitution of the GlcA and Man in the molecular core of the gum. A similar approach has been made using methylated cherry (*Prunus cerasus*) gum[18]. GLC analysis showed the linkages of the components to be → 3,4)-GlcA (1.3), → 4)-GlcA (1.1), → 2,3,4)-Man (1.8), and → 2,3)-Man (1), together with trace amounts of → 2)- and → 2,4)-Man, but there was no evidence for units derived from non-reducing terminal positions in the gum. Borodeuteride reduction was performed in order to label the reducing ends, and deuteriomethylation in order to indicate the positions from which sugar units had been released during the (incomplete) hydrolysis of methylated gum. Lithium aluminium deuteride reduction was carried out in order to convert GlcA derivatives into Glc ethers. GLC analysis (Table II) of the derived per-*O*-methylated alditol acetates obtained after hydrolysis indicated a 2:3 ratio of → 2)-linked, reducing end to → 2)-linked, in-chain Man, and the proportion of reducing-end GlcA to reducing-end Man was ~ 1:3. There were present T-Glc and → 4)-Glc (both from GlcA); from the proportion of reducing-end groups, the average chain length was ~ 5. Sites of attachment of sugars released during partial hydrolysis of methylated gum were then deduced from the mass spectra of the permethylated alditol acetates, on the argument that CD_3O groups label such sites whereas CH_3O groups indicate unsubstituted positions in the gum itself. The results of MS analysis (Table II), from which the intensities of relevant ions were employed to deduce CD_3O to CH_3O ratios on C-4 (for Man derivatives) and C-3 (for GlcA derivatives), showed

(a) In-chain, substituted GlcA and Man units in partial-hydrolysis product:



(b) Substituted Man units occurring as reducing ends in partial-hydrolysis product:

Fig. 2. Inter-sugar linkages in *E. longifolius* gum, determined by analysis of a fraction from partially hydrolysed, methylated gum. Relative proportions of the moieties given in parenthesis. Units labelled "sugar" are neutral or acidic, unless Man is specified.

that, within the accuracy of the procedure, (*a*) for Man appearing as reducing-end group in the partial hydrolysate under consideration, the ratio of → 2,3,4)- to → 2,3)-substitution was 2:1; (*b*) for the → 2)-linked, in-chain Man units, each had been substituted at position 3 and the great majority (8:1) also at 4; (*c*) for the → 4)-linked GlcA, position 3 was substituted by a sugar unit in 5 out of every 9 units. Fig. 2 summarises these findings.

As the 3-position is always deuteriomethylated in the permethylated alditol acetates of Man, the substituent sugars that had been on that position in the original gum must all have been released during partial hydrolysis of the methylated gum. Similarly, all GlcA had CH_3O on C-2 and, therefore, had not been substituted in that position. All T-GlcA was deuteriomethylated on C-4, showing that it had all been derived from 4-linked GlcA.

CONCLUSIONS

There are four aspects to the molecular structure of *E. longifolius* gum: (*a*) peripheral groups, (*b*) the assembly of sugars and uronic acid based on the

galactan framework, (*c*) points of attachment to glucuronic acid and mannose in the core, and (*d*) the core itself. The individual sugar units and their modes of substitution have been reported above. As no fractionation of the present sample of gum has been achieved by Cetavlon-precipitation experiments, the sugar sequences and other structural elements are treated as if emanating from a single, complex molecular entity which represents an average structure for the polysaccharide.

(*a*) Methylation analysis and the ease of removal by acid hydrolysis of much of the Ara defines Ara*f* as a prominent (8%) terminal, sugar unit, others (in diminishing proportion) being Rha*p* (3-*O*-MeRha*p*), Ara*p*, Xyl*p*, Gal*p*, and possibly Fuc*p*.

(*b*) Within the set of peripheral sugars, there is a branched galactan comprising ~ four 3-linked Gal*p* units to most of which are attached further Gal*p* at O-6; exterior to the latter are β-D-Glc*p*A units. The release of β-Glc*p*A-(1 → 6)-Gal at an early stage of hydrolysis of the autohydrolysed gum shows that such units occur towards the outside of the structure. The acid units are terminal or are substituted at O-4 by Rha*p*. Of the Rha*p* units, 50% are substituted (generally at O-4) by other Rha*p*, and some are 2,4-disubstituted. Some → 3)-Ara*p* is present, as shown by the isolation of β-Glc*p*A-(1 → 6)-β-Gal*p*-(1 → 3)-Ara (**2**) and the corresponding β-Gal*p*-(1 → 3)-Ara (**1**) on partial hydrolysis of the gum, the Ara being considered as pyranosyl on account of the production of 2,4-Me_2Ara on hydrolysis of methylated gum.

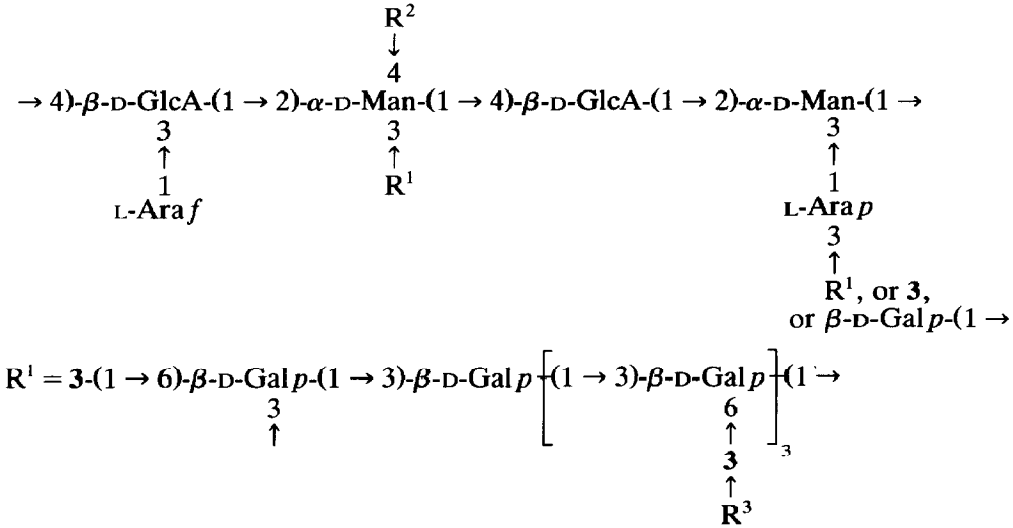


Fig. 3. Assemblies of sugar units for *E. longifolius* gum. Aldobiouronic acid moiety **3** defined in the text.

(*c*) This → 3)-Ara*p* unit may form the link between the substituted, acidic galactan, comprising ~ 15 units, as described in (*b*), and the inner core of Man and GlcA; this has been shown to be so in the analogously structured *Anogeissus* gums[19,20], from *A. latifolia* (gum ghatti) and *A. leiocarpus*. The number of Man residues and the composition of partial-hydrolysis products of molecular weight in the region of 2500 to 6000, however, indicates attachment of Gal*p* to O-3 of Man*p* as a prominent feature, and therefore an important link to the core.

(*d*) Evidence from examination of partial-acid hydrolysis products[21] from *Chorisia speciosa* gum shows that, in the *Encephalartos* gum, the inner, resistant core consists of → 4)-Glc*p*A and → 2)-Man*p* in similar proportions, alternating in the form of $[\rightarrow 4)$-β-Glc*p*A-$(1 \rightarrow 2)$-α-Man*p*-$1]_n$ where n is at least 4. The core is heavily substituted at O-3 of Man*p*, and there is some substitution at this position also of GlcA. The putative formula shown (Fig. 3) illustrates the points made. Chain kinking produced by regularly spaced → 2)-Man*p* units allows space for the attachment at O-3 of the large sugar and uronic acid-containing assemblies as well as substituents at O-4.

Structural examination of a segment of the exudate of *Encephalartos friderici-guilielmi* has recently been completed[22], and preliminary findings[2] regarding the composition of this gum have been reported.

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