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GUM HETEROPOLYSACCHARIDE AND FREE REDUCING MONO- AND OLIGOSACCHARIDES OF *ANADENANTHERA COLUBRINA*

CIRENE L. DELGOBO, PHILIP A. J. GORIN, CHRISTOPHER JONES* and MARCELLO IACOMINI†

Departamento de Bioquímica, Universidade Federal do Paraná, Caixa Postal 19046, 81531-990, Curitiba-PR, Brazil; * National Institute for Biological Standards and Control, South Mimms, Potters Bar, Herts. EN6 3QG, U.K.

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Key Word Index—*Anadenthera colubrina*; Mimosaceae; Angico branco; gum; polysaccharide; monosaccharides; oligosaccharides.

Abstract—The gum from *Anadenanthera colubrina* consists mainly of a complex high-arabinose heteropolysaccharide with a (1 → 3)-linked β-D-Gal*p* main-chain and many different side-chains. These contain β-D-Gal*p*-[(1 → 6)-β-D-Gal*p*]$_m$-(1 → 6)-, substituted in turn at O-3 by α-L-Ara*f*-[(1 → 3)-α-L-Ara*f*-]$_{0,2}$. Also present are (1) main-chain units substituted at O-4 and O-6 by α-L-Ara*f* units, (2) side-chains of Rha*p*-(1 → 4)-β-D-Glc*p*A-(1 → 6)-β-Gal*p*-groups, (3) α-L-Ara*p* non-reducing end-units linked (1 → 6) to D-Gal*p*, and (4) β-Ara*f* and β-Ara*p* structures. For the first time, a plant gum exudate was found to contain in the natural state, reducing low M_r carbohydrates. These were rhamnose (0.6%), arabinose (4.7%), mannose (0.1%), galactose (0.8%) and many oligosaccharides (0.6%; 11 with different R_fs, with the majority containing arabinose). They were all mixtures with the exception of α-Rha*p*-(1 → 4)-β-D-Glc*p*A-(1 → 6)-αβ-Gal and an incompletely identified hexasaccharide, probably having α-L-Ara*f*-(1 → 4)-β-D-Gal*p*- and -α-L-Ara*f*-(1 → 3)-β-D-Gal*p*- structures. The mono- and oligosaccharides do not appear to arise via *in situ* autohydrolysis of the gum. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

The leguminous tree Angico branco (*Anadenanthera colubrina*) is native to South American rain forests, growing at altitudes greater than 400 m. Its distribution is wide, varying in the north from Colombia and northern Brazilian States to the southern State of Paraná, where it is known as Monjoleiro. It is particularly useful as a component in the second phase of reforestation projects and its wood can be utilized in construction and carpentry, as well as in the production of charcoal. The bark of the tree is a possible source of tannin for the leather tanning industry and its gum exudate is reputed to be a remedy for respiratory problems.

In preliminary communications, the structure of the polysaccharide component of the gum was partially determined [1, 2], as were those of its free reducing oligosaccharides [2]. These and accompanying free reducing monosaccharides are now isolated and examined, with the objective of comparing their structures with those of different parts of the gum polysaccharide. This might indicate their source or mode of formation.

† Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

In preliminary analyses, the original ethanol-precipitated polysaccharide was found to have $[\alpha]_D$ −9° and contain arabinose, mannose, galactose, rhamnose and hexuronic acid, in a 63:1:20:6:10 molar ratio. The structure is extremely complex, as shown by examination by 500 MHz NMR, which revealed 26 H-1 and 20 C-1 signals. Fifteen of the former (Fig. 1A) and 13 of the latter (Fig. 1B) were detected with a 400 MHz spectrometer. In terms of arabinosyl signals, they were typical of α-Ara*f*, β-Ara*f*, and β-Ara*p* units; C-1 signals of the former were at δ 108.2 and 109.5 (major), and 108.6 and 109.9 (minor), as was that of C-5 (δ 61.2). The presence of signals at δ 83.4, 84.1, and 61.2 is consistent with interlinked residues and, according to Karácsonyi *et al.* [3], should arise from an α-Ara*f*-(1 → 3)-α-Ara*f*- structure. That of α-Ara*f*-(1 → 2)-α-Ara*f*- would furnish signals at δ 87.1, 84.1 and 61.2. A characteristic C-6 ^{13}C NMR signal of uronic acid (δ 175.6) was present but no OMe signals were found in its ^{1}H and ^{13}C DEPT spectra, eliminating the possibility of 4-Me-Glc*p*A residues. Gel filtration on Sepharose CL-4B showed a single peak with $M_r \sim 1 \times 10^6$.

Successive controlled Smith degradations provided data on main- and side-chain structures. After one

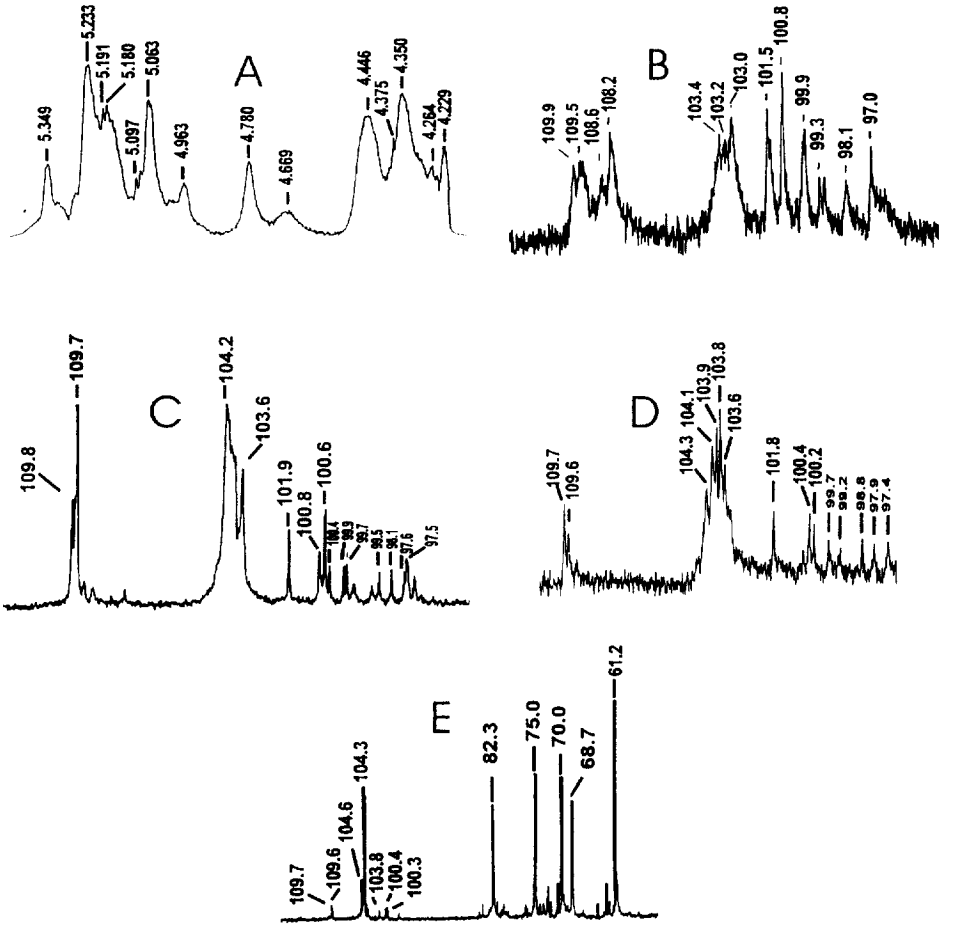


Fig. 1. (A) H-1 portion of ^{1}H NMR spectrum of Angico polysaccharide (400 MHz); (B) C-1 portion of ^{13}C NMR spectrum of Angico polysaccharide (100.6 MHz); (C) ^{13}C NMR spectrum of polysaccharide after—one controlled Smith degradations (C-1 region); (D)—after two degradations (C-1 region); and (E):—after three degradations (entire spectrum).

degradation, the C-1 region of the ^{13}C NMR spectrum (Fig. 1C) of the EtOH-insoluble product (31% yield; $M_r \sim 3 \times 10^5$; $[\alpha]_D$ +3°) was considerably simplified, with partial removal of α-Ara*f* side-chain units (C-1s at δ 109.7 > 109.8) and the appearance of 3-*O*-substituted β-Gal*p* residues (δ 82.3).

The resistance of the Ara*f* units to periodate oxidation persisted after a second Smith degradation, which gave a product (40%; yield, $[\alpha]_D$ +15°) with $M_r \sim 1.4 \times 10^5$ and gave rise to much smaller C-1 signals of α-Ara*f* units at δ 109.6 and 109.7 (Fig. 1D). The C-1 region also contained many signals at δ 103.6 to 104.3, mostly from β-Gal*p* units, showing that the degraded polysaccharide still had a complex structure. However, after a third Smith degradation, the spectrum of the product (Fig. 1E; 48% yield; $[\alpha]_D$ +17°) contained principal signals recognizable as those of a (1 → 3)-linked β-Gal*p* main-chain [4]. Very small C-1 signals were still present at δ 100.3, 100.4, 103.8, 104.6, 109.6 and 109.7. A further and final Smith degradation gave a product with $[\alpha]_D$ +11° (74% yield), whose spectrum still contained the minor signals, but in much decreased proportions. Thus, the maximum length of the (1 → 3)-linked Ara*f* side-chain units is three.

Methylation of the native polysaccharide, followed by conversion to partly *O*-methylated alditol acetates and GC-mass spectrometric examination on capillary columns of OV-225 and DB-210, showed a highly branched structure. This had non-reducing end-units of Ara*f* (40%), Rha*p* (4%), Ara*p* (2%) and Gal*p* (1%), 2-*O*-substituted Ara*p* (1%) and 3-*O*-substituted Ara*f* (12%) units, and those of Gal*p* that were 3-*O*- (1%), 6-*O*- (4%), 3,4- (5%) and 3,6-di-*O*- (24%), and 3,4,6-tri-*O*-substituted (7%) (Table 1). 4-*O*-Substituted Glc*p*A residues were demonstrated by the detection of deuterated 2,3-Me_2-glucitol acetate, derived from $LiAl^2H_4$-reduced per-*O*-methylated polysaccharide.

Partial hydrolysis of the polysaccharide (pH 1.0, 5 h, 100°) gave a core ($[\alpha]_D$ −24°; 27% yield) whose ^{13}C

Table 1. Methylation analyses of (1) Angico polysaccharide, (2) products after successive controlled Smith degradations, (3) partially hydrolyzed Angico polysaccharide (A.p. $-H^+$), and (4) product after one controlled Smith degradation

Acetylated alditol acetate	Angico poly (A.p)	% *Polysaccharide* Smx1	Smx2	Smx3	Smx4	A.p.-H^+	A.p.-H^+ Smx1
2,3,5-Me_3-Ara	40	20	10	5	2	4	2
2,3,4-Me_3-Rha	4	—	—	—	—	3	—
2,3,4-Me_3-Ara	2	—	—	—	—	2	—
2,5-Me_2-Ara	12	6	14	4	—	2	—
2,3,4,6-Me_4-Gal	1	10	22	15	2	19	20
3,4-Me_2-Ara	1	1	—	—	—	—	—
2,3-Me_2-Ara	—	2	—	—	—	—	—
2,4,6-Me_3-Gal	1	23	18	55	93	9	56
2,3,4-Me_3-Gal	4	4	6	8	—	32	5
2,6-Me_2-Gal	5	—	—	—	—	—	—
2,4-Me_2-Gal	24	33	30	17	3	27	18
2-Me-Gal	7	1	1	1	—	2	—

NMR spectrum showed that most of the α-Ara*f* units had been removed. It contained rhamnose, arabinose, galactose, glucose, and hexuronic acid in a 3:10:63:4:20 molar ratio. In the GC-mass spectrometric determination of neutral aldoses, acid hydrolysis gave rise to glucuronolactone, which was reduced with NaB^2H_4 to glucitol-$^2H_2$-6. Methylation analysis of the core polysaccharide (Table 1) showed a marked decrease of non-reducing (4%) and 3-*O*-substituted Ara*f* (2%), but not Ara*p* non-reducing end-units (2%), and the exposure of non-reducing end- (19%), 3-*O*- (9%) and 6-*O*-substituted (32%) Gal*p* units. The Glc*p*A units were 4-*O*-substituted.

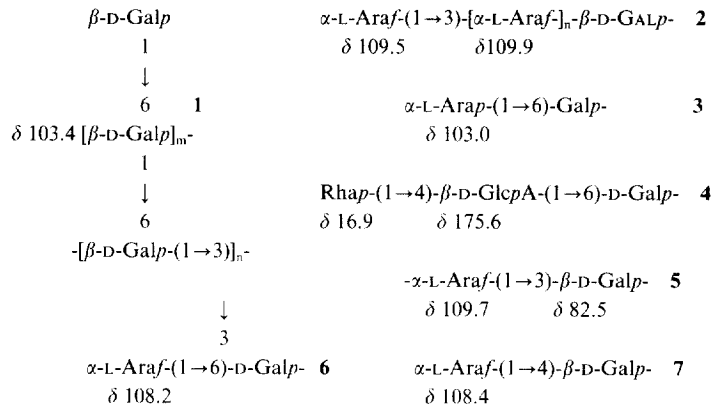
The partially hydrolysed polysaccharide was subjected to a controlled Smith degradation, which removed side-chain components of Glc*p*A and Rha*p*, giving a product (18% yield), with galactose and arabinose in a molar ratio of 93:7. Methylation analysis (Table 1) showed that the 6-*O*-substituted Gal*p* units were side-chain components (reduction from 32% to 5%), with the appearance of 3-*O*-substituted Gal*p* units (9% to 56%). The presence of a (1 → 3)-linked β-Gal*p* main-chain substituted at O-6 with β-Gal*p* was not inconsistent with ^{13}C NMR signals at δ 104.3 > 104.6 (C-1s), 82.3 (*O*-subst. C-3) and 69.6 (*O*-subst. C-6, DEPT). A further Smith degradation gave a product (59% yield; $[\alpha]_D$ −8°), whose ^{13}C NMR spectrum contained the signal at δ 104.3 in a greater proportion when compared with that at δ 104.6.

Further structural details of the native polysaccharide were revealed by examination of the oligosaccharides liberated on partial acid hydrolysis (M TFA, 1 h, 100°). These were isolated by successive charcoal and cellulose column chromatography, followed by purification on paper sheets. They had R_{Lact} 0.04, 0.07, 0.22, 0.25, 0.44, 0.76 and 1.34. The principal oligosaccharide (R_{Lact} 0.22) was 6-*O*-β-D-glucopyranosyl(uronic acid)-D-galactose, characterized as follows: (1) it gave galactose and a uronic acid on strong acid hydrolysis and its ^{13}C NMR spectrum had a uronic acid signal at δ 176.1, (2) reduction with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide followed by acid hydrolysis provided galactose and glucose (PC), (3) 6-*O*-substitution was shown since there was no C-6 signal at δ 62.8, whereas an inverted CH_2 resonance appeared in a DEPT spectrum at δ 71.5, and (4) a low-field C-1 signal at δ 104.5 showed a β-glycopyranosyl linkage. This structure was confirmed by its HMQC spectrum.

Most of the other oligosaccharides liberated on partial hydrolysis were characterised by their monosaccharide composition and NMR spectra. They had the following PC mobilities and structures: R_{Lact} 0.07, β-Glc*p*A-(1 → 6)-β-Gal*p*-(1 → 6)-αβ-Gal; R_{Lact} 0.22, β-Glc*p*A-(1 → 6)-αβ-Gal; R_{Lact} 0.25, β-Gal*p*-(1 → 6)-β-Gal*p*-(1 → 6)-αβ-Gal; R_{Lact} 0.44, mixture of 3-*O*- and 6-*O*-linked β-Gal*p* structures; R_{Lact} 0.76, β-Gal*p*-(1 → 6)-αβ-Gal; R_{Lact} 1.34, α-Ara*p*-(1 → 6)-αβ-Gal.

NMR correlation data obtained on the native polysaccharide at 70° at 500 MHz also showed the presence of β-Ara*p* and β-Ara*f* structures. High-arabinose arabinogalactans from plant gums have been found to contain Ara*f* and Ara*p* units, both of the α- and β-configuration [5–7]. However, in these investigations the proportion of α-Ara*p* units, when present, was generally lower.

A more detailed consideration of the data obtained on methylation analyses (Table 1) and NMR examination of the native polysaccharide, its successive controlled Smith degradation products and oligosaccharides formed on partial hydrolysis is as follows. They showed a main chain of (1 → 3)-linked β-D-Gal*p*



units (C-3; δ 82.3 in main chain) substituted at O-6 and O-4,6. Weak partial hydrolysis of the native polysaccharide removed the Ara*f* units of α-L-Ara*f*-(1 → 3)-[α-Ara*f*-(1 → 3)]$_{0\text{-}2}$-β-Gal*p* stuctures (**2**), exposing a high proportion of (1 → 6)-linked β-D-Gal*p* side-chains (**1**). α-L-Ara*f*-(1 → 4)-β-D-Gal*p* linkages were also cleaved (α-Ara*f* units were also removed by Smith degradation, see following NMR data). Not hydrolysed were α-L-Ara*p* units linked (1 → 6) to those of β-D-Gal*p* (**3**), agreeing with the formation of a corresponding disaccharide, on more vigorous acid hydrolysis. Rha*p* non-reducing end-units were also not hydrolysed, but a controlled Smith degradation removed them from L-Rha*p*-(1 → 4)-Glc*p*A-(1 → 6)-D-Gal*p*- groups (**4**), as shown by methylation analysis using lithium aluminum deuteride reduction of the per-*O*-methylated, degraded polysaccharide, which detected the formerly 4-*O*-substituted Glc*p*A units as terminal ones.

NMR examination at 400 MHz of the native polysaccharide in D_2O at 30° showed the presence of C-1 signals of α-Ara*f* units at δ 108.2, 108.6, 109.5 and 109.9 (Fig. 1B) and, after a controlled Smith degradation, the product gave signals at δ 109.7 > 109.8; the ones at δ 108.2 and 108.8 disappeared (Fig. 1C). The δ 109.7 signal is due to an α-L-Ara*f*-(1 → 3)-β-D-Gal*p*- structure (**5**), since in an HMBC spectrum (δ 5.22), its H-1′ signal correlated with that of *O*-substituted C-3 at δ 82.5. The δ 109.7 signal became progessively smaller after further Smith degradations indicating the successive removal of terminal units of α-L-Ara*f*-[(1 → 3)-α-L-Ara*f*-(1 → 3)]$_n$-β-D-Gal*p*- groups (**2**). The δ 108.2 signal could arise from α-Ara*f*-(1 → 6)-Gal*p* [**6**, 8] or α-Ara*f*-(1 → 4)-β-Gal*p* (**7**, δ 108.4), present in hexasaccharide W (see below). This structure also gave rise to a signal at δ 109.9 belonging to a → 3)-α-Ara*f*-(1 → 3)-β-Gal*p* group.

Of particular interest to us, was the presence in the Angico gum of free reducing low M_r carbohydrates, which have not been previously reported in gum exudates. These were rhamnose (0.6%), arabinose (4.7%), mannose (0.1%) and galactose (0.8%), and mixed oligosaccharides (0.6%). Attempts to isolate pure oligosaccharides were only partially successful, as of 11 of the oligosaccharide fractions with R_{Lact}s on PC of 0.05, 0.11, 0.13, 0.16, 0.25, 0.30, 0.34, 0.42, 0.58, 0.87 and 1.47, only two were pure according to NMR spectroscopy and HPLC examination of the fluorescently-labelled derivatives.

However, the oligosaccharide with R_{Lact} 0.58 was identified as α-L-Rha*p*-(1 → 4)-β-Glc*p*A-(1 → 6)-αβ-Gal by its 1D ^1H and ^{13}C NMR spectra (DEPT of the latter) and COSY plus HMQC examination. A similar examination of the R_{Lact} 0.16 component did not give sufficient connectivity in its 2D spectra to determine its complete structure. However, it was possible to show that its signal at δ 108.4 arose from α-L-Ara*f*-(1 → 4)-β-Gal*p*, whereas that at δ 109.7 was from -α-L-Ara*f*-(1 → 3)-β-D-Gal*p*. Most of the structures in the other oligosaccharide fractions were high-arabinose and, with one exception, gave low-field C-1 signals of α-Ara*f* units. These fractions will be purified and characterised in the future.

All of the free naturally occurring reducing monosaccharides, with the partial exception of galactose, are components of the side-chains of the native polysaccharide, as are the oligosaccharides, α-Rha*p*-(1 → 4)-β-Glc*p*A-(1 → 6)-αβ-Gal and the hexasaccharide. This is also true of the impure high-arabinose fractions.

In terms of the reason underlying these structural similarities, the most obvious was the formation of the low M_r components via *in situ* autohydrolysis of the polysaccharide. However, this is unlikely as a 20% aqueous solution of the gum has a pH of 4.2 and, even at higher concentrations, such as exist in the native gum, the acidity should be insufficient to cause hydrolysis. Even at pH 1.5 and 100° for 2 h, Ara*f* units were not liberated, let alone form reducing units of Gal*p* present in the some of the oligosaccharides. For liberation of arabinose, pH 1.0 at 100° for 5 h or M TFA at 100° was necessary. Possible explanations for

the presence of the mono- and oligosaccharides in the native gum are their formation as byproducts during biosynthesis of the polysaccharide or enzymolysis of the gum *in situ*.

EXPERIMENTAL

Preparation and properties of gum samples

These were freeze-dried and after 3 days the rubbery gum became crisp and was milled manually to a hygroscopic powder. However, after 24 h exposure to air, there was a tendency to rehydrate and the powder particles started to adhere to each other. At 20% gum in H_2O, the soln had pH 4.2; at 3%, it was 6.3. The polysaccharide was prepd from gum solns, following centrifugation to remove debris, by successive pptn with excess EtOH, redissolution in H_2O and freeze-drying to remove residual EtOH.

Determination of uronic acid contents

This was carried out by the method of Ref. [9].

Specific rotations

These were determined at 25° with solns of 0.3–0.5% in H_2O (oligosaccharide, original polysaccharide and partly acid-degraded product) and in H_2O containing 3% NaOH (other polysaccharides).

NMR spectroscopy

^1H and ^{13}C spectra were obtained at 400 MHz in D_2O solns maintained at 30°, except with the controlled Smith degradation products, which were dissolved in D_2O containing 3% NaOD. ^{13}C and ^1H spectra of the original polysaccharide and that obtained following one Smith degradation were also recorded at 500 MHz at 70°, using HMQC and HMQC-TOCSY pulse sequences. Among the 26 H-1 and 20 C-1 signals of the original polysaccharide, it was possible to recognize arabinosyl units, which gave correlated (HMQC) ^1H and ^{13}C shifts with δ 5.378 and 99.78 (β-Ara*f*), 5.335 and 100.00 (β-Ara*f*), 5.421 and 108.61 (α-Ara*f*), 5.408 and 109.92 (α-Ara*f*), 5.265 and 110.05 (α-Ara*f*), 5.20 and ? (Ara*f*), 5.090 and 100.22 (β-Ara*p*), and 5.060 and 100.22 (β-Ara*p*).

Estimation of monosaccharides

Gum (226.2 mg) and allitol (0.894 mg) in H_2O (20 ml) were stirred in the presence of $NaBH_4$ (50 mg). Bubbles on the surface of the gum showed that it was reacting. When it had dissolved completely after 18 h, a little more $NaBH_4$ was added and, after 3 h, the soln was adjusted to pH 7 with HOAc. It was then freeze-dried, dissolved in H_2O (1 ml) and added to EtOH (50 ml). The resulting ppt was removed by filtration and the filtrate evapd to a residue, which was dissolved in MeOH and evapd, the process being repeated twice. Acetylation was carried out with Ac_2O–pyridine (2 ml; 1:1) at 100° for 1 h, the mixt. treated with ice–H_2O for 1 h, and then extracted with $CHCl_3$, which was evapd. GC-MS showed acetates of rhamnitol, arabinitol, allitol, mannitol and galactitol in a 9:72:6:1:12 molar ratio. This corresponds to rhamnose (0.59%), arabinose (4.7%), mannose (0.10%) and galactose (0.79%) in the original gum. In another expt, gum (200.4 mg) with allitol (0.894 mg) were dissolved in H_2O (20 ml) for 15 h to complete soln. $NaBH_4$ (50 mg) was then added and, after 3 h, the soln was adjusted to pH 7 and conversion to alditol acetates effected as described above. GC-MS detected acetates of rhamnitol, arabinitol, allitol, mannitol and galactitol in a 6:70:10:2:12 molar ratio. This corresponds, in the original gum, to rhamnose (0.27%), arabinose (3.19%), mannose (0.10%) and galactose (0.55%).

Identification of enantiomers of monosaccharide components

The polysaccharide (0.5 g) was hydrolyzed with 2 M TFA at 100° for 8 h to give a product that was fractionated on a cellulose column (eluant: Me_2CO, then Me_2CO–H_2O, 10:1 v/v). Obtained were arabinose, recrystallised from EtOH, $[\alpha]_D$ +167° (initial value after 80 s) → +102° constant value), showing the L-isomer [10] and after mutarotation, a ^{13}C NMR spectrum typical of its α-βmixt. Other fractions contained D-galactose and a mixture of L-rhamnose and D-glucuronolactone (R_{Rham} 1.04). These gave typical ^{13}C NMR spectra and their enantiomeric forms were determined by GC-MS of their (−)-2-octyl glycoside acetates on a capillary column of Durowax-4 (30 m × 0.25 mm i.d.), program 50° (40° min^{-1}) → 230° [11].

Controlled Smith degradation

Angico polysaccharide (5.75 g) was dissolved in H_2O (200 ml), and $NaIO_4$ (15.0 g) added. After 42 h, ethylene glycol (3.0 ml) was introduced and the soln successively dialyzed for 2 days against tap H_2O and 1 day against dist. H_2O. $NaBH_4$ (2 g) was then added and, after 3 h, the soln acidified to pH 6 with HOAc and dialysed in the same way. The product contained glycerol, threitol, arabinose and galactose in a 27:5:31:37 molar ratio. The soln was evapd to 100 ml, adjusted to pH 2 with dil. H_2SO_4 and kept at 100° for 1 h, the solution neutralised ($BaCO_3$), filtered and the filtrate evapd to a small vol. Addition of excess EtOH gave insol. polysaccharide (1.84 g; arabinose–galactose ratio, 32:68; NMR data in Table 2 and Fig. 1C) and supernatant material (1.97 g). The ^{13}C NMR spectrum of the polysaccharide contained ^{13}C signals of α-Ara*f* at δ 109.7 > 109.8 (C-1), 81.7 (C-2), 76.9 (C-3) and 84.2 (C-4) [12]. Of the 2D spectra examined, that of HMBC the most useful, since it showed cor-

Table 2. ^{13}C NMR chemical shifts of signals obtained from (1) Angico polysaccharide, (2) polysaccharides obtained via successive controlled Smith degradations, (3) partially hydrolyzed Angico polysaccharide, (4) polysaccharide obtained after one controlled Smith degradation, and (5) oligosaccharides isolated from Angico gum

Material	Chemical shifts of most significant signals, δ in D_2O
Angico gum	175.6, 109.9, 109.5, 108.6, 108.2, 103.4, 103.2, 103, 101.5, 100.8, 99.9, 99.3, 98.1, 97, 84.1, 83.4, 82.1, 81.9, 81.3, 80.2, 79.9, 76.8–68.9, 63.2, 62.5, 61.2, 16.9.
Angico gum-H^+	175.6, 109.8, 109.6, 104.1, 103.8, 103.3, 103, 101, 81.7, 79.4, 75.8–69, 61.6, 61.4, 16.9
Angico gum-H^+, Smith × 1	104.6 < 104.3, 82.4, 77.8, 75.4, 72.9, 70.8, 70.3, 68.8, 68.7, 61.3
Angico gum. Smith × 1	109.8 < 109.7 (large), 103.6–104.2 (broad and large), 101.9, 100.8, 100.6, 100.4, 99.9, 99.7, 98.5, 98.1, 97.6, 97.5, 96.9, 84.2, 82.5, 82.3, 81.7, 63.5, 63.2, 62.9, 62.2, 61.6 and 61.4 (both large)
Angico gum, Smith × 2	109.7 > 109.6 > 109.3 (each one much smaller than before), 104.3, 104.1, 104, 103.9, 103.8, 103.6 (6 largest C-1 signals), 101.7, 100.4, 100.2, 99.7, 99.2, 98.4, 97.9, 97.3, 84, 82.6, 82.3 (large), 63.1 < 61.4.
Angico gum, Smith × 3	Largest peaks at δ 104.3, 82.3, 75, 70.6, 68.7, and 61.3, others small
Angico gum, Smith × 4	Peaks almost exclusively at δ 104.3, 82.3, 75, 70.6, 68.7 and 61.3.

relation between resonances of H-1 of α-Ara*f* (δ 5.22) and one component of the *O*-substituted C-3 region of β-Gal*p* units (δ 82.5), but not those of C-6s, indicating (1 → 3)-linkages.

A second controlled Smith degradation was carried out under identical conditions, giving a polyalcohol containing glycerol, arabinose and galactose in a 14:29:57 molar ratio, which was partially hydrolysed to EtOH-insol. (0.73 g; arabinose to galactose ratio, 24:76; NMR data: Table 2 and Fig. 1D) and sol. material (0.49 g).

A third controlled Smith degradation gave polyol-alcohol containing glycerol, threitol, arabinitol and galactitol in a 21:1:12:66 molar ratio. It was partially hydrolysed giving EtOH-insol. (0.35 g; ratio of arabinose to galactose, 15:85; NMR data: Table 2 and Fig. 1E) and sol. (0.26 g) material. It furnished ^{13}C NMR signals at δ 61.25 (C-6), 68.7 (C-4), 70.6 (C-2), 75.0 (C-5), 82.3 (C-3) and 104.3 (C-1) (values of 61.7, 69.4, 71.1, 75.9, 82.8 and 104.8, respectively, were found for internal signals of β-Gal*p*-[(1 → 3)-β-Gal*p*]$_4$-(1 → 4)-αβ-Glc in D_2O [4]).

A fourth and final controlled Smith degradation on polysaccharide (0.2 g) gave rise to EtOH-insol. (147 mg; arabinose to galactose ratio of 5:95; NMR data: Table 2) and sol. (50 mg) material.

Partial hydrolysis of polysaccharide to remove arabinofuranosyl units

Preliminary expts were carried out to determine optimum conditions for removal of α-Ara*f* units. Polysaccharide (9 g) was dissolved in H_2O (200 ml), which was adjusted to pH 1.5 with dil. H_2SO_4 and maintained at 100° for 2 h. The soln was neutralised ($BaCO_3$), filtered and the filtrate evapd to 50 ml, which was added to EtOH (250 ml). The resulting ppt. (5.8 g) was isolated and found to contain arabinose in a high proportion (rhamnose–arabinose–galactose molar ratio = 11:59:30). The ppt was dissolved in H_2O (100 ml), which was adjusted to pH 1 (dil. H_2SO_4) and kept at 100° for 1 h. EtOH (500 ml) was then added and the ppt isolated, dissolved in H_2O and dialysed (2.91 g). As it contained rhamnose, arabinose, galactose and glucose (from GlcA) in a 7:28:62:3 molar ratio, hydrolysis was continued for a further 4 h to remove Ara units. Polysaccharide (7 g) in H_2O (150 ml) at pH 1 (dil. H_2SO_4) was maintained at 100° for 5 h. The EtOH-insol. product (1.79 g) contained rhamnose, arabinose, galactose and glucose (from GlcA) in a 4:12:79:5 molar ratio and its ^{13}C NMR spectrum (Table 2) contained only small C-1 signals of Ara*f* at δ 109.6 and 109.8.

A portion (2.68 g) was submitted to a controlled Smith degradation, giving EtOH-insol. material (0.48 g; NMR data: Table 2), with arabinose and galactose in a 14:86 molar ratio. A further controlled Smith degradation was carried out giving polysaccharide (57 mg) precipitable with EtOH–Me_2O (1:1) and containing arabinose and galactose in a 9:91 molar ratio.

Partial hydrolysis of polysaccharide to form oligosaccharides

The polysaccharide (7 g) was dissolved in M TFA (100 ml), the soln maintained at 100° for 1 h and then evapd to dryness. The hydrolysate in H_2O was passed through a column of powdered charcoal–diatomaceous earth (1:1). Monosaccharides were eluted with H_2O and oligosaccharides with 30% v/v aq. EtOH. The oligosaccharide mixt. (1.47 g) gave rise to spots with R_{Lact} 0.04, 0.07, 0.22, 0.25, 0.44, 0.76 and 1.34 (solvent: *n*-BuOH–pyridine–H_2O, 1:1:1) and this was fractionated on a column of cellulose, using as successive eluants Me_2CO-H_2O mixts of 7:1, 4:1, 3:1 and 1.5:1. As purification was not complete, each fraction was applied to Whatman No. 3 filter paper (solvent: *n*-BuOH–pyridine–H_2O, 1:1:1). In order to determine the absence of OMe groups in the original polysaccharide, DEPT pulse sequences were used to invert possibly superimposed $\underline{C}H_2$ signals, using programs to (1) invert $\underline{C}H_2$ signals and (2) to eliminate them. The former sequence was also used with poly-

and oligosaccharides, in order to detect *O*-substituted $\underline{C}H_2$ signals.

Characterisation of oligosaccharides formed on partial hydrolysis

Monosaccharide compositions of the isolated oligosaccharides were determined by successive acid hydrolysis, NaB^2H_4 reduction and acetylation; the resulting alditol acetates analyzed by GC-MS. Using these and NMR data, the following structures were determined.

R_{Lact} 0.04. 52 mg. Gal > Ara, tr. Glc. C-1 signals at δ 104.5, 103.8, and 103 (1:2:1), 96.4 and 92.6, 3-*O*-substituted signals at δ 82.3 and 82.8, inverted DEPT signals at δ 69.7 and 69.9 (*O*-substituted C-6) and 61.3 (C-6): mixture with 3-*O*- and 6-*O*-substituted Gal*p*.

R_{Lact} 0.07. 43 mg. Gal > Glc (trideuteriated), tr. Ara. C-6 of uronic acid at δ 176.5, C-1 signals at 103 = 103.4 > 103.6, 96.4, and 92.3. Inverted DEPT signals at δ 69.5 = 69.6 > 69.9: β-D-Glc*p*A-(1 → 6)-β-D-Gal-(1 → 6)-$\alpha\beta$-D-Gal.

R_{Lact} 0.22. 127 mg. Uronic acid, Gal, Glc (trideuteriated). C-6 signals of GlcA at δ 176.1, C-1 signals at δ 102.69, 102.64, and 96.7 > 92.6. Inverted DEPT signals at δ 69.54 > 69.64: β-D-Glc*p*A-(1 → 6)-$\alpha\beta$-D-Gal.

R_{Lact} 0.25. 41 mg. Gal, tr. Ara and Glc. C-1 signals at δ 103.7 = 103.4 > 103.6, 96.7 > 92.6. Inverted *O*-substituted C-6 DEPT signals at δ 69.5 = 69.6 > 69.9. and free C-6 signal at δ 61.3: β-D-Gal*p*-(1 → 6)-β-D-Gal*p*-(1 → 6)-$\alpha\beta$-D-Gal.

R_{Lact} 0.44. 71 mg. Gal > Ara. Very complex with 12 C-1 signals.

R_{Lact} 0.76. 33 mg. Gal, tr. Ara. C-1 signals at δ 103.4 > 103.5, 96.7 > 92.6, inverted DEPT signals at δ 69.4 > 69.6 (C-6 *O*-substituted) Gal*p* and 61.3 (free C-6)- β-D-Gal*p*-(1 → 6)-$\alpha\beta$-D-Gal.

R_{Lact} 1.34. 70 mg, $[\alpha]_D$ +66°, Ara, Gal (9:10). C-1 signals at δ 103, 96.7 > 92.6, with inverted DEPT signals at δ 69.9 (C-6β of Gal) > 69.8 (C-6α of Gal), 67.7 (C-5′ of α-Ara*p* and not of β-Ara*p*, at ~3.5 ppm higher field [12]). The lowest-field signals in the *O*-substituted region were at δ 75.2 and 74.9. H-1 signals at δ 5.230, J = 3.6 Hz (H-1α), 4.571, J = 8.8 Hz (H-1β), partially superimposed on signal at δ 4.549, J = 8.8 Hz (H-1′)- α-L-Ara*p*-(1 → 6)-$\alpha\beta$-D-Gal.

Methylation analysis of polysaccharides

Polysaccharides were methylated successively by the methods of Haworth, Ciacunu (×2) and Kuhn (×5). The fully methylated products were treated with 3% MeOH–HCl at 70° for 3 h and M H_2SO_4 for 18 h at 100°. The resulting mixts were reduced with $NaBD_4$, acetylated and then examined by GC-MS on capillary columns of OV-225 and DB-210. Two columns were necessary because of the superimposition of key peaks.

On OV-225, there was superimposition of alditol acetates of 2,3,5-Me_3-Ara (447 *s*) and 2,3,4-Me_3-Rha (448 *s*) and 2,4-Me_2-Ara (545 *s*), 3,4-Me_2-Ara (542 *s*) and 2,3,4,6-Me_4-Gal (542 *s*). Those of 2,5-Me_2-Ara (509 *s*) and 3,5-Me_2-Ara (499 *s*) were resolved from the latter. That of 2,3-Me_2-Ara had 537 *s*. On DB-210, alditol acetates of 2,5-Me_2-Ara and 2,3,4,6-Me_4-Gal (533 *s*) are superimposed, but are resolved from those of 3,5-Me_2-Ara (519 *s*), 2,3-Me_2-Ara (577 *s*), 3,4-Me_2-Ara (571 *s*), 2,4-Me_2-Ara (558 *s*), 2,3,4,6-Me_4-Gal (533 *s*). Alditol acetates of of 2,3,5-Me_3-Ara (462 *s*) and 2,3,4-Me_3-Rha (469 *s*) were separable.

Isolation of oligosaccharides from gum

Freeze-dried gum (102.8 g) was crushed into smaller particles with an adjustable wrench and allowed to swell overnight in H_2O (500 ml) at pH 4.2. The gelatinous blobs were then dispersed in a blendor for 3 min and added to MeOH–EtOH (5 l; 3:2) and the ppt (86.5 g) isolated by filtration. It contained rhamnose, arabinose, mannose, galactose and glucose (2M TFA, 8 h, 100°, alditol acetates using $NaBD_4$) in a 5:52:1:39:3 molar ratio (the presence of an MS peak at m/z 141 incorporating -C^2H_2-6 indicated that some of the glucose arose from glucuronolactone). The mother liquor was evapd to a small vol. which was applied as an aq. soln to a column of activated charcoal powder–dietanaceous earth (1:1). The column was eluted with H_2O (2 l) and the eluate discarded, followed by 30% aq. EtOH (1 l) which was evapd to a glass (0.6 g). This was chromatographed on a cellulose column, using as eluants mixts (1 l each) of Me_2CO–H_2O 10:1, 7:1, 4:1, 3:1, 5:2 and 2:1. Six fractions were obtained and these were further fractionated on Whatman No. 3 filter paper (solvent: *n*-BuOH-pyridine-H_2O, 1:1:1) to give products with 1 PC spot. In some cases, heterogeneity was determined using a reductive amination method incorporating fluorescent-labelling and detection by HPLC [13].

Characterization of α-L-Rha*p*-(1 → 4)-β-D-Glc*p*A-(1 → 6)-$\alpha\beta$-D-Gal

This had R_{Lact} 0.58 and hydrolysis gave rhamnose, glucuronolactone and galactose, whereas $NaBH_4$-reduced material gave only rhamnose and glucuronolactone as reducing sugars. The ^{13}C NMR spectrum of the trisaccharide contained signals at δ 102.9 (C-1′, β-Glc*p*A), 101.2 (C-1″, α-Rha*p*), 96.9 and 92.9 (C-1α and C-1β of Gal*p* resp.). The Gal*p* units were 6-*O*-substituted, since no C-6 signals were present at δ ~61.3, but typical DEPT inverted ones were at δ 69.6 (C-6β) and 69.7 (C-6α). The signal at δ 79.7 would be expected from 4-*O*-substituted β-Glc*p*A units, by comparison with the C-4 resonance of Me β-Glc*p*A at δ 71.6 [12] (corrected by −0.7 ppm). Down-field α-shifts of signals of C-2 (δ 73.1) and C-3 (δ 75.8) would result in one at lower field than δ 79.7. This assignment was confirmed by COSY and HMQC data on α-L-Rha*p*-(1 → 4)-β-D-GlcA, isolated from cashew-tree

gum [14]. Other COSY- and HMQC-based assignments for the trisaccharide are δ 76.7 (C-5′), 74.8 (C-3′), 74.3 (C-5β), 73.7 (C-2′), 73.15 (C-3β), 72.5 (C-3′), 73.25 (C-2β), 17 (C-6″) 175.6 (broad; C-6′).

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