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ANALYTICAL AND STRUCTURAL STUDIES ON PLANT GUMS OF THE ACACIA GENUS

by

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TO MY PARENTS AND WINNIE

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SECTION I

INTRODUCTION

The polysaccharide gums exuded by a number of Acacia species have now been investigated, and have been found to differ widely in physical properties and chemical composition. Primary chemical structural studies of some of these exudates have shown that a variety of molecular structures exist for the polysaccharide gums of the Acacia genus. Thus, although the molecular structure of Acacia senegal gum[1] is very similar to that of Acacia laeta gum,[2,3] it differs significantly from that of Acacia arabica gum.[4]

In this thesis the gums of Acacia senegal, Acacia laeta, Acacia drepanolobium and Acacia seyal are examined. Section III deals with a comparative study of three samples of Acacia senegal gum. A Smith degradation study of Acacia laeta gum is detailed in Section IV. This confirms that Acacia laeta gum is very similar to Acacia senegal gum. Sections V and VI deal with chemical structural studies of Acacia drepanolobium and Acacia seyal gums respectively. The main structural features of these gums are compared with those of Acacia senegal gum and Acacia arabica gum. The experimental methods used in these experiments are outlined in Section II.

In Section VII the results from this thesis are compared with previous work on Acacia gums, and the chemical structure of Acacia gums are discussed with regard to the botanical classification of the Acacia Genus.[5,6]

SECTION II

EXPERIMENTAL METHODS

2.1) General Methods.

Weighings.- All accurate weighings were made within the range of the graticule scale (range, 0-30 mg.) on an aperiodic direct-reading balance having an accuracy of ±10 μg.

Dialyses of polysaccharides were carried out in cellophane tubing against running tap-water unless otherwise stated.

Electrodialyses of polysaccharides were carried out in a three compartment cell fitted with cellophane membranes.

Evaporations were carried out on a rotary evaporator at temperatures below 40°C.

Melting points were taken on a Kofler hot stage microscope; thermometers were corrected.

Specific rotations were measured for aqueous solutions (unless otherwise stated), using the sodium D-line as light source at 19±2°C. Values for concentrations used were corrected for non-carbohydrate material.

Reducing powers of carbohydrate solutions were determined by the alkaline ferricyanide method.[7]

Nitrogen, ash and moisture determinations.- Nitrogen was determined by a semi-micro Kjeldahl method, moisture by heating to constant weight at 105°C, and ash by heating in a muffle furnace to constant weight at 550°C.

Methoxyl contents were determined by a vapour-phase infrared method.[8,9]

Equivalent weight determinations on electrodialysed gum samples were carried out by direct titration with standard (ca. 0.01N) sodium hydroxide solution to a phenolphthalein end-point.

Uronic acid contents were determined by a vapour-phase infrared method, after decarboxylation.[9] All values for uronic acid content are expressed as the anhydride.

Rhamnose contents were determined, after periodate oxidation, as acetaldehyde by a vapour-phase infrared method.[10]

Quantitative estimation of sugars.- Sugars were separated by chromatography on washed Whatman No.3MM papers. After elution from the paper, sugars were estimated colorimetrically by the phenol-sulphuric acid method,[11] the optical density being read on a Unicam SP 1300 spectrophotometer using filter 2. Calibration curves were constructed for known weights of sugar.

Degree of polymerisation of homogeneous oligosaccharides were determined by the method of Timell.[12]

X-Ray powder photographs were taken with a 9 centimeter Unicam powder camera (Cu Kα radiation).

Viscosity determinations were carried out in M-sodium chloride solution (unless otherwise stated) in a suspended-level dilution viscometer at 25.00± 0.01°C. Solutions were filtered carefully before additions were made to the viscometer. Flow-times were measured to within 0.1 secs. using a stopwatch. The viscometer had a flow-time of 193.6 secs. for M-sodium chloride solution. The results were expressed in terms of the limiting viscosity number, $[\eta]$, where T is the flow-time in secs. of a solution of concentration c (g./ml.) and T_o is the flow-time in secs. of

the solvent.

$$[\eta] = \lim_{c \to 0} \frac{T - T_o}{cT_o}$$

Light-scattering measurements were carried out at 30.00 ± 0.02°C using a Photo Gonio Diffusometer. Unpolarised green light (5460 Å) was selected from a mercury lamp spectrum by use of a Wratten Kodak N61 filter.

Known weights of gum were dissolved in known volumes of M-sodium chloride solution; solutions were clarified through 0.80μ and 0.45μ "Millipore" filters using a stainless steel filter holder attached to a 20 ml. syringe. Concentrations of gum solutions were assumed to be unaltered by ultrafiltration.[13]

For each concentration the corrected scale readings, I_δ, were calculated[14] for angle δ as below :-

$$I_\delta = \frac{(I_{soln} - I_{sol}) \cdot \sin\delta}{1 + \cos^2\delta} \qquad \ldots\ldots\ldots\ldots\ldots (1)$$

where I_{soln} is scale reading for polymer solution

I_{sol} is scale reading for solvent (M-sodium chloride solution).

In an ideal solution the molecular weight, M, is obtained from the formula:-

$$\frac{1}{M} = \frac{2\pi^2 n_o^2}{\lambda^4 \cdot N \cdot R} \cdot [dn/dc]^2 \cdot I_B \cdot [c/I_\delta]_{c=0}^{\delta=0} \qquad \ldots\ldots (2)$$

where n_o = refractive index of solvent

n = refractive index of solution

N = Avogadro's number

λ = wavelength of incident light (5460 Å)

R = Rayleigh constant ($16.3 \ 10^{-6}$ for green light)

c = concentration in g./ml.

I_B = intensity diffused, selected for standard benzene.

This formula can be used in an extrapolation method developed by Zimm.[15] In this investigation unsymmetrical Zimm plots were obtained, and so this method could not be used.[16,17]

If it is assumed that in equation (2) molecular weight, M, is independent of concentration, c, then equation (3) is obtained.

$$\frac{1}{M} = \frac{2\pi^2 n_o^2}{\lambda^4 \cdot N \cdot R} \cdot [dn/dc]^2 \cdot I_B \cdot c \cdot [1/I_o] \quad \ldots\ldots\ldots\ldots (3)$$

$$\text{ie. } M = \frac{R}{\frac{2\pi^2 n_o^2}{\lambda^4 \cdot N} \cdot [dn/dc]^2 \cdot I_B \cdot c \cdot [1/I_o]} \quad \ldots\ldots\ldots\ldots (4)$$

The reciprocal corrected scale reading, $1/I_\delta$, is plotted against $\sin^2\delta/2$. Extrapolation of the linear portion of this graph to $\delta = 0^o$ gives a value for $[1/I_o]$. The graphs obtained in these experiments have a downward curvature at low angles. This is thought to be caused by scatter from dust particles suspended in solution.[16]

2.2) Chemical Methods.

Small-scale polysaccharide hydrolyses were carried out with N-sulphuric acid for 7 hours on a boiling water bath, unless otherwise stated. These conditions do not cause any extensive hydrolysis of uronosyl linkages in Acacia gums; this was taken into account when determining proportions of galactose. Hydrolysate solutions were neutralised with barium carbonate, filtered, deionised with Amberlite resin IR120 (H), and concentrated on a rotary evaporator.

Small-scale polysaccharide methylations.-

(a) Haworth's method.[18]- Methylations were carried out in an atmosphere of nitrogen at room temperature. Dimethyl sulphate (2 ml.) and sodium hydroxide solution [2 ml., 30% (w/v)] were added dropwise with stirring to the

polysaccharide (100 - 500 mg.) in water (10 ml.) over a period of 1 hour. Acetone (5 ml.) was then added to the reaction mixture, and six further additions of dimethyl sulphate (12 ml.) and sodium hydroxide solution (17 ml.) were made, allowing 3 hours for each addition. The reaction was stopped by heating at 60°C for 30 mins., with nitrogen bubbling through the solution. After cooling, the reaction mixture was neutralised with 4N-sulphuric acid, and made slightly acidic (pH 4.0). The methylated product was extracted into chloroform (four 100 ml. extractions) and the extract shaken with saturated sodium chloride solution (ca. 100 ml.). The chloroform layer was separated, dried over anhydrous sodium sulphate, concentrated on a rotary evaporator and poured into light petroleum (b.p. 60° - 80°, 300 ml.) with stirring. The methylated product was isolated as a white amorphous powder.

(b) Purdie's method.[19]- The partially methylated product (100 - 400 mg.) was dissolved in methanol (10 ml.) and methyl iodide (10 ml.). Silver oxide (1 g.) was added in four batches of ca. 250 mg. every 1.5 hours; the mixture was refluxed for 6 hours in a dry flask fitted with a water condenser and a calcium chloride tube. The mixture was cooled, filtered through sintered glass, and the residue extracted six times with hot chloroform (ca. 50 ml.). The combined filtrate and extracts were concentrated to a small volume and poured into light petroleum (b.p. 60°- 80°, 300 ml.) with stirring. The methylated product was isolated as a white amorphous powder.

(c) Kuhn's method.[20]- The partially methylated product (100 - 400 mg.) was dissolved in N,N-dimethylformamide (5 ml.) and methyl iodide (5 ml.). Silver oxide (2.5 g.) was added and the mixture was shaken at room

temperature in the dark for 18 hours. The mixture was filtered, the residue extracted with chloroform, and the combined filtrate and extracts were reduced to a syrup on a rotary evaporator. The syrup was dissolved in a small volume of chloroform, and the concentrated chloroform solution was poured into light petroleum (b.p. 60° - 80°, 300 ml.) with stirring. The methylated product was isolated as a white amorphous powder.

Small-scale oligosaccharide methylations.[20,21] - The oligosaccharide (0.5 - 2.0 mg.) was shaken with methyl iodide (0.2 ml.), N,N-dimethylformamide (0.2 ml.) and silver oxide (0.2 g.) at room temperature in the dark for 18 hours. The mixture was filtered and the residue washed with chloroform. The combined filtrate and washings were reduced to a syrup on a rotary evaporator.

Methanolyses were carried out under reflux for 6 hours with methanolic 5% hydrogen chloride (unless otherwise stated). Solutions were neutralised with silver carbonate and filtered. The residue was washed with methanol and the combined filtrate and washings were reduced to a syrup on a rotary evaporator.

Demethylations[22] were carried out by heating the O-methyl sugar (ca. 5 mg.) with hydriodic acid (1 ml.) in a sealed tube for 5 mins. at 100°C. The solution was diluted with water and neutralised with silver carbonate. After filtration and washing, the combined filtrate and washings were reduced to a syrup on a rotary evaporator.

Periodate oxidation of polysaccharides were carried out at room temperature unless otherwise stated.

(a) Consumption of periodate.- The amount of periodate consumed by a polysaccharide was estimated by back-titration of excess periodate.

Potassium iodide was added in excess to the periodate solution, and the iodine released was titrated, after addition of sodium bicarbonate (ca. 200 mg.), with standard arsenite solution (ca. 0.06N) using Thyodene as indicator.[23]
(b) Formic acid released was estimated titrimetrically.[24] Excess periodate was destroyed by addition of ethylene glycol. Formic acid was titrated with standard sodium hydroxide (ca. 0.1N) to a methyl red end-point.
(c) Formaldehyde released was estimated colorimetrically with chromotropic acid.[25]

Periodate oxidation of O-methyl sugars.[26]- The O-methyl sugar (1-2 mg.) was treated with 0.05M-sodium metaperiodate solution (0.2 ml.) at 0°C for 1 hour. After destruction of excess periodate by addition of ethylene glycol, the solution was made alkaline by addition of 0.5N-sodium hydroxide solution. The solution was reduced to dryness and extracted with acetone. The acetone extract was reduced to a syrup on a rotary evaporator.

2.3) Chromatographic and Zone Electrophoretic Separations.

Molecular-sieve chromatography.[27,28,29]- Separations were carried out on columns (45 × 5.0 cm.) of Bio-Gel P300 using M-sodium chloride as eluant. Thymol (10 mg.) was added to M-sodium chloride (5 l.) as a bacteriostatic agent. To prevent deformation by "wall-effects", columns were pretreated with 1% dimethyldichlorosilane in benzene at 60°C. After oven-drying, columns were packed with gel which had been allowed to swell in M-sodium chloride solution for 2 days. A thin layer of glass beads at the bottom of the column was used to support the gel; care was taken to keep the "dead-space" to a minimum. In order to stabilise the soft top surface of the "P300" gel, 1 cm. layers of Bio-Gel P200 and Bio-Gel P10 were added

successively to the column. Eluant was allowed to flow through the columns for 2 days before they were used. Each column was checked for evenness of packing by observing the passage through it of a band of "blue-dextran". Columns were calibrated using dextran fractions (Pharmacia Ltd.) of known number-average molecular weight, $\overline{M}_n$. Columns were eluted continuously with M-sodium chloride solution when not in use.

Polysaccharide (ca. 10 mg.) dissolved in 2M-sodium chloride solution (1 ml.) was applied to the column by careful layering beneath the M-sodium chloride solution. Fractions (2 ml.) collected by an automatic collector, were screened directly using the phenol-sulphuric acid method.[11] The optical density was read on a Unicam SP1300 spectrophotometer using filter 2. Collection of fractions was commenced as soon as the polysaccharide had been applied to the top of the column. Elution volumes, V_e, were estimated from an elution diagram.

Ion-exchange chromatography on D.E.A.E.-cellulose.[30]- D.E.A.E-cellulose powder (Whatman DE32, microgranular form, 10g.) was mixed with 0.5N-hydrochloric acid (250 ml.) for 30 mins. After filtration and washing until the effluent pH was ca. 4, the exchanger was mixed with 0.5N-sodium hydroxide solution (250 ml.) for 30 mins. After further filtration and washing until the effluent was neutral, columns (45 x 1.3 cm.) were packed. Columns were equilibrated with 0.02M-acetate buffer (pH 4.1). Samples (50 mg.) of gum were dissolved in buffer (2 ml.), and washed into the column with excess of buffer. Elution of the acidic polysaccharide was performed by use of a sodium chloride concentration gradient (0 → 0.5M) in 0.02M-acetate buffer (pH 4.1). Fractions (10 ml.) collected by an automatic collector, were sampled (1 ml.); the samples were screened by

the phenol-sulphuric acid method.[11] The optical density was read on a Unicam SP1300 spectrophotometer using filter 2.

Cellulose column chromatography.- Cellulose columns were packed with dry Whatman cellulose powder CF1 and washed with water; they were then washed with the solvent system to be used for separating the sugars. After excess of the solvent had been removed from the top of the column, sugar mixtures were added in minimum amounts of eluant and allowed to soak into the cellulose at the top of the column. Fractions (20 ml.) were collected by an automatic collector. Samples (ca. 5 ml.) from every tenth tube were reduced to dryness and examined for sugars by paper chromatography. Tubes containing the same sugars were combined. Solutions were reduced to dryness, and the residues were extracted with warm acetone-water mixture (1:1, v/v), treated with charcoal and filtered. Filtrates were reduced to dryness, dried in a vacuum desiccator, and weighed.

Paper chromatography of sugars was carried out on Whatman No.1 papers using the following solvent systems (v/v):-

(a) benzene - butan-1-ol - pyridine - water (1:5:3:3, upper layer).

(b) ethyl acetate - acetic acid - formic acid - water (18:3:1:4).

(c) ethyl acetate - acetic acid - formic acid - water (18:8:3:9).

(d) butan-1-ol - ethanol - water (4:1:5, upper layer).

(e) butan-1-ol - acetic acid - water (4:1:5, upper layer).

(f) ethyl acetate - pyridine - water (10:4:3).

(g) butan-2-one - water - ammonia (d. 0.880)(200:17:1).

(h) butan-2-one - acetic acid - water (9:1:1, satd. with boric acid).

The following spray reagents were used:-

(1) Silver nitrate.- Chromatograms were dipped in a saturated solution of

silver nitrate in acetone, prepared by adding saturated aqueous silver nitrate (1 ml.) to acetone (100 ml.). After drying, chromatograms were sprayed with aqueous ethanolic M-sodium hydroxide solution [water : ethanol, 1:9 (v/v)]. Chromatograms were preserved by treating with sodium thiosulphate solution and washing with water.

(2) Aniline oxalate.- Chromatograms were sprayed with a saturated solution [water : ethanol, 1:1 (v/v)] of aniline oxalate, then heated for ca. 5 mins. at 150°C.

(3) Aniline phthalate.- Chromatograms were sprayed with a saturated solution, in butan-1-ol, of aniline phthalate, then heated for ca. 5 mins. at 150°C.

(4) Periodate-permanganate reagent.- Chromatograms were sprayed with a mixture of 4 parts of 2% (w/v) sodium metaperiodate and 1 part (by volume) of 1% (w/v) potassium permanganate in 2% (w/v) sodium carbonate solution. After 5 mins. excess permanganate was removed by washing with water.

(5) Glucose was detected by an enzymic method. Chromatograms were sprayed, in succession, with (i) 0.5M-phosphate buffer pH 7.0; (ii) 0.05% peroxidase; (iii) 0.05% glucose oxidase and (iv) 0.1% o-dianisidine in 90% (v/v) aqueous ethanol. Glucose oxidase specifically oxidises D-glucose releasing hydrogen peroxide, which is coupled to o-dianisidine by peroxidase to give a purple spot.

R_F values of sugars refer to distances moved relative to that of the solvent front. R_{Gal} values of sugars refer to distances moved relative to that of galactose. R_{Rha} values of sugars refer to distances moved relative to that of rhamnose. R_G values of O-methyl sugars refer to distances moved relative to that of 2,3,4,6-tetra-O-methyl-D-glucose.

Gas-liquid partition chromatography (Chromatograph Type S3A fitted with flame ionisation detectors, supplied by Gas Chromatography Ltd.) of mixtures of O-methyl sugars[31,32] was carried out at nitrogen flow-rates of ca. 100 ml./min. on columns of:-

(i) 15% by weight of butan-1,4-diol succinate polyester on 60 - 70 mesh Celite (5 ft. x 1/4 in.) at 175°C.

(ii) 15% by weight of ethylene glycol adipate polyester on 60 - 70 mesh Celite (3 ft. x 1/4 in.) at 160°C.

Retention times, T, are quoted relative to methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside as standard.

Zone electrophoresis (Shandon Universal Electrophoresis Apparatus, Mark II, fed by a Vokam power unit, capable of providing constant voltage or constant current) of polysaccharides was carried out on strips (18 x 5 cm.) of cellulose acetate film using either 0.1M-ammonium carbonate buffer (pH 8.9) or 0.1M-acetate buffer (pH 4.7) as electrolytes. Electrophoresis was carried out at a field strength of between 15 - 20 volts/cm. for 2 - 4 hours. Polysaccharide bands were located by a modification of the periodate-rosaniline hydrochloride method.[33]

Zone electrophoresis of monosaccharides was carried out on Whatman No.1 paper in 0.05M-borate buffer at pH 9.2. Papers were sprayed with aniline oxalate. M_G values refer to the true migration of the sugar relative to the true migration of glucose (i.e. the observed migration distances are corrected for electroendosmotic flow movement by reference to 2,3,4,6-tetra-O-methyl-D-glucose, which does not form a borate complex).

2.4) Preparation of Derivatives.

Preparation of phenylosazones.- The sugar (ca. 10 mg.) was heated on

a boiling water bath with phenylhydrazine (0.01 ml.), glacial acetic acid (0.01 ml.), water (0.25 ml.) and a drop of saturated sodium bisulphite solution. On cooling and adding water (ca. 1.5 ml.), the phenylosazone precipitated; it was recrystallised from the stated solvents.

Preparation of aniline derivatives.- The O-methyl sugar (10 - 50 mg.) was refluxed with equimolar amounts of freshly distilled aniline in dry ethanol (5 ml.) for 1 hour in darkness. After removal of solvents, syrups usually crystallised; recrystallisations were carried out from the stated solvents.

Preparation of aldonolactones.- The O-methyl sugar (10—50 mg.) was oxidised with an excess of bromine water for 1 day. Excess of bromine was removed by aeration. The solution was neutralised with silver carbonate, filtered and evaporated to dryness. The organic material was extracted with warm acetone-water mixture (1:1, v/v).

Preparation of aldonamides.- The aldonolactone (10 - 50 mg.) was treated with dry methanolic ammonia (5 ml.) at 0°C for 1 day. After removal of solvent, the aldonamide was crystallised from the stated solvents.

SECTION III

A COMPARATIVE STUDY OF THREE SAMPLES OF GUM FROM ACACIA SENEGAL (L.) WILLD.

3.1) Introduction.

Acacia senegal gum is an acidic polysaccharide containing D-galactose, L-arabinose, L-rhamnose, D-glucuronic acid and 4-O-methyl-D-glucuronic acid.[1] In early investigations, prolonged acid hydrolysis of the gum has led to the isolation[34,35,36] and characterisation[37,38,39] of the aldobiouronic acid, 6-O-(β-D-glucopyranosyluronic acid)-D-galactose. The aldobiouronic acid, 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose has been isolated and characterised after acid hydrolysis of *A.senegal* gum.[40] Experiments utilising partial acid hydrolysis have shown that 3-O-α-D-galactopyranosyl-L-arabinose,[41,42,43] 3-O-β-D-galactopyranosyl-D-galactose[43,44] and 3-O-β-L-arabinopyranosyl-L-arabinose[45] are constituent units of the gum. Recently the isolation and characterisation from carboxyl-reduced gum of 4-O-α-L-rhamnopyranosyl-D-glucose[46] and O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-D-galactose[47] have given evidence for the attachment of some, if not all, of the L-rhamnose residues to the 4-position of D-glucuronic acid units.

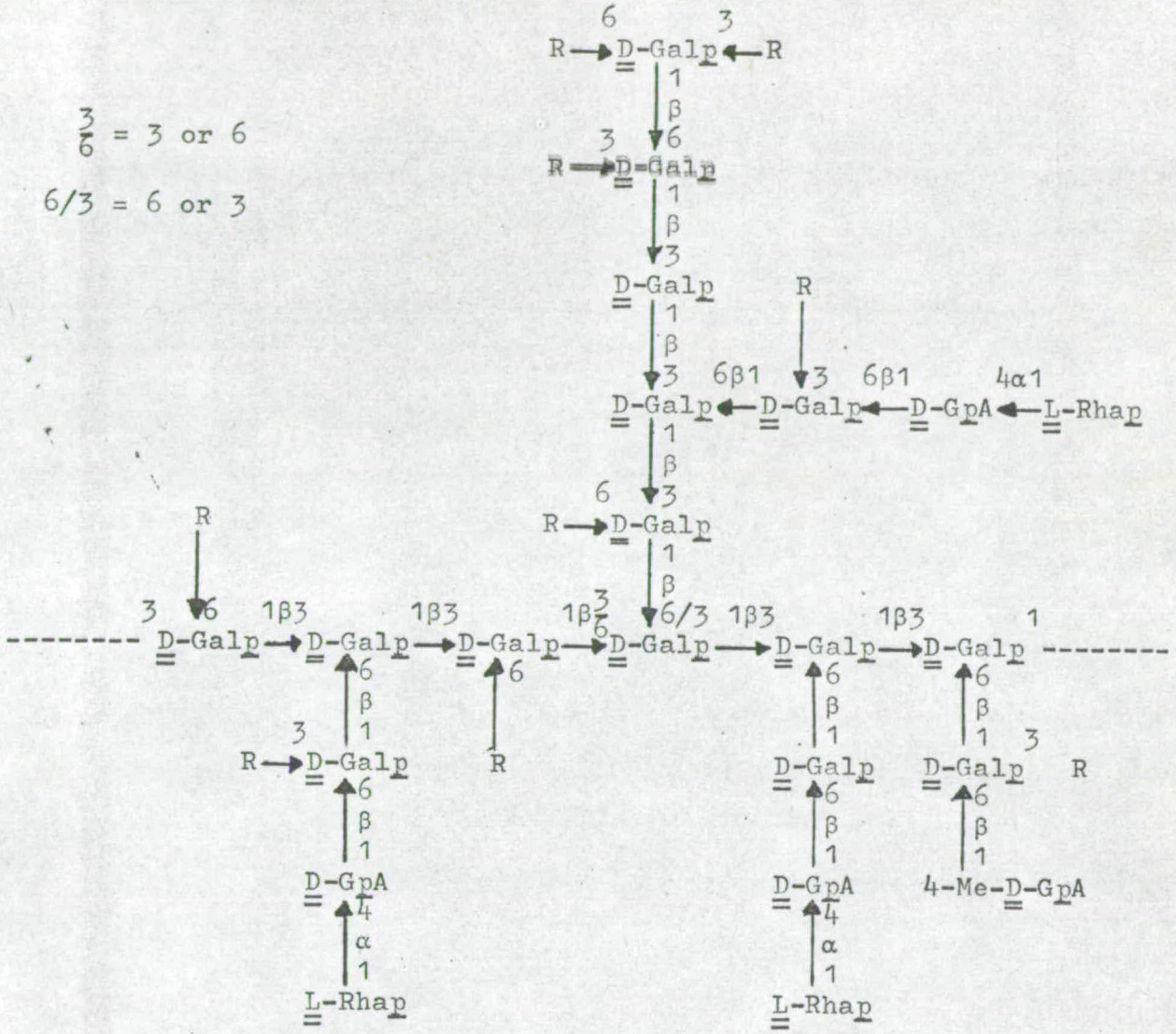
Important structural features have been deduced from a study of the methylated whole gum[48] and of the methylated degraded gum,[49,50] obtained after autohydrolysis and methylation. Hydrolysis of the methylated degraded gum gave 2,3,4,6-tetra-, 2,3,4-tri- and 2,4-di-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-glucuronic acid in the approximate molar proportions 1:5:3:3. The isolation of 6-O-(2,3,4-tri-O-methyl-β-D-gluco-

pyranosyluronic acid)-2,3,4-tri-O-methyl-D-galactose[50] from the methylated degraded gum by controlled acid hydrolysis limited the number of possible structural formulae which could be advanced for the degraded gum. These structural formulae were based on branched frameworks of 1,3- and 1,6-linked D-galactose residues. Results from four successive Barry degradations[51] on the whole gum,[52,53] and from a Smith degradation on the autohydrolysed gum[54] led to the idea that the molecular structure of A.senegal gum was based on a "main chain" of 1,3-linked D-galactose units.

In a recent reinvestigation[1] of A.senegal gum, the whole gum was subjected to seven successive Smith degradations and the methylated autohydrolysed gum was examined. As a result of these investigations, the workers concluded that unequivocal proof for a "main chain" of β1,3-linked D-galactose units was lacking and suggested Figure 1 as a possible structural fragment in A.senegal gum.

It has become apparent that gum obtained from different trees of the same Acacia species show significant analytical differences. In a study of nine gum nodules obtained from nine individual trees of A.nilotica,[55] the nitrogen content ranged from 0.01% to 0.03%, the methoxyl content from 0.71% to 1.30% and the specific rotation from +106° to +109°. A study has also been made on authenticated gum samples from single trees of A.laeta.[2] Analysis on the purified material showed that the nitrogen content ranged from 0.56% to 0.94%, the methoxyl content from 0.26% to 0.56% and the specific rotation from -29° to -42°.

In this Section three samples of A.senegal gum are compared. Periodate oxidation and methylation analysis are used in order to ascertain whether analytical differences between the samples are



R represents D-galactopyranose-, L-arabinopyranose-, and L-arabinofuranose-containing side chains. These side chains may be up to four units long and contain ····^{3}L-Araf^{1}···· residues. They may be terminated by L-Araf^{1}····, L-Arap$^{1\beta3}$L-Araf^{1}····, or D-Galp$^{1\alpha3}$L-Araf^{1}···· residues.

Figure 1. Possible structural fragment in Acacia senegal gum.

accompanied by differences in molecular structure.

3.2) Results.

Purification of the gum samples.- All the samples were collected by the Gum Research Officer, Department of Forests, Republic of Sudan. The nodules of A.senegal (L.) Willd. gum were collected at Quala en Nahal, Kassala Province, as the first collection of the 1960 gum season. The nodules of A.senegal Hennawi gum were collected at Goz el Gonzara, Kordofan Province, in March 1963, as natural exudate from the main stem of trees. The sample of A.senegal insect-damaged gum was collected at Goz el Gonzara, Kordofan Province, in January 1961.

A.senegal gum (25 g.) was dissolved in water (500 ml.), filtered, dialysed and electrodialysed. The polysaccharide, Sample I, was isolated as the freeze-dried product. A.senegal Hennawi gum (25 g.) was purified in the same way to yield the polysaccharide, Sample II, as the freeze-dried product. The yields of Samples I and II were 23 g. and 23.5 g. respectively.

A.senegal insect-damaged gum (180 g.) was received in the form of hard dark brown masses of gum mixed with wood borings and debris. Half the crude material was stirred with water (750 ml.) for 48 hours, filtered, dialysed and electrodialysed. The polysaccharide, Sample IIIa, was isolated as the freeze-dried product (33.4 g.). The rest of the crude material was stirred with water (750 ml.) for 24 hours and filtered. The first extract was dialysed and electrodialysed, and the polysaccharide, Sample IIIb, isolated as the freeze-dried product (27.7 g.). The residue of the crude material was extracted further with water (500 ml.), filtered,

Table 1.

Analytical data for Samples I - IIIc.

	I	II	IIIa	IIIb	IIIc
Moisture %	12.8	10.0	7.0	8.3	7.9
Ash %	0.04	0.01	0.02	0.015	0.02
Nitrogen %	0.33	0.35	0.25	0.21	0.28
Protein % (N% x 6.25)	2.2	2.2	1.6	1.3	1.8
Intrinsic viscosity[a] ($cm^3 g^{-1}$)	19.9	20.6	16.4	15.8	20.1
$[\alpha]_D$ (c, 1.0 water)[a]	-31.0°	-27.6°	-30.5°	-31.3°	-31.5°
Methoxyl %[b,c]	0.25	0.25	0.25	0.25	0.25
Equivalent weight[b]	1085	1047	1066	1065	1060
Uronic acid %[b] (17600/E.W.)	16.2	16.8	16.5	16.5	16.6
Uronic acid %[b,c]	17.2	17.5	17.4	17.3	17.5
Formic acid released on[b] periodate oxidation (mmoles/g.)	1.62	1.69	1.76	1.85	1.90
Periodate consumed on[b] periodate oxidation (mmoles/g.)	5.07	5.18	5.23	5.34	5.42
$\frac{\text{Periodate consumption}}{\text{Formic acid released}}$[b]	3.13	3.06	2.97	2.89	2.85
Galactose %[b]	41	46	43	43	44
Arabinose %[b]	28	30	27	27	26
Rhamnose %[b,c]	13.1	6.2	13.0	12.6	12.8
Glucuronic acid %[b]	15.5	16.5	15.5	15.5	15.5
4-O-methylglucuronic[b] acid %	1.5	1.5	1.5	1.5	1.5

a. Values are corrected for moisture contents.
b. Values are corrected for moisture and protein contents; sugar percentages are for the anhydro-form.
c. Determined by vapour-phase infra-red method.

Table 2.

Data for O-methyl derivatives of Samples I—IIIc.

	I	II	IIIa	IIIb	IIIc
Yield (mg.)	250	240	250	260	240
$[\alpha]_D$ (c, 1.0 $CHCl_3$)	-46.5°	-42.0°	-45.3°	-46.0°	-45.6°
Methoxyl %	41.5	42.0	42.1	41.6	41.5

dialysed and electrodialysed. The polysaccharide, Sample IIIc, was isolated as the freeze-dried product (8.9 g.). The total amount of purified A.senegal insect-damaged gum obtained (70 g.) represents a 39% yield.

Samples I - IIIc migrated as single bands on electrophoresis on cellulose acetate film in both 0.1M-ammonium carbonate buffer (pH 8.9) and 0.1M-acetate buffer (pH 4.7), and had the same mobility when run under comparable conditions. Samples I - IIIc were chromatographed on a D.E.A.E.-cellulose column (45 x 1.3 cm.).[30] Each Sample, on gradient elution with sodium chloride solution (0→0.5M) in 0.02M-acetate buffer (pH 4.1), yielded a single slightly asymmetric peak for its elution pattern.

Analytical comparison of Samples I - IIIc.- Hydrolysis of Samples I - IIIc with N-sulphuric acid followed by chromatographic examination of the hydrolysates, showed the presence of galactose, arabinose, rhamnose and two aldobiouronic acids with the chromatographic mobilities of 6-O-(β-D-glucopyranosyluronic acid)-D-galactose [R_{Gal} 0.23 in solvent (b)] and 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose [R_{Gal} 0.57 in solvent (b)].

Analytical data for Samples I - IIIc are shown in Table 1. If the methoxyl groups are taken to be located solely in 4-O-methyl-D-glucuronic acid units, the percentage of 4-O-methyl-D-glucuronic acid in the

Table 3.

Examination of methanolysis and hydrolysis products from O-methyl derivatives of Samples I - IIIc.

O-Methyl sugars	Relative retention times, T, of methyl glycosides on columns:- (i)	(ii)	R_G in solvent (d)
2,3,4-Me_3-L-rhamnose	0.48	(0.51)[a]	1.01
2,3,5-Me_3-L-arabinose	0.58; 0.72	(0.51)[a]; 0.64	0.97
2,3,4-Me_3-L-arabinose	1.00	0.85	0.79
2,5-Me_2-L-arabinose	1.79; 3.16	1.29; (2.21)[a]	0.82
2,3,4,6-Me_4-D-galactose	1.68	1.64	0.88
2,4,6-Me_3-D-galactose	3.77; 4.22	3.01; 3.51	0.74
2,3,4-Me_3-D-galactose	6.35	5.14	0.74
2,4-Me_2-D-galactose	14.6; 16.4	9.7; 11.0	0.53
2,3,4-Me_3-D-glucuronic acid[b]	2.35; 2.94	(2.21)[a]; 2.65	-
2,3-Me_2-D-glucuronic acid[b]	7.9; 9.3	5.8; 6.9	-
2-Me-D-galactose	-	-	0.34
Unknown sugars	1.18; 1.44	0.98; 1.13; 2.50	-

a. Figures in parentheses indicate T values of components not completely resolved.
b. As methyl ester methyl glycoside.

polysaccharides can be calculated. The percentages of the five constituent sugars in the polysaccharides, calculated as anhydro-sugar residues, are therefore shown in Table 1. Periodate oxidation of Samples I - IIIc were followed by simultaneous estimation of formic acid and sodium metaperiodate in aliquots (2 ml.) of oxidation mixtures at increasing times of oxidation. Oxidation of all the Samples was complete after 48 hours.

Methylation of Samples I - IIIc.- Samples I - IIIc (300 mg. polysaccharide) were methylated successively with dimethyl sulphate and

sodium hydroxide solution, and with methyl iodide and silver oxide. Yields, specific rotations and methoxyl contents of the methylated products are shown in Table 2. Methoxyl contents were not raised on further attempted methylation. On methanolysis and g.l.c. examination of the mixture of methyl glycosides, the methylated product of each Sample gave identical traces using columns (i) and (ii), except that the methylated product of A.senegal Hennawi gum yielded less 2,3,4-tri-O-methyl-L-rhamnose and more 2,3,4-tri-O-methyl-D-glucuronic acid than the other methylated products. Results of g.l.c. examination are shown in Table 3; retention times on columns (i) and (ii) were comparable with those for methyl glycosides from authentic O-methyl sugars. A portion of each methanolysate was hydrolysed with N-sulphuric acid for 4 hours on a boiling water bath. The cooled solutions were neutralised with barium carbonate, filtered, treated with Amberlite resin IR120 (H) and concentrated. Paper chromatographic examination of each hydrolysate in solvents (d) and (g) showed the presence of 2-O-methylgalactose in addition to those O-methyl sugars already identified by g.l.c. examination of their methyl glycosides (Table 3).

Smith degradation[56,57] of Samples I - IIIc.- Samples I - IIIc (2.0 g. polysaccharide) were dissolved in water (50 ml.), and 0.25M-sodium meta-periodate (50 ml.) was added. The oxidation mixtures were left in darkness at room temperature for 48 hours. The reactions were stopped by addition of ethylene glycol (2 ml.), and the solutions were dialysed against running tap water for 48 hours. Sodium borohydride (0.5 g.) was added to each solution, and the solutions were kept at room temperature for 30 hours, then dialysed for a further 48 hours. The five polyalcohols were hydrolysed in N-sulphuric acid at room temperature for 48 hours, after which the

Table 4.

Analytical data for the Smith-degraded polysaccharides of Samples I - IIIc.

	I	II	IIIa	IIIb	IIIc
Moisture %	11.2	10.7	10.3	11.5	11.0
Nitrogen %	0.00	0.01	0.00	0.00	0.01
Yield of polysaccharide[a]	1.34 g.	1.26 g.	1.34 g.	1.32 g.	1.31 g.
Yield %[a]	67	63	67	66	65
$[\alpha]_D$ (c, 1.0 water)[a]	-28.2°	-28.0°	-29.1°	-28.0°	-28.5°
$\overline{M_n}$[b]	98000	96000	93000	96000	93000
Uronic acid %[a,c]	3.7	1.6	4.4	3.8	4.3
Galactose %[a]	70	71	70	70	69
Arabinose %[a]	26	27	26	26	27
Formic acid released on[a] periodate oxidation (mmoles/g.)	1.85	1.80	1.83	1.78	1.75
Periodate consumed on[a] periodate oxidation (mmoles/g.)	5.53	5.45	5.31	5.29	5.42
$\frac{\text{Periodate consumption}}{\text{Formic acid released}}$[a]	2.99	3.03	2.89	2.97	3.09

a. Values are corrected for all non-carbohydrate material.
b. Estimated by molecular-sieve chromatography.
c. Determined by a vapour-phase infra-red method.

solutions were dialysed against running tap water for 48 hours to remove sulphuric acid and small carbohydrate fragments. The Smith-degraded polysaccharides were isolated as freeze-dried products. Yields and analytical data for the Smith-degraded polysaccharides are shown in Table 4. The number-average molecular weights, $\overline{M_n}$, of the Smith-degraded polysaccharides were estimated by molecular-sieve chromatography on a previously calibrated Bio-Gel P300 column (45 x 5.0 cm.).[27,28,29]

Table 5.

Data for O-methyl derivatives of Smith-degraded polysaccharides of Samples I - IIIc.

	I	II	IIIa	IIIb	IIIc
Yield (mg.)	220	212	220	215	225
$[\alpha]_D$ (c, 1.0 $CHCl_3$)	-41.3°	-40.0°	-41.4°	-41.0°	-42.1°
Methoxyl %	40.5	40.9	41.1	40.9	40.5

Hydrolysis of the Smith-degraded A.senegal Hennawi gum with N-sulphuric acid on a boiling water bath, followed by paper chromatographic examination of the hydrolysate, showed the presence of galactose and arabinose. Hydrolysis of the other Smith-degraded polysaccharides, followed by paper chromatographic examination of the hydrolysates, showed the presence of galactose, arabinose and a trace of an aldobiouronic acid with the chromatographic mobility of 6-O-(β-D-glucopyranosyluronic acid)-D-galactose [R_{Gal} 0.23 in solvent (c)]. Mild acid hydrolysis of the Smith-degraded polysaccharides (0.5N-sulphuric acid for 1 hour on a boiling water bath) followed by paper chromatographic examination, showed the presence of galactose, arabinose and two neutral disaccharides with the chromatographic mobilities of 3-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.49 in solvent (a), and 0.53 in solvent (f)], and 6-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.29 in solvent (a), and 0.38 in solvent (f)].

Methylation of the Smith-degraded polysaccharides.- Smith-degraded Samples I - IIIc (300 mg., polysaccharide) were methylated successively with dimethyl sulphate and sodium hydroxide solution, and with methyl iodide and silver oxide. Yields, specific rotations and methoxyl contents of the methylated products are shown in Table 5. Methoxyl contents were not

Table 6.

Examination of methanolysis and hydrolysis products from methylated Smith-degraded polysaccharides.

O-Methyl sugars	Relative retention times, T, of methyl glycosides on columns:- (i)	(ii)	R_G in solvent (d)
2,3,5-Me_3-L-arabinose	0.57; 0.73	0.50; 0.64	0.97
2,5-Me_2-L-arabinose	1.79; 3.15	1.30; (2.21)[a]	0.82
2,3,4,6-Me_4-D-galactose	1.68	1.65	0.88
2,4,6-Me_3-D-galactose	3.78; 4.22	2.99; 3.50	0.74
2,3,4-Me_3-D-galactose	6.34	5.14	0.74
2,6-Me_2-D-galactose	9.1	6.5; 6.9; 7.7	0.59
2,4-Me_2-D-galactose	14.6; 16.4	9.7; 11.0	0.53
2,3,4-Me_3-D-glucuronic acid[b]	2.35; 2.95	(2.21)[a]; 2.66	-
2-Me-D-galactose	-	-	0.34
Unknown sugars	1.08; 1.52	0.86; 2.48	-

a. Figures in parentheses indicate T values of components not completely resolved.
b. As methyl ester methyl glycoside.

raised on further attempted methylation. On methanolysis and g.l.c. examination of the mixtures of methyl glycosides, the methylated product from each Smith-degraded polysaccharide gave identical traces using columns (i) and (ii), except that the methylated product from Smith-degraded A.senegal Hennawi gum yielded no 2,3,4-tri-O-methyl-D-glucuronic acid. Results of g.l.c. examination are shown in Table 6. A portion of each methanolysate was hydrolysed with N-sulphuric acid for 4 hours on a boiling water bath. The cooled solutions were neutralised with barium carbonate, filtered, treated with Amberlite resin IR120 (H) and concentrated. Paper

chromatographic examination of the hydrolysates in solvents (d) and (g) showed the presence of 2-O-methylgalactose in addition to those O-methyl sugars already identified by g.l.c. examination of their methyl glycosides (Table 6).

3.3) Discussion.

The analytical data show that the A.senegal insect-damaged gum Samples (Samples IIIa - IIIc) are almost identical to normal A.senegal gum. The nitrogen contents and the intrinsic viscosities, $[\eta]$, of the insect-damaged gum Samples are slightly lower than those for normal A.senegal gum. However for properties which are dependent on the polysaccharide part of the gum, the insect-damaged gum Samples and normal A.senegal gum are very similar.

The A.senegal Hennawi gum Sample is interesting because its rhamnose content is half that of normal A.senegal gum. The relatively high specific rotation of the Hennawi Sample could be connected with this low rhamnose content. The other analytical data for the Hennawi Sample show little difference from those of normal A.senegal gum.

Methylation of the different Samples followed by methanolysis and g.l.c. examination gave identical results, except that the content of 2,3,4-tri-O-methyl-L-rhamnose was lower, and the content of 2,3,4-tri-O-methyl-D-glucuronic acid was higher for the O-methyl derivative of the Hennawi Sample. The presence of 2-O-methylgalactose is ascribed to undermethylation. In spite of these differences, methylation analysis shows that the overall molecular structures of the insect-damaged gum Samples and the Hennawi gum Sample are identical to that of normal

A.senegal gum.

On Smith degradation Samples I - IIIc gave similar yields of degraded polysaccharide. The Smith-degraded polysaccharides of A.senegal gum and the three A.senegal insect-damaged gum Samples are analytically identical, and have uronic acid contents in the region of 4%. The isolation and characterisation, after deacetylation, of 4-O-α-L-rhamnopyranosyl-D-glucose from acetolysis of diborane-reduced acetylated A.senegal gum, established that some L-rhamnopyranose residues are glycosidically linked to C-4 of D-glucuronic acid.[46] Complete oxidation of all glucuronic acid residues would be expected during treatment with periodate; the incomplete oxidation observed may result from steric hindrance.[58] The Smith-degraded product from A.senegal Hennawi gum contains no uronic acid residues. Steric hindrance to periodate oxidation is probably smaller in the case of A.senegal Hennawi gum, because of the lower rhamnose content. The number-average molecular weights, $\overline{M_n}$, of the five Smith-degraded products, as estimated by molecular-sieve chromatography, are of the same order. Methylation analysis of the five Smith-degraded products gave identical results, except that the O-methyl derivative of Smith-degraded A.senegal Hennawi gum contained no 2,3,4-tri-O-methyl-D-glucuronic acid. This confirms that the overall molecular structures of Samples I - IIIc are identical. The presence of 2,6-di- and 2-O-methyl-D-galactose in the methanolysates of the O-methyl derivatives of the Smith-degraded products is ascribed to undermethylation. Undermethylation at C-4 of D-galactose residues has been found previously in a Smith degradation study of A.senegal gum.[1]

Polymeric carbohydrates such as starch, mucilages and gums, cannot be

expected to be translocated in plants.[59] Thus the biosynthesis of gums must occur near the site of exudation. The low rhamnose content of A.senegal Hennawi gum (natural exudate from the main stem of trees) compared with that of normal A.senegal gum (natural or tapped exudate from branches of trees), suggests that the synthesising enzymes for the exudate gum differ in different parts of the tree. The situation is reminiscent of the different types of glycogen found in mammalian tissues. Liddle and Manners[60] found that the average chain length of rabbit muscle glycogen was less than that of rabbit liver glycogen. Slight differences in the synthesising enzymes of glycogen from rabbit muscle and rabbit liver probably cause this structural variation.

The small differences found between A.senegal Hennawi gum and normal A.senegal gum suggests that normal A.senegal gum might be to some extent heterogeneous, since gum exuded by old branches might have different rhamnose contents from gum exuded by young branches. An analytical comparison of A.senegal gum nodules obtained from branches of different ages would give an indication whether such structural differences occur. Gum from other Acacia species should also be examined to ascertain whether it is general that gum exuded by main stems is depleted in rhamnose.

SECTION IV

SMITH DEGRADATION STUDY OF THE GUM EXUDATE OF ACACIA LAETA VAR. HASHAB

4.1) Introduction.

Acacia laeta R. Br. ex Benth. is a natural hybrid of *Acacia senegal* (L.) Willd. and *Acacia mellifera* (Vahl.) Benth., and it occurs in two varieties: *Acacia laeta* var. *hashab* (which resembles *Acacia senegal*) and *Acacia laeta* var. *mellifera* (which resembles *Acacia mellifera*). *A.laeta* var. *hashab* gum is an acidic polysaccharide containing D-galactose, L-arabinose, L-rhamnose, D-glucuronic acid and 4-O-methyl-D-glucuronic acid. Prolonged acid hydrolysis of the gum has led to the isolation and characterisation[2] of two aldobiouronic acids, viz., 6-O-(β-D-glucopyranosyluronic acid)-D-galactose and 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose. Experiments utilizing partial acid hydrolysis have shown that 3-O-α-D-galactopyranosyl-L-arabinose, 3-O-β-L-arabinopyranosyl-L-arabinose, 3-O-β-L-arabinofuranosyl-L-arabinose, 3-O-β-D-galactopyranosyl-D-galactose and 6-O-β-D-galactopyranosyl-D-galactose are constituent units of the gum.[3]

Study of methylated whole gum and of methylated autohydrolysed gum has revealed important structural features. Methanolysis of the methylated autohydrolysed gum gave the methyl glycosides of 2,3,5- (trace) and 2,3,4-tri-O-methyl-L-arabinose (trace), 2,3,4,6-tetra- (++), 2,4,6- (++) and 2,3,4-tri- (++), 2,4- (+++) and 2,6-di- (trace) and 2-O-methyl-D-galactose (+), and 2,3,4-tri-O-methyl-D-glucuronic acid (+++).[3] This is evidence for direct attachment of arabinofuranosyl and arabinopyranosyl residues to the

galactan framework. Only arabinofuranosyl residues are attached directly to the galactan framework of A.senegal gum.[1] Smith degradation of the autohydrolysed gum[3] gave results similar to those obtained in an identical experiment[61] on autohydrolysed A.senegal gum.

The structural evidence obtained in these experiments show the close similarity of A.laeta gum and A.senegal gum. In a recent investigation,[1] A.senegal gum was subjected to seven successive Smith degradations, and the degraded polysaccharides were examined by partial acid hydrolysis and methylation analysis. In this Section a similar investigation of A.laeta gum is carried out, with a view to determine any differences in overall molecular structure between A.laeta gum and A.senegal gum.

The sample of gum from A.laeta var. hashab used in this investigation was collected by the Gum Research Officer to the Republic of Sudan, from botanically authenticated trees at the El Ain Forest Reserve in November 1962.

4.2) Results.

Purification of A.laeta gum.- The same sample of purified gum was used in this study as was used in the previous structural study of A.laeta gum.[2,3] The gum had $[\alpha]_D$ -42° (c, 1.0)(Found: limiting viscosity number, 20.7; N, 0.65%; OMe, 0.35%; equivalent weight, 1250; uronic acid, 14.0%; galactose, 44%; arabinose, 29%; rhamnose, 13%; all sugar percentages are for the anhydro-forms and are corrected for moisture and protein contents).

The gum migrated as a single band on electrophoresis on strips (18 x 5cm.) of cellulose acetate film in both 0.1M-ammonium carbonate buffer (pH 8.9) and 0.1M-acetate buffer (pH 4.7) at field strengths between 15 and 20

Table 1.

Examination of methanolysis and hydrolysis products from the O-methyl derivative of A.laeta gum.

O-Methyl sugars	Relative retention times, T, of methyl glycosides on columns:- (i)	(ii)	R_G in solvent (d)
2,3,4-Me_3-L-rhamnose	0.50	(0.52)[a]	1.01
2,3,5-Me_3-L-arabinose	0.60; 0.72	(0.52)[a]; 0.62	0.98
2,3,4-Me_3-L-arabinose	1.01	0.82	0.78
2,5-Me_2-L-arabinose	1.81; 3.18	1.27; (2.16)[a]	0.80
2,3,4,6-Me_4-D-galactose	1.70	1.67	0.88
2,4,6-Me_3-D-galactose	3.83; 4.27	2.92; 3.45	0.72
2,3,4-Me_3-D-galactose	6.66	5.09	0.72
2,4-Me_2-D-galactose	14.7; 16.6	9.8; 11.2	0.52
2,3,4-Me_3-D-glucuronic acid[b]	2.34; 2.91	(2.16)[a]; 2.70	-
2,3-Me_2-D-glucuronic acid[b]	7.8; 9.5	5.8; 6.8	-
2-Me-D-galactose	-	-	0.32
Unknown sugars	1.19; 1.45	0.98; 1.13	-

a. Figures in parentheses indicate T values of components not completely resolved.
b. As methyl ester methyl glycoside.

volts/cm. for 2-4 hours. The gum was chromatographed on a D.E.A.E.-cellulose column (35 x 1.5 cm.).[30] Gradient elution with sodium chloride solution (0.02M → 0.50M) buffered at pH 6, yielded a single slightly asymmetric peak for its elution pattern.[3] On molecular-sieve chromatography using a previously calibrated Bio-Gel P300 column,[27,28,29] A.laeta gum was eluted at the void volume. The number-average molecular weight is therefore over 200,000.

Methylation of A.laeta gum.- The gum (500 mg.) was methylated to give a product (410 mg.), $[\alpha]_D$ -49° (c, 1.0 in $CHCl_3$)(Found: OMe, 42.1%, not raised on further attempted methylation). Methanolysis followed by g.l.c. examination of the mixture of methyl glycosides gave the results shown in Table 1. Hydrolysis of the methyl glycosides followed by paper chromatography in solvent (d) and (g) indicated the presence of 2-O-methyl-D-galactose, in addition to those O-methyl sugars already characterised by g.l.c. examination of their methyl glycosides.

Preparation, partial acid hydrolysis and methylation of polysaccharide I.- The gum (28.3 g. polysaccharide) was dissolved in water (700 ml.), mixed with 0.25M-sodium metaperiodate solution (700 ml.) and kept at room temperature for 48 hours. The amount of formic acid released in this time was 1.40 mmoles/g. The corresponding amount of periodate consumed was 5.24 mmoles/g. The reaction was stopped by addition of ethylene glycol (15 ml.), and the solution dialysed against running tap water for 48 hours. Sodium borohydride (7.5 g.) was added, and the mixture kept at room temperature for 30 hours, then dialysed for a further 48 hours. The polyalcohol was hydrolysed in N-sulphuric acid at room temperature for 48 hours. After dialysis against running tap water for 48 hours, polysaccharide I was isolated as the freeze-dried product (17.0 g.; yield, 60%), $[\alpha]_D$ -18° (c, 0.82)(Found: uronic acid, 4%; galactose, 68%; arabinose, 28%). Hydrolysis of polysaccharide I with N-sulphuric acid for 7 hours on a boiling water bath gave a trace of 6-O-(β-D-glucopyranosyluronic acid)-D-galactose [R_{Gal} 0.22 in solvent (b)] in addition to galactose and arabinose. Molecular-sieve chromatography of polysaccharide I (20 mg.) on a previously calibrated Bio-Gel P300 column

(45 x 4.8 cm.) gave $\overline{M}_n$ *ca.* 91,000.

Polysaccharide I (20 mg.) was hydrolysed with 0.5N-sulphuric acid (10 ml.) for 1 hour on a boiling water bath. Paper chromatographic examination of the hydrolysate indicated the presence of galactose, arabinose and two neutral disaccharides with the mobilities of 3-*O*-β-*D*-galactopyranosyl-*D*-galactose [R_{Gal} 0.49 in solvent (a), and 0.54 in solvent (f), minor component] and 6-*O*-β-*D*-galactopyranosyl-*D*-galactose [R_{Gal} 0.31 in solvent (a), and 0.38 in solvent (f), major component].

Polysaccharide I (200 mg.) was methylated to give a product (125 mg.), $[\alpha]_D$ -43° (*c*, 1.0 in $CHCl_3$)(Found: OMe, 39.5%, not raised on further attempted methylation). Methanolysis of a sample of this product, followed by g.l.c. examination of the mixture of methyl glycosides, gave the results shown in Table 2. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g) indicated the presence of 2-*O*-methylgalactose, in addition to those *O*-methyl sugars already characterised by g.l.c. of their methyl glycosides (Table 2).

Preparation, partial acid hydrolysis and methylation of polysaccharide II.- Borohydride reduction of periodate oxidised polysaccharide I (16.5 g.) followed by controlled acid hydrolysis at room temperature for 48 hours, gave polysaccharide II (8.75 g.; yield, 53%), $[\alpha]_D$ +4° (*c*, 0.96)(Found: galactose, 91%; arabinose, 9%). The amount of formic acid released during periodate oxidation was 2.06 mmoles/g. The corresponding amount of periodate consumed was 5.00 mmoles/g. Molecular-sieve chromatography indicated that polysaccharide II had $\overline{M}_n$ *ca.* 53,000.

Partial acid hydrolysis of polysaccharide II (20 mg.), followed by paper chromatographic examination of the hydrolysate indicated the presence

of galactose, arabinose and equal amounts of 3-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.47 in solvent (a), and 0.53 in solvent (f)] and 6-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.30 in solvent (a), and 0.38 in solvent (f)].

Polysaccharide II (193 mg.) was methylated to give a product (120 mg.), $[\alpha]_D$ -33° (c, 1.0 in $CHCl_3$)(Found: OMe, 41.3%, not raised on further attempted methylation). Methanolysis of a sample of this product, followed by g.l.c. examination of the mixture of methyl glycosides, gave the results shown in Table 2. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g) indicated the presence of 2-O-methylgalactose, in addition to those O-methyl sugars already characterised by g.l.c. of their methyl glycosides (Table 2).

Preparation, partial acid hydrolysis and methylation of polysaccharide III.- Borohydride reduction of periodate oxidised polysaccharide II (8.35 g.), followed by controlled acid hydrolysis at room temperature for 48 hours, gave polysaccharide III (5.0 g.; yield, 60%), $[\alpha]_D$ +20° (c, 1.65)(Found: galactose, 97%; arabinose, 3%). The amount of formic acid released during periodate oxidation was 1.62 mmoles/g. The corresponding amount of periodate consumed was 4.14 mmoles/g. Molecular-sieve chromatography indicated that polysaccharide III had $\overline{M_n}$ ca. 26,000.

Partial acid hydrolysis of polysaccharide III (20 mg.), followed by paper chromatographic examination of the hydrolysate indicated the presence of galactose, arabinose, two disaccharides with mobilities of 3-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.48 in solvent (a), and 0.54 in solvent (f), major component] and 6-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.31 in solvent (a), and 0.38 in solvent (f), minor component], and

two trisaccharides with mobilities of the β1,3-linked galactotriose [R_{Gal} 0.22 in solvent (a), and 0.23 in solvent (f), major component] and the β1,6-linked galactotriose [R_{Gal} 0.15 in solvent (a), and 0.18 in solvent (f), minor component].

Polysaccharide III (165 mg.) was methylated to give a product (80 mg.), $[\alpha]_D$ -11° (c, 0.5 in $CHCl_3$)(Found: OMe, 39.1%, not raised on further attempted methylation). Methanolysis of a sample of this product, followed by g.l.c. examination of the mixture of methyl glycosides, gave the results shown in Table 2. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g) indicated the presence of 2-O-methylgalactose, in addition to those O-methyl sugars already characterised by g.l.c. of their methyl glycosides (Table 2).

Preparation, partial acid hydrolysis and methylation of polysaccharide IV.- Borohydride reduction of periodate oxidised polysaccharide III (4.6 g.), followed by controlled acid hydrolysis at room temperature for 48 hours, gave polysaccharide IV (3.0 g.; yield, 65%), $[\alpha]_D$ +26.5° (c, 1.58)(Found: galactose, 97%; arabinose, 3%). The amount of formic acid released during periodate oxidation was 1.25 mmoles/g. The corresponding amount of periodate consumed was 2.81 mmoles/g. Molecular-sieve chromatography indicated that polysaccharide IV had $\overline{M_n}$ ca. 20,000.

Partial acid hydrolysis of polysaccharide IV (10 mg.), followed by paper chromatographic examination of the hydrolysate indicated the presence of galactose, arabinose and the same oligosaccharides as detected after mild acid hydrolysis of polysaccharide III.

Polysaccharide IV (158 mg.) was methylated to give a product (85 mg.), $[\alpha]_D$ -1° (c, 0.5 in $CHCl_3$)(Found: OMe, 37.2%, not raised on further

Table 2.

O-Methyl sugars present in methylated polysaccharides I - VI and VIII.

O-Methyl sugar	I	II	III	IV	V	VI	VIII	Approximate relative molar proportions in V	VI	VIII
2,3,5-Me_3-L-arabinose	+	+	+	+						
2,5-Me_2-L-arabinose	+	+	+							
2,3,4,6-Me_4-D-galactose	+	+	+	+	+	+	+	1	1	1
2,4,6-Me_3-D-galactose	+	+	+	+	+	+	+	14	16	18
2,3,4-Me_3-D-galactose	+	+	+	+	+	+	+	trace	trace	trace
2,6-Me_2-D-galactose	+	+	+	+	+	+	+	3	1	trace
2,4-Me_2-D-galactose	+	+	+	+	+	+	+	1	1	1
2,3,4-Me_3-D-glucuronic acid	*									

attempted methylation). Methanolysis of a sample of this product, followed by g.l.c. examination of the mixture of methyl glycosides, gave the results shown in Table 2. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g) indicated the presence of 2-O-methylgalactose, in addition to those O-methyl sugars already characterized by g.l.c. of their methyl glycosides (Table 2).

Preparation, partial acid hydrolysis and methylation of polysaccharide V.- Borohydride reduction of periodate oxidised polysaccharide IV (2.6 g.), followed by controlled acid hydrolysis at room temperature for 48 hours, gave the galactan, polysaccharide V (1.9 g.; yield, 73%), $[\alpha]_D$ +28° (c, 0.92). The amount of formic acid released during periodate oxidation was 0.69 mmoles/g. The corresponding amount of periodate consumed was 1.47 mmoles/g. Molecular-sieve chromatography indicated that polysaccharide V had $\overline{M_n}$ ca. 16,000.

Partial acid hydrolysis of polysaccharide V (10 mg.), followed by paper chromatographic examination of the hydrolysate, indicated the presence of galactose, 3-O-β-D-galactopyranosyl-D-galactose, 6-O-β-D-galactopyranosyl-D-galactose and the β1,3-linked galactotriose.

Polysaccharide V (184 mg.) was methylated to give a product (76 mg.), $[\alpha]_D$ -1° (c, 0.5 in $CHCl_3$)(Found: OMe, 37.1%, not raised on further attempted methylation). Methanolysis of a sample of this product, followed by g.l.c. examination of the mixture of methyl glycosides, gave the results shown in Table 2. Hydrolysis of the methyl glycosides, followed by paper chromatography in solvents (d) and (g), indicated the presence of 2-O-methylgalactose, in addition to those O-methyl sugars already characterized by g.l.c. of their methyl glycosides (Table 2).

Preparation, partial acid hydrolysis and methylation of polysaccharide VI.- Borohydride reduction of periodate oxidised polysaccharide V (1.55 g.), followed by controlled acid hydrolysis at room temperature for 48 hours, gave polysaccharide VI (1.2 g.; yield, 77%), $[\alpha]_D$ +28° (c, 0.90). The amount of formic acid released during periodate oxidation was 0.63 mmoles/g. The corresponding amount of periodate consumed was 1.43 mmoles/g. Molecular-sieve chromatography indicated that polysaccharide VI had $\overline{M}_n$ ca. 14,000.

Partial acid hydrolysis of polysaccharide VI (10 mg.), followed by paper chromatographic examination of the hydrolysate, indicated the presence of galactose, 3-O-β-D-galactopyranosyl-D-galactose, 6-O-β-D-galactopyranosyl-D-galactose (a trace), and the β1,3-linked galactotriose.

Polysaccharide VI (180 mg.) was methylated to give a product (65 mg.), $[\alpha]_D$ +1° (c, 0.5 in $CHCl_3$)(Found: OMe, 36.8%, not raised on further

attempted methylation). Methanolysis of a sample of this product, followed by g.l.c. examination of the mixture of methyl glycosides, gave the results shown in Table 2. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g), indicated the presence of 2-O-methylgalactose, in addition to those O-methyl sugars already characterized by g.l.c. of their methyl glycosides (Table 2).

Preparation of polysaccharides VII and VIII.- Borohydride reduction of periodate oxidised polysaccharide VI (0.86 g.), followed by controlled acid hydrolysis at room temperature for 48 hours, gave polysaccharide VII (0.68 g.; yield, 79%). The amount of formic acid released during periodate oxidation was 0.67 mmoles/g. The corresponding amount of periodate consumed was 1.44 mmoles/g. A similar degradation of polysaccharide VII (0.63 g.) gave polysaccharide VIII (0.49 g.; yield, 78%), $[\alpha]_D$ +27° (c, 0.42). The amount of formic acid released during periodate oxidation was 0.43 mmoles/g. The corresponding amount of periodate consumed was 0.95 mmoles/g. Molecular-sieve chromatography indicated that polysaccharides VII and VIII had $\overline{M}_n$ ca. 12,000 and 9,500 respectively.

Partial acid hydrolysis and methylation of polysaccharide VIII.- Partial acid hydrolysis of polysaccharide VIII (10 mg.), followed by paper chromatography in solvents (a) and (f), indicated the presence of galactose, 3-O-β-D-galactopyranosyl-D-galactose, 6-O-β-D-galactopyranosyl-D-galactose (a trace), and the β1,3-linked galactotriose.

Polysaccharide VIII (250 mg.) was methylated to give a product (81 mg.), $[\alpha]_D$ +1° (c, 0.5 in $CHCl_3$)(Found: OMe, 36.5%, not raised on further attempted methylation). Methanolysis of a sample of this product, followed by g.l.c. examination of the mixture of methyl glycosides, gave the results shown in Table 2. Hydrolysis of the methyl glycosides followed

by paper chromatography in solvents (d) and (g) indicated the presence of 2-O-methylgalactose, in addition to those O-methyl sugars already characterized by g.l.c. of their methyl glycosides (Table 2).

Preparation and partial acid hydrolysis of polysaccharide V'.- A.laeta gum (30 g.) was subjected to five successive Smith degradations, without isolation of the intermediate polysaccharides, to obtain polysaccharide V' (2.2 g.). This polysaccharide was hydrolysed with 0.5N sulphuric acid (500 ml.) for 1 hour on a boiling water bath. The hydrolysate was chromatographed on Whatman 3MM papers in solvent (a), and a pure trisaccharide was isolated.

The trisaccharide (44 mg.) had $[\alpha]_D$ +51° (c, 0.88), and yielded galactose and the β1,3-linked galactobiose as the only disaccharide on partial acid hydrolysis. The trisaccharide was crystallised from aqueous ethanol, m.p. and mixed m.p. 237-242° (Lit. m.p.[62] 240-245°). It had R_{Gal} values of 0.22 in solvent (a) and 0.23 in solvent (f).

4.3) Discussion.

Purified A.laeta var. hashab gum was shown to be an acidic polysaccharide containing residues of D-galactose (44%), L-arabinose (29%), L-rhamnose (13%), D-glucuronic acid (10.5%) and 4-O-methyl-D-glucuronic acid (3.5%). A.laeta gum was examined by zone electrophoresis on cellulose acetate film, and by ion-exchange chromatography on D.E.A.E.-cellulose.[30] Since no sharp discontinuities in the properties of the molecular species were indicated by these experiments, it seems probable that A.laeta gum exhibits the same kind of heterogeneity as A.senegal gum[1,61] and may be considered to contain a continuous spectrum of related

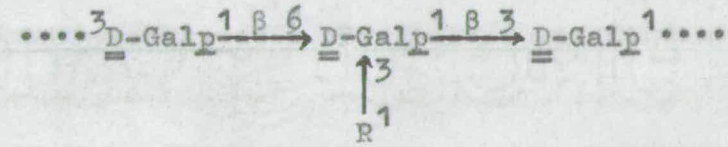
molecular species.

On molecular-sieve chromatography using a previously calibrated Bio-Gel P300 column,[27,28,29] A.laeta gum was eluted at the void volume. Since the viscosity properties of A.laeta gum are similar to those of A.senegal gum, its number-average molecular weight, $\overline{M}_n$, is probably close to the value of 250,000 which Oakley obtained for A.senegal gum.[63,64,65]

In a recent investigation,[3] experiments utilizing partial acid hydrolysis have shown that 6-O-(β-D-glucopyranosyluronic acid)-D-galactose, 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose, 3-O-α-D-galactopyranosyl-L-arabinose, 3-O-β-L-arabinopyranosyl-L-arabinose, 3-O-β-L-arabinofuranosyl-L-arabinose, 3-O-β-D-galactopyranosyl-D-galactose and 6-O-β-D-galactopyranosyl-D-galactose are constituent units of A.laeta gum. Partial acid hydrolyses have shown that 6-O-(β-D-glucopyranosyluronic acid)-D-galactose,[34-39] 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose,[40] 3-O-α-D-galactopyranosyl-L-arabinose,[41,42,43] 3-O-β-L-arabinopyranosyl-L-arabinose,[45] 3-O-β-L-arabinofuranosyl-L-arabinose,[66] 3-O-β-D-galactopyranosyl-D-galactose[1,43,44] and 6-O-β-D-galactopyranosyl-D-galactose[1] are structural units of A.senegal gum.

In the same investigation[3] autohydrolysed A.laeta gum was prepared. This contained galactose (76%), arabinose (3%) and uronic acid (21%). Molecular-sieve chromatography gave an estimated value of 5,700 for the number-average molecular weight of the autohydrolysed gum. The decrease in number-average molecular weight from ca. 250,000 to ca. 5,700 is much greater than can result from the simple removal of arabinose and rhamnose from the molecular periphery, and does not appear to have been caused by the presence of any internal acid-labile arabinofuranosyl residues, since

acid hydrolysis of borohydride reduced autohydrolysed gum yielded galactitol but no arabinitol. There is no evidence for the presence of any galactofuranose residues in the gum, thus, as in the case of A.senegal gum,[1,61] certain galactopyranosidic bonds must be unusually sensitive towards the mild conditions of autohydrolysis.[67,68] Methylation analysis of A.laeta autohydrolysed gum[3] suggested that it had a larger proportion of 1,3-glycosidic links than A.senegal autohydrolysed gum. This could be the reason why autohydrolysed A.laeta gum has a lower specific rotation (-28°) than autohydrolysed A.senegal gum (-11°). Methylation analysis of Smith degraded autohydrolysed A.laeta gum indicated a trace of 2,3,4-tri-O-methyl-D-galactose.[3] This indicates that I is a possible structural



where R = L-Arap^{1}... ; L-Araf^{1}... ; D-Galp^{1}... ; ...^{6}D-Galp^{1}... .

I

fragment of autohydrolysed A.laeta gum.

The methylated gum was subjected to methanolysis, and the mixture of methyl glycosides was analysed by gas-liquid partition chromatography (Table 1). The methyl glycosides of 2,3,4-tri-O-methyl-L-rhamnose, 2,3,5- and 2,3,4-tri- and 2,5-di-O-methyl-L-arabinose, 2,3,4,6-tetra-, 2,4,6- and 2,3,4-tri- and 2,4-di-O-methyl-D-galactose, and 2,3,4-tri- and 2,3-di-O-methyl-D-glucuronic acid were identified. The g.l.c. traces were virtually identical to those obtained by Anderson, Hirst and Stoddart[1] for a methanolysate of methylated A.senegal gum. This indicates there is a strong similarity between the gums of A.senegal and A.laeta var. hashab.

A sample of A.laeta gum was subjected to eight successive Smith

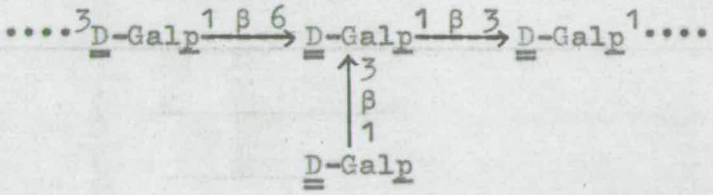
degradations.[56,57] The first Smith degradation yielded polysaccharide I, which contained galactose (68%), arabinose (28%) and glucuronic acid (4%). Acid hydrolysis of polysaccharide I yielded a small amount of 6-O-(β-D-glucopyranosyluronic acid)-D-galactose. The first Smith degradation therefore removed all the rhamnose and 4-O-methylglucuronic acid residues, together with some of the galactose, arabinose and glucuronic acid. Complete oxidation of all glucuronic acid residues would be expected during the first treatment with periodate; the incomplete oxidation observed may result from steric hindrance.[58] On methanolysis, the O-methyl derivative of polysaccharide I gave the methyl glycosides of 2,3,5-tri- and 2,5-di-O-methyl-L-arabinose, and 2,3,4,6-tetra-, 2,4,6- and 2,3,4-tri-, and 2,6- and 2,4-di-, and 2-O-methyl-D-galactose, and 2,3,4-tri-O-methyl-D-glucuronic acid. Thus all the rhamnopyranosyl and arabinopyranosyl residues are removed by one Smith degradation. The presence of 2,6-di- and 2-O-methyl-D-galactose may result from undermethylation at the C-4 position of certain galactose residues. Partial acid hydrolysis of polysaccharide I afforded 3-O-β-D-galactopyranosyl-D-galactose and 6-O-β-D-galactopyranosyl-D-galactose.

A second Smith degradation yielded polysaccharide II, which contained galactose (91%) and arabinose (9%). On methanolysis, the O-methyl derivative of polysaccharide II gave the methyl glycosides of 2,3,5-tri- and 2,5-di-O-methyl-L-arabinose, and 2,3,4,6-tetra-, 2,4,6- and 2,3,4-tri-, 2,6- and 2,4-di- and 2-O-methyl-D-galactose. A Smith degradation of polysaccharide II gave polysaccharide III, which contained galactose (97%) and arabinose (3%). On methanolysis, the O-methyl derivative of polysaccharide III gave the methyl glycosides of 2,3,5-tri- and 2,5-di-

O-methyl-L-arabinose, and 2,3,4,6-tetra-, 2,4,6- and 2,3,4-tri-, 2,6- and 2,4-di- and 2-O-methyl-D-galactose. A Smith degradation of polysaccharide III gave polysaccharide IV, which contained galactose (97%) and arabinose (3%). On methanolysis, the O-methyl derivative of polysaccharide IV gave the methyl glycosides of 2,3,5-tri-O-methyl-L-arabinose, 2,3,4,6-tetra-, 2,4,6- and 2,3,4-tri-, 2,6- and 2,4-di- and 2-O-methyl-D-galactose. Successive Smith degradations then gave polysaccharides V and VI. On methylation and methanolysis, both polysaccharides V and VI gave the methyl glycosides of 2,3,4,6-tetra-, 2,4,6- and 2,3,4-tri-, 2,6- and 2,4-di- and 2-O-methyl-D-galactose. Partial acid hydrolysis of polysaccharides II, III and IV afforded 3-O-β-D-galactopyranosyl-D-galactose, 6-O-β-D-galactopyranosyl-D-galactose, the β1,3-linked galactotriose and the β1,6-linked galactotriose. Partial acid hydrolysis of polysaccharides V and VI afforded 3-O-β-D-galactopyranosyl-D-galactose, 6-O-β-D-galactopyranosyl-D-galactose and the β1,3-linked galactotriose. The amounts of the β1,6-linked galactobiose detected, decreased from polysaccharides II - VI.

Successive Smith degradations of polysaccharide VI gave polysaccharides VII and VIII. On methylation and methanolysis, polysaccharide VIII gave the methyl glycosides of 2,3,4,6-tetra-, 2,4,6- and 2,3,4-tri-, 2,6- and 2,4-di- and 2-O-methyl-D-galactose. Partial acid hydrolysis of polysaccharides VII and VIII afforded the same oligosaccharides as polysaccharides V and VI. The β1,6-linked galactobiose is unlikely to have arisen as a result of acid reversion, since it was not detectable on paper chromatography after prolonged acid hydrolysis. The presence of trace amounts of 2,3,4-tri-O-methyl-D-galactose in the

methanolysate of the O-methyl derivative of polysaccharide VIII indicates that there are a few D-galactose residues which are only 6-O-substituted in polysaccharide VIII. This shows that II is a possible structural



II

fragment of polysaccharide VII. The Smith degraded polysaccharides were quite difficult to methylate as is seen by the relatively low methoxyl contents of the methylated products. The presence of 2,6-di- and 2-O-methyl-D-galactose after methanolysis and hydrolysis of these products is therefore ascribed to undermethylation.

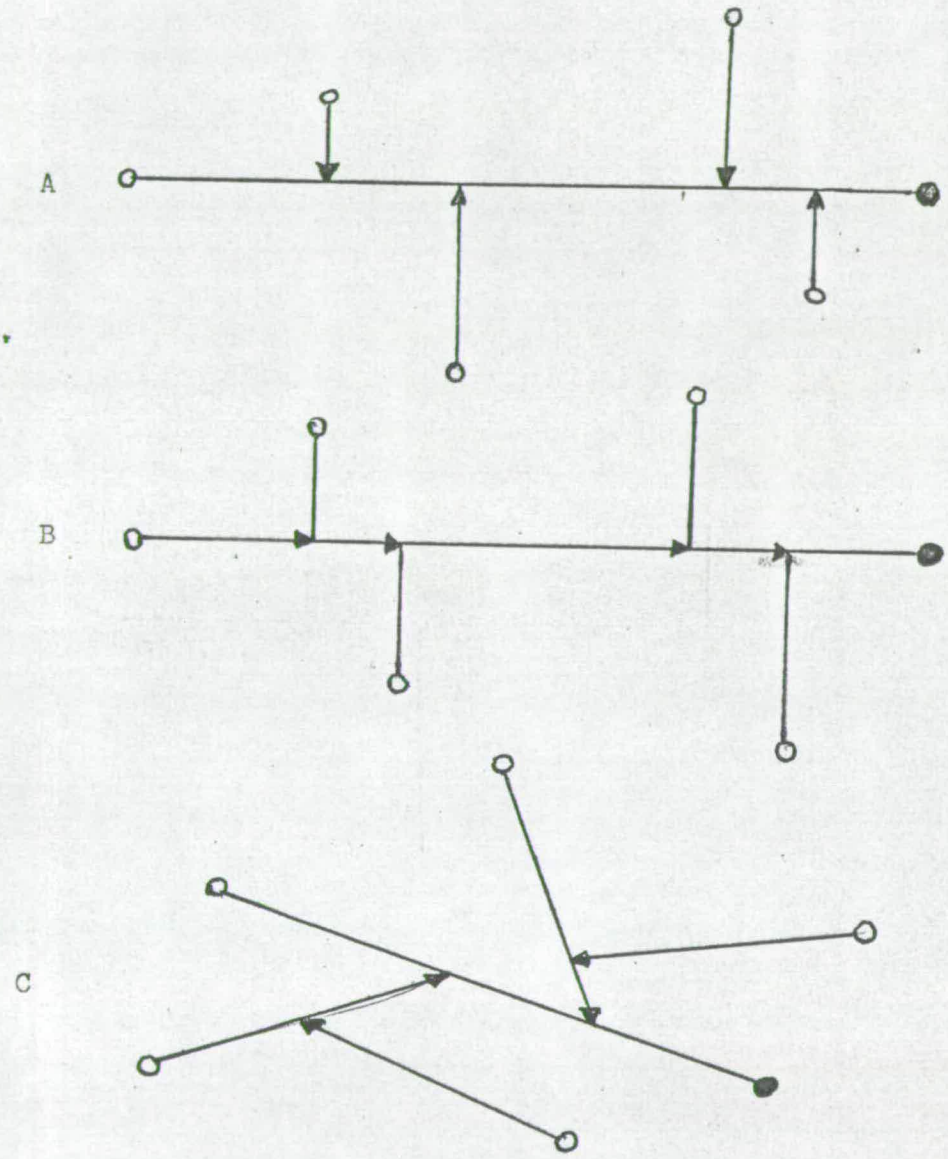
Molecular-sieve chromatography on a previously calibrated Bio-Gel P300 column enabled the approximate number-average molecular weights, $\overline{M_n}$, of polysaccharides I - VIII to be estimated. There is a significant drop in $\overline{M_n}$ from ca. 250,000 for A.laeta gum to ca. 91,000 for polysaccharide I, after the first Smith degradation. There is a subsequent drop to ca. 14,000, 12,000 and 9,500 for polysaccharides VI, VII, and VIII respectively.

Partial acid hydrolysis of polysaccharide V' (obtained after five successive Smith degradations of A.laeta gum in a duplicate experiment) led to the isolation of the β1,3-linked galactotriose. It was characterized by crystallization from aqueous ethanol. The β1,3-linked galactotriose has been characterized from A.senegal gum.[1]

On the basis of the available evidence, the structural interpretations for polysaccharides V - VIII are those which have been put forward for

polysaccharide E, the fifth Smith degraded product of A.senegal gum.[1] Methylation evidence indicates that polysaccharides V - VIII are not simple linear β1,3-linked galactans. Although 2,4,6-tri-O-methyl-D-galactose is the most predominant O-methyl sugar obtained from methylated polysaccharides V - VIII, the identification of some 2,4-di- and 2,3,4,6-tetra-O-methyl-D-galactose indicates occasional branching at C-3 and C-6 positions. The presence of small amounts of the β1,6-linked galactobiose in the partial acid hydrolysates of these polysaccharides is further support for a branched structure. If there is a "main chain" of β1,3-linked galactose units in polysaccharides V - VIII, it must carry short side-chains of β1,3-linked galactose residues; the branch points for these would be through β1,6-linkages (Figure 1, A). Alternatively there could be occasional β1,6-linkages along a "main chain" of β1,3-linked galactose units; here the branch points for the short side-chains would be through β1,3-linkages to β1,6-linked residues in the "main chain" (Figure 1, B). A randomly branched structure of β1,3-linked chains with β1,6 branch points would also satisfy the available structural evidence (Figure 1, C).

The ratio of the amounts of 2,4,6-tri-O-methyl-D-galactose to 2,3,4,6-tetra-O-methyl-D-galactose and 2,4-di-O-methyl-D-galactose, as judged from the peak areas obtained for their methyl glycosides on gas-liquid partition chromatography, increased from polysaccharides V - VIII. This is evidence for a decrease in branching from polysaccharides V - VIII. Similarly the ratio of 2,4,6-tri-O-methyl-D-galactose to 2,3,4,6-tetra-O-methyl-D-galactose and 2,4-di-O-methyl-D-galactose is greater for polysaccharide V than for polysaccharide E of A.senegal gum.[1] This suggests that the galactan framework of A.laeta gum has a higher proportion



—— = chains of β1,3-linked D-galactose residues.

↓ = β1,6-linkage at branch point.

● = reducing end group.

○ = non-reducing end group.

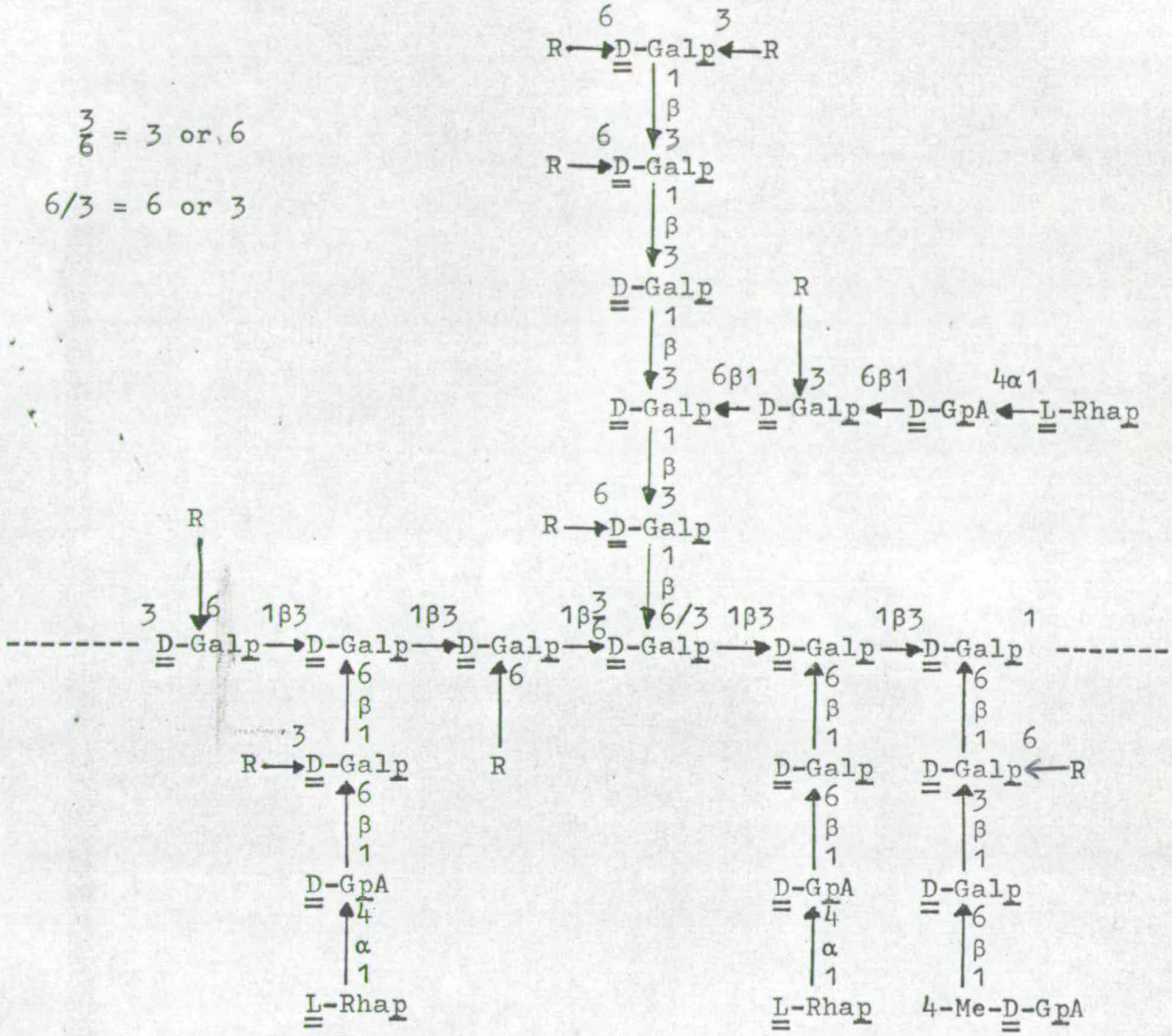
Figure 1. Representation of some of the possible structures for the periodate-resistant galactan framework of Acacia laeta gum.

of β1,3-linkages than that of A.senegal gum. This could be the reason why A.laeta gum has a lower specific rotation (-42°) than A.senegal gum (-31.5°).

Zone electrophoresis and ion-exchange chromatography have indicated that A.laeta gum exhibits the same kind of heterogeneity as A.senegal gum.[1,61] The polysaccharide entities that comprise A.laeta gum are probably polymer systems which have, in addition to molecular weight distribution, a variation in monosaccharide composition and a distribution in the mode of linking and branching of certain monosaccharide units. Figure 2 shows the polysaccharide array which represents a possible structural fragment from the internal chains of one of these entities. Any of the galactan frameworks shown in Figure 1 may be considered to form the basis for fragments of this general type.

Four Smith degradations were required to remove all the arabinose residues from A.senegal gum.[1] Thus the longest arabinose containing side-chains in A.senegal gum contain four units. However five Smith degradations were required to remove all the arabinose residues from A.laeta gum, thus some of the arabinose containing side-chains in A.laeta gum contain five units. Methylation analysis of autohydrolysed A.senegal gum showed that arabinofuranose units were the only arabinose residues attached directly to the galactan framework.[1] In contrast, both arabinofuranose and arabinopyranose units have been shown to be attached directly to the galactan framework of A.laeta gum.[3]

Apart from the differences discussed above, A.laeta gum is closely similar to A.senegal gum on an overall molecular basis. As in the case of A.senegal gum, there is no unequivocal proof for a "main chain" or "backbone" in A.laeta gum.



R represents D-galactopyranose-, L-arabinopyranose-, and L-arabinofuranose-containing side chains. These side chains may be up to five units long and contain ····^{3}L-Araf^{1}···· residues. They may be terminated by L-Araf^{1}····, L-Arap$^{1\beta3}\rightarrow$L-Araf^{1}····, or D-Galp$^{1\alpha3}\rightarrow$L-Araf^{1}···· residues.

Figure 2. Possible structural fragment in Acacia laeta gum

SECTION V

STRUCTURAL STUDY OF THE GUM EXUDATE OF ACACIA DREPANOLOBIUM HARMS EX SJÖSTED

5.1) Introduction.

Acacia drepanolobium Harms ex Sjösted is a bush or small tree (1 - 5 meters) with short radiating branches from a main stem, sometimes spreading at the top. It is both widely distributed and variable, and it is not clear how much this is due to heredity and how much to causes such as exposure and burning.[69]

A preliminary study[70] showed that Acacia drepanolobium gum is a further example of an Acacia gum having a positive specific rotation and a low content of L-rhamnose that does not bear a unit molar correspondence to the uronic acid content. It was also shown that A.drepanolobium gum does not dissolve completely in cold water,[70] giving ca. 20% of a water insoluble gel that can be dispersed in cold, dilute alkaline solution. Chromatographic evidence for the presence of four aldobiouronic acids, 6-O-(β-D-glucopyranosyluronic acid)-D-galactose, 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose, 4-O-(α-D-glucopyranosyluronic acid)-D-galactose and 4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose, has been reported.[40] The results of a more detailed examination of A.drepanolobium gum are reported and discussed in this Section.

The nodules of gum from A.drepanolobium Harms ex Sjösted used in this study were collected by Mr. W. M. C. Bagshawe, Provincial Forest Officer, at Tabora, Western Province, Tanganyika, in July, 1961.

5.2) Results.

(A). Comparative study of Fractions A, B and C of A.drepanolobium gum.

Fractionation procedure.- Three portions of crushed, bark free A.drepanolobium gum (20 g., 20 g., and 45 g. respectively) were stirred with distilled water (500 ml.) for 12 hours; the soluble Fractions A were collected by filtration, dialysed against tap water and electrodialysed. The residues were stirred with M-sodium chloride solution (500 ml.) for 12 hours; the salt-soluble Fractions B were collected by filtration, dialysed against tap water and electrodialysed. The residual insoluble gels (Fractions C) were dialysed against tap water. Fractions A, B and C were freeze dried. The percentage yields of Fractions A, B and C from the first two extractions are shown in Table 1. In the third extraction, A.drepanolobium gum (45 g.) gave Fraction A (35 g.), Fraction B (3.5 g.) and Fraction C (5.0 g.).

Fractions A, B and C migrated as single bands on electrophoresis on cellulose acetate film in both 0.1M-ammonium carbonate buffer (pH 8.9) and 0.1M-acetate buffer (pH 4.7), and had the same mobility when run under comparable conditions. Fractions A and B were chromatographed on a D.E.A.E.-cellulose column (45 x 1.3 cm.).[30] On gradient elution with sodium chloride solution (0.00 → 0.50M) in 0.02M-acetate buffer (pH 4.1), both Fractions yielded a single, slightly asymmetric peak for its elution pattern.

Fractions A, B and C from the first two extractions were compared analytically. Various solvents were used in an attempt to dissolve Fraction C of the third extraction. Fractions A and B, and solubilised Fraction C from the third extraction were compared using Smith

Table 1.

Analytical data for A.drepanolobium gum, Fractions A, B and C.[a]

	First extraction			Second extraction		
	A	B	C	A	B	C
Recovery %	79	8	13	80	8	12
Ash %	0.00	0.00	2.78	0.00	0.00	2.56
Nitrogen %	1.11	1.21	0.58	1.18	1.24	0.61
$[\eta]$, $cm.^3 g.^{-1}$	17.8	19.5	22.8	15.0	17.2	20.2
$[\alpha]_D$ (c, 1.0 in water)[b]	+77.8°	+77.2°	-	+82.2°	+82.6°	-
$[\alpha]_D$ (c, 1.0 in M-NaOH)[b]	+77.2°	+78.2°	+78.0°	+81.5°	+81.9°	+80.0°
Methoxyl %[b,c]	0.43	0.43	0.41	0.42	0.42	0.42
Equivalent weight[b]	1976	1997	-	1983	1980	-
Uronic acid %[b] (17600/E.W.)	8.9	8.8	-	8.8	8.8	-
Uronic acid %[b,c]	9.6	9.9	9.7	9.5	9.8	9.8
Formic acid released on[b] periodate oxidation (mmoles/g.)	1.07	1.05	1.02	1.06	1.08	1.05
Periodate consumed[b] (mmoles/g.)	3.96	3.97	3.93	3.95	3.98	3.96
Periodate consumption[b] / Formic acid released	3.70	3.78	3.85	3.72	3.69	3.77
Galactose %[b]	38	39	41	37	37	39
Arabinose %[b]	52	51	49	53	53	51
Rhamnose %[b,c]	1	1	1	1	1	1
Glucuronic acid %[b]	7	7	7	7	7	7
4-O-methylglucuronic[b] acid %	2	2	2	2	2	2

a. All data corrected to a dry-weight basis.
b. Values are corrected for moisture and protein contents; sugar percentages are for the anhydro-form.
c. Determined by vapour-phase infra-red method.

Table 2.

Solubility of Fraction C in different solvents.

Solvent	Percentage of Fraction C dissolved
Distilled water	1 %
3M-sodium chloride solution	1.5 %
3M-magnesium chloride solution	1.5 %
7M-urea solution	2 %
2% disodium ethylenediaminetetra-acetate solution	17.5 %
2% sodium hexametaphosphate solution	47 %
1% sodium borohydride solution	69 %
0.1M-phosphate buffer, pH 9.0	10.5 %
3% sodium borate solution	7 %

degradation and methylation analysis.

Comparative analytical study of Fractions A, B and C.- Hydrolysis (N-sulphuric acid for 7 hours at 100°) of each Fraction, followed by paper chromatographic examination, showed the presence of galactose, arabinose and four acidic disaccharides with the chromatographic mobilities of 6-O-(β-D-glucopyranosyluronic acid)-D-galactose, 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose, 4-O-(α-D-glucopyranosyluronic acid)-D-galactose and 4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose. Examination of paper chromatograms in ultraviolet light, after spraying with aniline oxalate, revealed that a trace of rhamnose was present in each Fraction. Hydrolysis (2N-sulphuric acid for 7 hours at 100°) of each Fraction, followed by paper chromatography in solvent (b), showed that glucuronic acid, glucurono-6,3-lactone and 4-O-methylglucuronic acid

Table 3.

Analytical data for Soluble Fraction C.[a]

	Soluble Fraction C
Nitrogen %	0.85
Intrinsic viscosity, $[\eta]$, $cm.^3 g.^{-1}$	11.1
$[\alpha]_D$ (c, 1.0 in water)[b]	$+79.5^o$
Equivalent weight[b]	1955
Uronic acid % (176000/E.W.)[b]	9.0
Galactose %[b]	39
Arabinose %[b]	51
Rhamnose %[b,c]	1
Formic acid released on periodate oxidation (mmoles/g.)[b]	1.05
Periodate consumed on periodate oxidation (mmoles/g.)[b]	3.95
$\frac{\text{Periodate consumption}}{\text{Formic acid released}}$[b]	3.76

a. All data corrected to a dry-weight basis.
b. Values are corrected for moisture and protein contents; sugar percentages are for the anhydro-form.
c. Determined by vapour-phase infra-red method.

were present in each hydrolysate.

Analytical data for the Fractions are shown in Table 1. Methylated neutral sugars were not detected in the hydrolysates; if the methoxyl content of the Fractions is assumed to be located solely in the residues of 4-O-methyl-D-glucuronic acid, calculation shows that this acid is present in each Fraction to the extent of 2%.

Because of the water-insolubility of Fraction C, the specific rotation

Table 4.

Data for the O-methyl derivatives of Fractions A and B, and Soluble Fraction C.

	Fraction A	Fraction B	Soluble Fraction C
Yield (mg.)	255	262	256
$[\alpha]_D$ (c, 1.0 in $CHCl_3$)	+60°	+61°	+60°
Methoxyl %	40.8	40.7	40.1

and intrinsic viscosity of Fraction C were measured using M-sodium hydroxide solution as solvent. Each Fraction (1 g.) was oxidised by addition of 0.125M-sodium metaperiodate solution (50 ml.). Periodate oxidation was followed by simultaneous determination (2 ml. aliquots) of formic acid and periodate. Oxidation of all Fractions was complete after 48 hours, although the oxidation mixtures of Fraction C did not become homogeneous until after 18 hours.

Dissolution of Fraction C.- Although Fraction C is soluble in M-sodium hydroxide solution to a strength of 1% (w/v), this method of dissolution is not entirely satisfactory, since in such strongly alkaline conditions polysaccharide degradation will probably occur. Different methods of dissolution were therefore attempted. Samples of Fraction C (120 mg., dry weight) were stirred with (i) distilled water; (ii) 3M-sodium chloride solution; (iii) 3M-magnesium chloride solution; (iv) 7M-urea solution; (v) 2% disodium ethylenediaminetetra-acetate solution (w/v); (vi) 2% sodium hexametaphosphate solution (w/v); (vii) 1% sodium borohydride solution (w/v); (viii) 3% sodium borate solution (w/v); (ix) 0.1M-phosphate buffer (pH 9.0). Each extraction was filtered, dialysed against tap water for 48 hours, and isolated as the freeze dried product. Table 2 shows the percentage of Fraction C dissolved by these solvents. All the

freeze dried products were water soluble.

The best solvent for dissolving Fraction C is undoubtedly 1% sodium borohydride solution (w/v). Fraction C (3.75 g.) was therefore extracted with 1% sodium borohydride solution (400 ml.), filtered, dialysed against tap water for 48 hours, and electrodialysed to give Soluble Fraction C (2.25 g.) as the freeze dried product. The analytical data for Soluble Fraction C are shown in Table 3. Hydrolysis (N-sulphuric acid for 7 hours at 100°) of Soluble Fraction C, followed by paper chromatography, indicated the presence of galactose, arabinose, rhamnose and the same four aldobiouronic acids which were detected in the hydrolysates of Fractions A, B and C.

In order to check whether Fraction C had been degraded by treatment with 1% sodium borohydride solution (w/v), Fractions A and B, and Soluble Fraction C were examined by molecular-sieve chromatography using a previously calibrated Bio-Gel P300 column (45 x 4.8 cm.).[27,28,29] Fractions A and B were eluted at the elution volume of Blue Dextran 2000 (Pharmacia). In contrast, Soluble Fraction C was eluted before the elution of Blue Dextran 2000. This suggests that Soluble Fraction C has a higher molecular weight than Fractions A and B. Light-scattering measurements gave values of weight-average molecular weights, $\overline{M}_w$, for Fractions A and B, and Soluble Fraction C of 9.5×10^5, 10.2×10^5, and 22.0×10^5 respectively.

Methylation analysis of Fractions A and B, and Soluble Fraction C.- The three Fractions (300 mg.) were methylated exhaustively using the Haworth[18] and Purdie[19] proceedures. Yields, specific rotations and methoxyl contents of the methylated products are shown in Table 4.

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Table 5.

Examination of methanolysis and hydrolysis products from the O-methyl derivatives of Fractions A and B, and Soluble Fraction C.

O-Methyl sugars	Relative retention times, T, of methyl glycosides on columns:- (i)	(ii)	R_G in solvent (d)
2,3,4-Me_3-L-rhamnose	0.48	(0.50)[a]	-
2,3,5-Me_3-L-arabinose	0.55; 0.73	(0.50)[a]; 0.64	0.97
2,3,4-Me_3-L-arabinose	(1.05)[a]	(0.82)[a]	0.79
2,5-Me_2-L-arabinose	1.80; 3.20	1.26; (2.20)[a]	0.82
3,5-Me_2-L-arabinose	(1.05)[a]; (2.36)[a]	(0.82)[a]; 1.76	0.82
3,4-Me_2-L-arabinose	1.98	1.43	0.56
2,3,4,6-Me_4-D-galactose	1.68	1.65	0.88
2,3,6-Me_3-D-galactose	(3.05)[a]; (3.89)[a]; (4.22)[a]	2.44; (3.00)[a]; (3.44)[a]	0.73
2,4,6-Me_3-D-galactose	(3.89)[a]; (4.22)[a]	(3.00)[a]; (3.44)[a]	0.73
2,3,4-Me_3-D-galactose	6.42	5.08	0.73
2,4-Me_2-D-galactose	14.6; 16.4	9.9; 11.4	0.52
2,3,4-Me_3-D-glucuronic acid[b]	(2.36)[a]; (3.05)[a]	(2.20)[a]; 2.75	-
2-Me-D-galactose	-	-	0.32
4-Me-L-arabinose	-	-	0.35

a. Figures in parentheses indicate T values of components not completely resolved.
b. As methyl ester methyl glycoside.

Methoxyl contents were not raised on further attempted methylation. On methanolysis and g.l.c. examination of the mixture of methyl glycosides, the methylated product of each Fraction gave identical traces using columns (i) and (ii). Results of g.l.c. examination are shown in Table 5;

Table 6.

Analytical data for Smith-degraded Fractions A and B, and Soluble Fraction C.

	Fraction A	Fraction B	Soluble Fraction C
Moisture %	11.9	10.5	10.8
Nitrogen %[a]	1.01	0.98	0.32
Protein %[a] (N% x 6.25)	6.32	6.13	2.00
Yield of polysaccharide (g.)[a,b]	1.11	1.14	1.12
Yield %[a,b]	55.5	57	56
Galactose %[a,b,c]	43	44	44
Arabinose %[a,b,c]	57	56	56
$[\alpha]_D$ (c, 1.0 in water)[b,a]	+45°	+45°	+45°
Formic acid released on periodate oxidation (mmoles/g.)[a,b]	0.94	0.96	0.97
Periodate consumed on periodate oxidation (mmoles/g.)[a,b]	3.38	3.47	3.45
Periodate consumption / Formic acid release[a,b]	3.59	3.62	3.56

a. Values corrected for moisture content.
b. Values corrected for protein content.
c. Sugar percentages are for the anhydro-form.

retention times were comparable with those for methyl glycosides from authentic O-methyl sugars. A portion of methanolysate from each methylated product was hydrolysed with N-sulphuric acid for 4 hours on a boiling water bath. The cooled solutions were neutralised with barium carbonate, filtered, treated with Amberlite resin IR120 (H^+) and concentrated. Paper chromatographic examination of the hydrolysates in solvents (d) and (g) showed the presence of 2-O-methylgalactose and 4-O-methylarabinose in addition to those O-methyl sugars already

Table 7.

Data for methylated Smith-degraded Fractions A and B, and Soluble Fraction C.

	Fraction A	Fraction B	Soluble Fraction C
Yield (mg.)	125	120	124
$[\alpha]_D$ (*c*, 1.0 in $CHCl_3$)	+39°	+39°	+38°
Methoxyl %	40.1	40.0	40.1

characterized by g.l.c. of their methyl glycosides (Table 5).

Smith degradation of Fraction A and B, and Soluble Fraction C.- The purified Fractions (1.5 g. polysaccharide) were dissolved in water (37.5 ml.) and 0.25M-sodium metaperiodate solution (37.5 ml.) was added. The oxidation mixtures were left in darkness at room temperature for 48 hours. The reactions were stopped by addition of ethylene glycol (2 ml.), and the solutions were dialysed against running tap water for 48 hours. Sodium borohydride (0.5 g.) was added to each solution, and the mixtures were kept at room temperature for 30 hours, then dialysed for a further 48 hours. The three polyalcohols were hydrolysed in N-sulphuric acid at room temperature for 48 hours, and the hydrolysates were neutralised with barium carbonate, filtered and deionised with Amberlite resin IR120 (H^+). The solutions were each dialysed against distilled water (1 l.) for 24 hours, and then dialysed against running tap water for 48 hours. The three Smith-degraded polysaccharides were isolated as freeze dried products, and the dialysates were concentrated to syrups.

Yields and analytical data for the three Smith-degraded polysaccharides are shown in Table 6. Hydrolysis of the Smith-degraded polysaccharides (N-sulphuric acid, for 7 hours at 100°), followed by paper chromatography, indicated the presence of only galactose and arabinose. The Smith-degraded

Table 8.

Examination of methanolysis and hydrolysis products from methylated Smith-degraded Fractions A and B, and Soluble Fraction C.

O-Methyl sugars	Relative retention times, T, of methyl glycosides on columns:- (i)	(ii)	R_G in solvent (d)
2,3,5-Me_3-L-arabinose	0.56; 0.74	0.50; 0.63	0.97
2,5-Me_2-L-arabinose	1.80; 3.22	1.26; 2.20	0.82
3,5-Me_2-L-arabinose	1.06; 2.36	0.81; 1.76	0.82
2,3,4,6-Me_4-D-galactose	1.68	1.65	0.88
2,4,6-Me_3-D-galactose	3.90; 4.22	3.00; 3.45	0.73
2,3,4-Me_3-D-galactose	6.45	5.08	0.73
2,4-Me_2-D-galactose	14.6; 16.4	9.9; 11.4	0.52
2,6-Me_2-D-galactose	-	-	0.55
2-Me-D-galactose	-	-	0.32
Unknown sugar	1.53	1.05	-

polysaccharides were hydrolysed with 0.5N-sulphuric acid for 1 hour on a boiling water bath. Paper chromatographic examination of the hydrolysates indicated the presence of galactose, arabinose and two neutral disaccharides with the chromatographic mobilities of 3-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.49 in solvent (a), and 0.54 in solvent (f), minor component] and 6-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.31 in solvent (a), and 0.38 in solvent (f), major component].

Chromatographic examination of the three dialysates in solvents (a) and (f) showed the presence of glycollic aldehyde and glycerol, and traces of ethylene glycol and threitol. The traces of ethylene glycol would be expected from non-reducing arabinopyranose end-groups. The traces of

threitol would be expected from D-galactopyranose residues with a substituent on C-4, as in the α1,4-linked aldobiouronic acids previously detected in the hydrolysates of Fractions A and B, and Soluble Fraction C. Glycerol was characterized from the dialysate of the Smith degradation of Fraction A, after purification by thick paper chromatography in solvent (a), by preparation of its tribenzoate derivative, m.p. 75° - 76° (Lit. m.p.[71] 76°).

Methylation analysis of Smith-degraded Fractions A and B, and Soluble Fraction C.- The three Smith-degraded products (200 mg.) were methylated exhaustively using the methods of Haworth[18] and Purdie.[19] Yields, specific rotations and methoxyl contents of the methylated products are shown in Table 7. Methoxyl contents were not raised on further attempted methylation. On methanolysis and g.l.c. examination of the mixture of methyl glycosides using columns (i) and (ii), the three methylated products gave identical results. Results of g.l.c. examination are shown in Table 8. A portion of each methanolysate was hydrolysed (N-sulphuric acid for 4 hours at 100°). The cooled solutions were neutralised with barium carbonate, filtered, treated with Amberlite resin IR120 (H^+) and concentrated. Paper chromatographic examination of the hydrolysates in solvents (d) and (g) showed the presence of 2,6-di- and 2-O-methylgalactose in addition to those O-methyl sugars already characterized by g.l.c. of their methyl glycosides (Table 8).

(B). Structural study of A.drepanolobium gum Fraction A.

Purification of A.drepanolobium gum Fraction A.- Two portions of A.drepanolobium gum (180 g. and 160 g.) were extracted with cold water,

as described previously, to yield two portions of Fraction A (148 g. and 130 g.). The polysaccharide content of both portions of Fraction A was 83%. Zone electrophoresis and ion-exchange chromatography on D.E.A.E.-cellulose[30] showed that both portions were homogeneous. The first portion of Fraction A was used in a Smith degradation study, while other structural evidence was obtained using the second portion.

Separation and characterization of neutral sugars.- The polysaccharide (0.75 g.) was hydrolysed with N-sulphuric acid for 7 hours on a boiling water bath. The cooled solution was neutralised with barium carbonate, filtered, treated with Amberlite resin IR120 (H^+) and concentrated to a syrup. The hydrolysate was fractionated on Whatman 3MM sheets for 36 hours in solvent (a) to yield crystalline D-galactose and L-arabinose.

D-galactose (114 mg.) had $[\alpha]_D$ +81°(equil.)(c, 1.0) and, after recrystallisation from ethanol, had m.p. and mixed m.p. 168°. Its X-ray diffraction pattern was identical to that of an authentic specimen of D-galactose.

L-arabinose (180 mg.) had $[\alpha]_D$ +105°(equil.)(c, 1.0) and, after recrystallisation from aqueous ethanol, had m.p. and mixed m.p. 159°. Its X-ray diffraction pattern was identical to that of an authentic specimen of L-arabinose. The derived phenylosazone, after recrystallisation from water, had m.p. and mixed m.p. 165°.

The polysaccharide (3.0 g.) was hydrolysed with N-sulphuric acid for 7 hours on a boiling water bath. The hydrolysate was fractionated on Whatman 3MM sheets for 16 hours in solvent (a). The position of rhamnose was located by spraying side-strips where L-rhamnose had been run as a marker. Elution of the appropriate zone with water gave L-rhamnose (21 mg.)

having $[\alpha]_D$ +6°(equil.)(c, 0.21). After recrystallisation from aqueous ethanol, the L-rhamnose had m.p. and mixed m.p. 93° - 94°.

Isolation of two pairs of aldobiouronic acids.- The polysaccharide (10 g.) was hydrolysed with N-sulphuric acid for 7 hours on a boiling water bath. Paper chromatography of the hydrolysate in solvent (b) indicated the presence of galactose, arabinose and two acidic fractions (1 and 2) having R_{Gal} values of 0.26 and 0.59. Fractionation of the hydrolysate on Whatman 3MM sheets in solvent (b) led to the isolation, as syrups, of these two acidic fractions.

Fraction 1 (147 mg.) was examined by paper chromatography in solvent (b) for 72 hours; two components were observed having R_{Gal} values of 0.23 and 0.29 respectively. Similarly, paper chromatography in solvent (c) for 24 hours indicated two components having R_{Gal} values of 0.56 and 0.61. Fraction 1 was hydrolysed with 2N-sulphuric acid for 7 hours on a boiling water bath. Paper chromatography of the hydrolysate in solvent (b) indicated ca. equal amounts of galactose and glucurono-6,3-lactone, accompanied by traces of glucuronic acid and unhydrolysed aldobiouronic acids. Fractionation of the hydrolysate on Whatman 3MM sheets in solvent (b) yielded crystalline D-galactose and D-glucurono-6,3-lactone. D-galactose (50 mg.) had $[\alpha]_D$ +80°(equil.)(c,1.0) and after recrystallisation from ethanol, had m.p. and mixed m.p. 167°. D-glucurono-6,3-lactone (45 mg.) had $[\alpha]_D$ +19°(equil.)(c, 0.90) and, after recrystallisation from aqueous ethanol, had m.p. and mixed m.p. 176°. Its X-ray diffraction pattern was identical to that of an authentic specimen of D-glucurono-6,3-lactone.

Fraction 2 (78 mg.) was examined by paper chromatography in solvent

(b) for 72 hours; two components were observed having R_{Gal} values of 0.56 and 0.66 respectively. Similarly, paper chromatography in solvent (c) for 24 hours indicated two components having R_{Gal} values of 0.90 and 0.94. Fraction 2 was hydrolysed with 2N-sulphuric acid for 7 hours on a boiling water bath. Paper chromatography of the hydrolysate indicated ca. equal amounts of galactose and 4-O-methylglucuronic acid, accompanied by traces of unhydrolysed aldobiouronic acids. Fractionation of the hydrolysate on Whatman 3MM sheets in solvent (b) yielded crystalline D-galactose and pure 4-O-methyl-D-glucuronic acid. D-galactose (25 mg.) had $[\alpha]_D$ +80° (equil.)(c, 0.50) and m.p. and mixed m.p. 168°. 4-O-methyl-D-glucuronic acid (31 mg.) had $[\alpha]_D$ +36°(equil.)(c, 0.62). 4-O-methyl-D-glucuronic acid (29 mg.) was converted into the methyl ester methyl glycoside by refluxing with methanolic 5% hydrogen chloride for 8 hours. The resulting solution was neutralised with silver carbonate and filtered, and the residue was washed with warm methanol. The extract was evaporated to a syrup and dissolved in distilled water (10 ml.). Sodium borohydride (0.25 g. in 25 ml. water) was added, and the mixture was left for 16 hours. Excess borohydride was destroyed by addition of Amberlite resin IR120 (H^+). After filtration, the product was evaporated to dryness, and borate was removed by repeated distillation with methanol. After hydrolysis of the methyl glycoside, the product was fractionated on Whatman 3MM sheets in solvent (a) to yield 4-O-methyl-D-glucose (14 mg.) as a syrup [R_{Gal} 1.92 and R_{Rha} 0.90 in solvent (a); R_{Gal} 2.19 and R_{Rha} 0.84 in solvent (b); and R_{Gal} 1.74 and R_{Rha} 0.93 in solvent (d)]. The derived phenylosazone, after recrystallisation from aqueous acetone, had m.p. 156° - 158° (Lit. m.p.[72] 158° - 159°).

Isolation and characterization of aldobiouronic acids.- The N-sulphuric acid hydrolysate of the polysaccharide was fractionated on Whatman 3MM sheets for 96 hours in solvent (b). Four aldobiouronic acids [(i), (ii), (iii) and (iv)] were isolated as syrups.

Acid (i) (102 mg.) had R_{Gal} 0.23 in solvent (b) and R_{Gal} 0.56 in solvent (c), and was chromatographically identical to 6-O-(β-D-gluco-pyranosyluronic acid)-D-galactose in these solvents. The syrup had $[\alpha]_D$ -3° (c, 1.02); this suggests the presence of a β-D-linkage. The syrup (5 mg.) was hydrolysed (2N-sulphuric acid for 7 hours at 100°), and paper chromatography in solvent (b) revealed ca. equal amounts of galactose and glucurono-6,3-lactone. Sodium borohydride (25 mg. in 5 ml. water) was added to Acid (i) (10 mg.) and the mixture was left for 18 hours. Excess borohydride was destroyed by addition of Amberlite resin IR120 (H^+). The resin and solvent were removed, and the borate was volatilised as methyl borate. The methyl ester was formed by refluxing the product with methanolic 5% hydrogen chloride for 4 hours, and, after neutralisation and evaporation to dryness, was reduced with sodium borohydride (25 mg. in 5 ml. water) for 18 hours. The reduced product was hydrolysed (N-sulphuric acid for 7 hours at 100°), and paper chromatography in solvent (h) indicated ca. equal amounts of glucose and galactitol. (Glucose was detected by the enzymic method outlined in Section II). The oxidation of known weights (3-5 mg.) of Acid (i) with excess of sodium metaperiodate did not give formaldehyde. Acid (i) was methylated by the Kuhn method. Methanolysis followed by g.l.c. examination of the mixture of methyl glycosides indicated the presence of 2,3,4-tri-O-methyl-D-glucuronic acid [(i),T 2.33, 2.96; (ii),T 2.08, 2.70], 2,3,4-tri- [(i),T 6.59; (ii),T 5.25]

and 2,3,5-tri-O-methyl-D-galactose [(i),T 4.09, 4.50; (ii),T 3.44, 4.14]. A portion of the methanolysate was reduced with sodium borohydride; after removal of methyl glycosides by mild acid hydrolysis, the products were examined chromatographically in solvent (d). This revealed 2,3,4-tri-O-methyl-D-galactose (red spot, R_G 0.72) and a red-black spot (R_G 0.84) corresponding to 2,3,4-tri-O-methyl-D-glucose and 2,3,5-tri-O-methyl-D-galactose. Double development in solvent (g) separated the 2,3,4-tri-O-methyl-D-glucose (red spot) from 2,3,5-tri-O-methyl-D-galactose (black spot). These experiments led to the identification of Acid (i) as 6-O-(β-D-glucopyranosyluronic acid)-D-galactose.

Acid (ii) (91 mg.) had R_{Gal} 0.29 in solvent (b) and R_{Gal} 0.61 in solvent (c), and was chromatographically identical to 4-O-(α-D-glucopyranosyluronic acid)-D-galactose in these solvents. The syrup had $[\alpha]_D$ +106° (c, 0.91); this suggests an α-D-linkage is present. The syrup (5 mg.) was hydrolysed (2N-sulphuric acid for 7 hours at 100°), and paper chromatography in solvent (b) revealed ca. equal amounts of galactose and glucurono-6,3-lactone. Acid (ii) (10 mg.) was reduced in two stages with sodium borohydride, as described for Acid (i). The product was hydrolysed (N-sulphuric acid for 7 hours at 100°), and paper chromatography in solvent (h) indicated ca. equal amounts of glucose and galactitol. The oxidation of known weights (3-5 mg.) of Acid (ii) with excess of sodium metaperiodate gave 1.01 moles of formaldehyde per mole of Acid (ii). Acid (ii) (10 mg.) was methylated by the Kuhn method.[20,21] Methanolysis followed by g.l.c. examination of the mixture of methyl glycosides indicated the presence of 2,3,4-tri-O-methyl-D-glucuronic acid [(i),T 2.40, (2.99); (ii),T 2.04, 2.70] and 2,3,6-tri-O-methyl-D-galactose [(i),T (2.99), 3.88, 4.23;

(ii), τ 2.42, 3.07, 3.41]. A portion of the methanolysate was reduced with sodium borohydride; after removal of methyl glycosides by mild acid hydrolysis, the products were examined chromatographically in solvent (d). This revealed 2,3,6-tri-O-methyl-D-galactose (R_G 0.76) and 2,3,4-tri-O-methyl-D-glucose (R_G 0.84). These experiments led to the identification of Acid (ii) as 4-O-(α-D-glucopyranosyluronic acid)-D-galactose.

Acid (iii) (42 mg.) had R_{Gal} 0.56 in solvent (b) and R_{Gal} 0.90 in solvent (c), and was chromatographically identical to 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose in these solvents. The syrup had $[\alpha]_D$ +3° (c, 0.42); this suggests the presence of a β-D-linkage. The syrup (5 mg.) was hydrolysed (2N-sulphuric acid for 7 hours at 100°), and paper chromatography in solvent (b) revealed ca. equal amounts of galactose and 4-O-methylglucuronic acid. Acid (iii) (5 mg.) was reduced in two stages with sodium borohydride as described for Acid (i). The product was hydrolysed (N-sulphuric acid for 7 hours at 100°), and paper chromatography in solvents (a) and (h) indicated ca. equal amounts of galactitol and 4-O-methylglucose. The remainder of the hydrolysate was oxidised by the method of Lemieux and Bauer,[26] and the products were examined chromatographically in solvent (d). Development with aniline hydrogen phthalate[73] revealed 2-O-methylerythrose, a characteristic product of 4-O-methylglucose, as a yellow-green spot[26] (R_F 0.55). Known weights (3-5 mg.) of Acid (iii) did not give formaldehyde when oxidised with excess of sodium metaperiodate. On methylation, Acid (iii) gave the same O-methyl sugars as Acid (i). These experiments led to the identification of Acid (iii) as 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose.

Acid (iv) (31 mg.) had R_{Gal} 0.66 in solvent (b) and R_{Gal} 0.94 in

solvent (c), and was chromatographically identical to 4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose in these solvents. The syrup had $[\alpha]_D$ +98° (c, 0.31); this suggests the presence of an α-D-linkage. The syrup (5 mg.) was hydrolysed (2N-sulphuric acid for 7 hours at 100°), and paper chromatography in solvent (b) revealed ca. equal amounts of galactose and 4-O-methylglucuronic acid. Acid (iv) (5 mg.) was reduced in two stages with sodium borohydride as described for Acid (i). The product was hydrolysed (N-sulphuric acid for 7 hours at 100°), and paper chromatography in solvents (a) and (h) indicated ca. equal amounts of galactitol and 4-O-methylglucose. The remainder of the hydrolysate was oxidised by the method of Lemieux and Bauer,[26] and paper chromatography of the products in solvent (d) revealed the presence of 2-O-methylerythrose (R_F 0.55). The oxidation of known weights (3-5 mg.) of Acid (iv) with excess of sodium metaperiodate gave 0.96 moles of formaldehyde per mole of Acid (iv). On methylation, Acid (iv) gave the same O-methyl sugars as Acid (ii). These experiments led to the identification of Acid (iv) as 4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose.

Determination of conditions for autohydrolysis of A.drepanolobium gum Fraction A.- In a trial experiment, a 4% polysaccharide solution (2 g. in 50 ml. water) was heated on a boiling water bath for 120 hours. Aliquots (2 ml.) were removed at various times, filtered and diluted to 10 ml. The specific rotation of the diluted aliquots were determined, assuming that the concentration of carbohydrate present in the autohydrolysis solution remained constant. The reducing powers of the aliquots were measured using the alkaline ferricyanide method,[7] and was expressed as mg. of arabinose per ml. of autohydrolysis solution. The

Table 9.

Measurements taken at different times during autohydrolysis.

Time (hr.)	Specific rotation $[\alpha]_D$	Reducing power in mg. of arabinose per ml. of autohydrolysate	Intrinsic viscosity $[\eta]$
0	+82.1°	1.56	25.1
2	+83.8°	5.20	-
5	-	-	18.4
9	+88.8	12.72	-
10	-	-	13.2
24	+91.1°	22.20	7.5
48	+91.9°	29.72	5.2
72	+92.2°	32.62	3.6
96	+91.7°	32.80	3.5
120	+89.5°	33.00	-

results are shown in Table 9. In a parallel trial experiment, a 4% polysaccharide solution (3 g. in 75 ml. water) was heated on a boiling water bath for 120 hours. At various times aliquots (6 ml.) were removed and filtered. Filtered aliquots (5 ml.) were added to 2M-sodium chloride solution (5 ml.) to give a 2% carbohydrate solution in M-sodium chloride solution. Using these solutions the intrinsic viscosity, $[\eta]$, at different times of autohydrolysis was obtained. The results are shown in Table 9.

Reducing powers of autohydrolysis solutions increase to a constant value, and intrinsic viscosities of autohydrolysis solutions decrease to a constant value, with increasing time of autohydrolysis. From these results autohydrolysis was taken to be complete after 84 hours.

Autohydrolysis of Fraction A.- A 4% solution of Fraction A (50 g. polysaccharide in 1.25 l. water) was heated on a boiling water bath. After 84 hours the solution was cooled, filtered and dialysed against distilled water (4 x 4 l.). Dialysis was completed against running tap water, and freeze drying gave degraded gum A (11 g. polysaccharide; yield, 22%), $[\alpha]_D$ +31° (c, 1.0)(Found: uronic acid, 17%; galactose, 80%; arabinose, 3%; OMe, 0.73%). Hydrolysis (N-sulphuric acid for 7 hours at 100°) of degraded gum A indicated the presence of the four aldobiouronic acids which had been characterized from the whole gum.

The diffusate was concentrated to a syrup (30 g.). Chromatographic examination of the syrup showed the presence of galactose, arabinose, rhamnose (a trace), and two neutral disaccharides with the mobilities of 3-O-β-L-arabinofuranosyl-L-arabinose [R_{Gal} 1.25 in solvent (b) and 1.47 in solvent (g)] and 3-O-β-L-arabinopyranosyl-L-arabinose [R_{Gal} 0.77 in solvent (a) and 0.71 in solvent (b)].

The first arabinobiose was isolated by fractionation of a portion of the diffusate on Whatman 3MM papers in solvent (g) to give a syrup (32 mg.). This had $[\alpha]_D$ +89.7° (c, 0.64) and yielded arabinose on acid hydrolysis. It had the same paper chromatographic mobility [R_{Gal} 1.25 in solvent (b) and 1.47 in solvent (g)] as an authentic specimen of 3-O-β-L-arabinofuranosyl-L-arabinose. A portion (10 mg.) was methylated. G.l.c. examination of the methanolysis products revealed peaks with retention times corresponding to the methyl glycosides of 2,3,5-tri- [(i),T 0.56, 0.73; (ii),T 0.47, 0.63] and 2,5- [(i),T 1.82, 3.24; (ii),T 1.27, 2.20] and 2,4-di-O-methyl-L-arabinose [(i),T 2.07, 2.17; (ii),T 1.46, 1.57]. Hydrolysis of the methyl glycosides followed by

paper chromatography in solvent (d) confirmed the presence of 2,3,5-tri- (R_G 0.98), and 2,5- (R_G 0.84) and 2,4-di-O-methyl-L-arabinose (R_G 0.65). The phenylosazone of the disaccharide was prepared and, after recrystallisation from water, had m.p. 196° - 198° (Lit. m.p.,[74] 200°).

The second arabinobiose was isolated by fractionation of a portion of diffusate on Whatman 3MM papers in solvent (b) to give a syrup (50 mg.). This had $[\alpha]_D$ +195° (c, 1.00) and yielded arabinose on acid hydrolysis. It had the same paper chromatographic mobility [R_{Gal} 0.77 in solvent (a) and 0.71 in solvent (b)] as an authentic specimen of 3-O-β-L-arabino-pyranosyl-L-arabinose. A portion (15 mg.) was methylated. G.l.c. examination of the methanolysis products revealed peaks with retention times corresponding to the methyl glycosides of 2,3,4-tri- [(i),T 1.01; (ii),T 0.82] and 2,5- [(i),T 1.81, 3.25; (ii),T 1.26, 2.18] and 2,4-di-O-methyl-L-arabinose [(i),T 2.08, 2.18; (ii),T 1.48, 1.56]. Hydrolysis of the methyl glycosides followed by paper chromatography in solvent (d) confirmed the presence of 2,3,4-tri- (R_G 0.79), and 2,5- (R_G 0.84) and 2,4-di-O-methyl-L-arabinose (R_G 0.65). The phenylosazone of the disaccharide was prepared and, after recrystallisation from water, had m.p. 233° - 234° (decomp.)(Lit. m.p.,[74] 233° - 235°).

Partial acid hydrolysis of degraded gum A.- Degraded gum A (5 g.) was hydrolysed with 0.5N-sulphuric acid (1 l.) for 1 hour on a boiling water bath. The cooled solution was neutralised with barium carbonate, filtered, treated with Amberlite resin IR120 (H^+) and concentrated to a syrup (4.8 g.). The syrup was chromatographed on Whatman 3MM papers in solvent (a) to give two pure disaccharides and two pure trisaccharides.

Fraction 1 (62 mg.) had $[\alpha]_D$ +60° (c, 0.62) and yielded galactose on

acid hydrolysis. It had the same paper chromatographic mobility [R_{Gal} 0.48 in solvent (a) and 0.53 in solvent (f)] as an authentic specimen of 3-O-β-D-galactopyranosyl-D-galactose. A portion (15 mg.) was methylated. G.l.c. examination of the methanolysis products revealed peaks with retention times corresponding to the methyl glycosides of 2,3,4,6-tetra- [(i),T 1.68; (ii),T 1.66] and 2,4,6- [(i),T 3.76, 4.23; (ii),T 3.00, 3.45] and 2,5,6-tri-O-methyl-D-galactose [(i),T 3.98; (ii),T 3.26]. Hydrolysis of the methyl glycosides followed by paper chromatography in solvent (d) confirmed the presence of 2,3,4,6-tetra- (R_G 0.90), and 2,4,6- (R_G 0.72) and 2,5,6-tri-O-methyl-D-galactose (R_G 0.88). The disaccharide was crystallised from aqueous acetone to give needles of 3-O-β-D-galacto-pyranosyl-D-galactose monohydrate, which had m.p. and mixed m.p. 156° - 159° (Lit. m.p.,[74] 159° - 160°).

Fraction 2 (94 mg.) had $[\alpha]_D$ +29° (c, 0.94) and yielded galactose on acid hydrolysis. It had the same paper chromatographic mobility [R_{Gal} 0.29 in solvent (a) and 0.37 in solvent (f)] as an authentic specimen of 6-O-β-D-galactopyranosyl-D-galactose. A portion (20 mg.) was methylated. G.l.c. examination of the methanolysis products revealed peaks with retention times corresponding to the methyl glycosides of 2,3,4,6-tetra- [(i),T 1.68; (ii),T 1.66], and 2,3,5- [(i),T 4.05, 5.39; (ii),T 3.28, 4.37] and 2,3,4-tri-O-methyl-D-galactose [(i),T 6.45; (ii),T 5.30]. Hydrolysis of the methyl glycosides followed by paper chromatography in solvent (d) confirmed the presence of 2,3,4,6-tetra- (R_G 0.90), and 2,3,5- (R_G 0.86) and 2,3,4-tri-O-methyl-D-galactose (R_G 0.71). The phenylosazone of the disaccharide was prepared and, after recrystallisation from water, had m.p. 200° - 203° (Lit. m.p.,[74] 203° - 204°).

Fraction 3 (9 mg.) had $[\alpha]_D$ +46° (c, 0.18) and yielded galactose and the β1,3-linked galactobiose as the only disaccharide on partial acid hydrolysis. Its degree of polymerisation, as determined by the method of Timell, was 2.90. It had the same paper chromatographic mobility [R_{Gal} 0.22 in solvent (a) and 0.23 in solvent (f)] as an authentic specimen of the β1,3-linked galactotriose. A portion (4 mg.) was methylated. G.l.c. examination of the methanolysis products revealed peaks with retention times corresponding to the methyl glycosides of 2,3,4,6-tetra- [(i),T 1.68; (ii),T 1.66], and 2,4,6- [(i),T 3.75, 4.23; (ii),T 3.00, 3.45] and 2,5,6-tri-O-methyl-D-galactose [(i),T 3.98; (ii),T 3.24].

Fraction 4 (19 mg.) had $[\alpha]_D$ +20° (c, 0.38) and yielded galactose and the β1,6-linked galactobiose as the only disaccharide on partial acid hydrolysis. Its degree of polymerisation, as determined by method of Timell, was 2.95. It had the same paper chromatographic mobility [R_{Gal} 0.15 in solvent (a) and 0.18 in solvent (f)] as an authentic specimen of the β1,6-linked galactotriose. A portion (8 mg.) was methylated. G.l.c. examination of the methanolysis products revealed peaks with retention times corresponding to the methyl glycosides of 2,3,4,6-tetra- [(i),T 1.68; (ii),T 1.66], and 2,3,5- [(i),T 4.08, 5.40; (ii),T 3.28, 4.40] and 2,3,4-tri-O-methyl-D-galactose [(i),T 6.46; (ii),T 5.28]. Hydrolysis of the methyl glycosides followed by paper chromatography in solvent (d) confirmed the presence of 2,3,4,6-tetra- (R_G 0.90), and 2,3,5- (R_G 0.86) and 2,3,4-tri-O-methyl-D-galactose (R_G 0.71).

Borohydride reduction of degraded gum A.- Degraded gum A (500 mg.) was dissolved in water (100 ml.), and sodium borohydride (400 mg.) was added. The solution was kept for 24 hours at room temperature before

further sodium borohydride (100 mg.) was added. After the solution had been stirred for 6 hours, it was dialysed against running tap water for 2 days. The freeze dried product was hydrolysed to yield galactose, arabinose and the same four aldobiouronic acids obtained from degraded gum A. In addition, paper chromatography in solvent (h) indicated the presence of galactitol. No arabinitol was detected.

Degraded gum A gave no formaldehyde on periodate oxidation. The production of formaldehyde with time for reduced degraded gum A (40.4 mg. polysaccharide) was as follows: 0.25 hours, 180 μg.; 0.5 hours, 230 μg.; 1 hour, 235 μg.; 2 hours, 245 μg.; 6 hours, 245 μg.; 24 hours, 250 μg. Assuming the production of one molecule of formaldehyde per average polymer unit, a value for $\overline{M_n}$ of 4,800 was calculated for degraded gum A. Molecular-sieve chromatography[27,28,29] using a previously calibrated Bio-Gel P300 column (45 x 4.8 cm.) gave an estimated $\overline{M_n}$ for degraded gum A of 5,200.

Methylation of degraded gum A.- Degraded gum A (1.34 g. polysaccharide) was exhaustively methylated to give a product (974 mg.), $[\alpha]_D$ +18° (*c*, 1.00 in $CHCl_3$)(Found: OMe, 40.1%, not raised on further attempted methylation). Methanolysis of a sample of this product followed by g.l.c. examination of the mixture of methyl glycosides gave the results shown in Table 10. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g) indicated the presence of 2,6-di- and 2-*O*-methyl-*D*-galactose in addition to those *O*-methyl sugars already identified by g.l.c. examination of their methyl glycosides (Table 10).

Methylated degraded gum A (790 mg.) was heated under reflux for 8 hours with methanolic 5% hydrogen chloride (80 ml.). The resulting mixture

Table 10.

Examination of methanolysis and hydrolysis products from methylated degraded gum A.

O-Methyl sugar	Approximate relative molar proportions	Relative retention times of methyl glycosides on:- column (i)	column (ii)	R_G in solvent (d)
2,3,5-Me_3-L-arabinose	trace	0.56; 0.73	0.49; 0.63	0.98
2,3,4-Me_3-L-arabinose	trace	1.01	0.82	0.79
2,3,4,6-Me_4-D-galactose	++	1.68	1.66	0.90
2,3,6-Me_3-D-galactose	+/2	(3.00)[a]; (3.76)[a]; (4.25)[a]	2.45; (3.04)[a]; (3.50)[a]	0.72
2,4,6-Me_3-D-galactose	++	(3.76)[a]; (4.25)[a]	(3.04)[a]; (3.50)[a]	0.72
2,3,4-Me_3-D-galactose	+++	6.45	5.30	0.72
2,4-Me_2-D-galactose	+++	14.5; 16.3	9.9; 11.3	0.53
2,3,4-Me_3-D-glucuronic acid[b]	++	2.38; (3.00)[a]	2.20; 2.75	-
2,6-Me_2-D-galactose	+	-	-	0.59
2-Me-D-galactose	+	-	-	0.34

a. Figures in parenthesis indicate T values of components which are not completely resolved.
b. As methyl ester methyl glycoside.

of methyl glycosides was hydrolysed with N-sulphuric acid (100 ml.) on a boiling water bath for 4 hours. The cooled solution was neutralised with barium carbonate, filtered, treated with Amberlite resin IR120 (H^+) and concentrated to a syrup (750 mg.). The mixture of O-methyl sugars was separated on Whatman 3MM papers by double development in solvent (g) to give the following fractions.

Fraction 1 (58 mg.) had $[\alpha]_D$ +117° (c, 0.58). It had the same paper chromatographic mobility [R_G 0.89 in solvent (d) and 0.72 in solvent (g)] as an authentic specimen of 2,3,4,6-tetra-O-methyl-D-galactose. It was characterized by conversion into N-phenyl-2,3,4,6-tetra-O-methyl-D-galactosylamine which, after recrystallisation from ethyl acetate, had m.p. 192° - 195° [Lit. m.p.,[62] 192° - 197°].

Fraction 2 (39 mg.) had $[\alpha]_D$ +100° (c, 0.39). Demethylation gave galactose and arabinose. A portion (5 mg.) was refluxed with methanolic 5% hydrogen chloride for 7 hours. G.l.c. examination of the mixture of methyl glycosides indicated the presence of 2,3,5- (+/2) [(i),T 0.56, 0.73; (ii),T 0.48, 0.63] and 2,3,4-tri-O-methyl-L-arabinose (+) [(i),T 1.00; (ii),T 0.82], and 2,3,4,6-tetra-O-methyl-D-galactose (++) [(i),T 1.68; (ii),T 1.66].

Fraction 3. On chromatographic examination by double development in solvent (g), this fraction was found to contain three components. Fraction 3 was therefore refractionated on Whatman 3MM papers, by double development in solvent (g), to give Fractions 3a, 3b and 3c.

Fraction 3a (26 mg.) had $[\alpha]_D$ +96° (c, 0.26). Examination by double development in solvent (g) indicated the presence of 2,3,6- (++) and 2,4,6-tri-O-methyl-D-galactose (+). A portion (5 mg.) was refluxed with methanolic 5% hydrogen chloride for 7 hours. G.l.c. examination of the mixture of methyl glycosides indicated the presence of 2,3,6- [(i),T 3.04, (3.76), (4.24); (ii),T 2.50, (3.04), (3.50)], and 2,4,6-tri-O-methyl-D-galactose [(i),T (3.76), (4.24); (ii),T (3.04), (3.50)].

Fraction 3b (54 mg.) had $[\alpha]_D$ +95° (c, 0.54). A portion (5 mg.) was refluxed with methanolic 5% hydrogen chloride for 7 hours. G.l.c.

examination of the methyl glycosides indicated the presence of 2,4,6- [(i),τ 3.76, 4.24; (ii),τ 3.04, 3.50] and a trace of 2,3,4-tri-O-methyl-D-galactose [(i),τ 6.46; (ii),τ 5.29]. Fraction 3b is therefore almost pure 2,4,6-tri-O-methyl-D-galactose. It was characterized by conversion into N-phenyl-2,4,6-tri-O-methyl-D-galactosylamine, which after recrystallisation twice from ethyl acetate and once from acetone - ether - light petroleum [1:1:1 (v/v)] had m.p. 163° - 165° [Lit. m.p.,[62] 170° - 180°].

Fraction 3c (31 mg.) had $[\alpha]_D$ +110° (c 0.31). It had the same paper chromatographic mobility [R_G 0.71 in solvent (d)] as an authentic specimen of 2,3,4-tri-O-methyl-D-galactose. A portion (5 mg.) was refluxed with methanolic 5% hydrogen chloride for 7 hours. G.l.c. examination of the methyl glycosides indicated the presence of 2,3,4-tri-O-methyl-D-galactose [(i),τ 6.44; (ii),τ 5.30]. It was characterized by conversion into N-phenyl-2,3,4-tri-O-methyl-D-galactosylamine which, after recrystallisation from ethyl acetate, had m.p. 164° - 167° [Lit. m.p.,[62] 164° - 169°].

Fraction 4 (22 mg.) had $[\alpha]_D$ +80° (c 0.44). It had the same paper chromatographic mobility [R_G 0.54 in solvent (d) and 0.19 in solvent (g)] as 2,6-di-O-methyl-D-galactose. A portion (5 mg.) was submitted to periodate oxidation. Paper chromatographic examination of the oxidation products in solvent (d) revealed a bright yellow spot (p-anisidine hydrochloride spray) with the same mobility (R_G 0.20) as 2-O-methyl-malondialdehyde.

Fraction 5 (42 mg.) had $[\alpha]_D$ +88° (c, 0.42). It had the same paper chromatographic mobility [R_G 0.49 in solvent (d) and 0.12 in solvent (g)] as an authentic specimen of 2,4-di-O-methyl-D-galactose. Recrystallisation of the O-methyl sugar from acetone containing 1% of water gave 2,4-di-O-

methyl-D-galactose monohydrate, m.p. 96° - 97°; its X-ray diffraction pattern was identical to that of an authentic specimen of 2,4-di-O-methyl-D-galactose monohydrate. The derived N-phenyl-2,4-di-O-methyl-D-galactosylamine, after recrystallisation from acetone, had m.p. 216° - 219° [Lit. m.p.,[62] 214° - 228°].

Fraction 6 (28 mg.) had $[\alpha]_D$ +81° (c, 0.56). It had the same paper chromatographic mobility [R_G 0.31 in solvent (d)] as an authentic specimen of 2-O-methyl-D-galactose. Recrystallisation of the O-methyl sugar from glacial acetic acid gave crystals with m.p. 151° - 154° [Lit. m.p.,[62] 145° - 148°].

Fraction 7 (76 mg.) had $[\alpha]_D$ +53° (c, 0.76). A portion (5 mg.) was refluxed with methanolic 5% hydrogen chloride for 7 hours. G.l.c. examination of the methyl glycosides indicated the presence of the methyl ester of 2,3,4-tri-O-methyl-D-glucuronic acid [(i),T 2.35, 3.05; (ii),T 2.20, 2.76]. The rest of the fraction was refluxed with methanolic 5% hydrogen chloride for 7 hours. The derived methyl ester methyl glycoside was reduced with sodium borohydride (200 mg. in 10 ml. water) for 12 hours. Excess borohydride was destroyed by addition of Amberlite resin IR120 (H^+), and borate was removed as methyl borate. Thick paper chromatography of the product yielded pure 2,3,4-tri-O-methyl-D-glucose (26 mg.). A portion of this (5 mg.) was refluxed with methanolic 5% hydrogen chloride for 7 hours. G.l.c. examination of the methyl glycosides indicated the presence of 2,3,4-tri-O-methyl-D-glucose [(i),T 2.44, 3.43].

Reduction of methylated degraded gum A.- Lithium aluminium hydride (50 mg.) was added to methylated degraded gum A (50 mg.) dissolved in tetrahydrofuran (10 ml.); the mixture was refluxed for 3 hours. After

Table 11.

Examination of methanolysis and hydrolysis products from reduced methylated degraded gum A.[a]

O-Methyl sugar	Approximate relative molar proportions	Relative retention times of methyl glycosides on:- column (i)	column (ii)	R_G in solvent (d)
2,3,5-Me_3-L-arabinose	trace	0.57; 0.73	0.49; 0.63	0.98
2,3,4-Me_3-L-arabinose	trace	(1.00)	0.82	0.79
2,3,4,6-Me_4-D-glucose	++	(1.00); 1.40	1.00; 1.39	1.00
2,3,4,6-Me_4-D-galactose	++	1.68	1.66	0.90
2,3,6-Me_3-D-galactose	+/2	2.96; (3.75); (4.25)	2.45; (3.04); (3.50)	0.72
2,4,6-Me_3-D-galactose	++	(3.75); (4.25)	(3.04); (3.50)	0.72
2,3,4-Me_3-D-galactose	+++	6.45	5.30	0.72
2,4-Me_2-D-galactose	+++	14.6; 16.4	9.9; 11.3	0.53
2,6-Me_2-D-galactose	+	-	-	0.59
2-Me-D-galactose	+	-	-	0.34

a. Figures in parenthesis indicate T values of components which are not completely resolved.

cooling the excess hydride was destroyed by addition of ethyl acetate and water. The mixture was reduced to dryness and extracted with chloroform. The chloroform extract was concentrated to a syrup, which was methylated with methyl iodide (1 ml.) and silver oxide (1 g.) in N,N-di-methylformamide (1 ml.) to give a product (36 mg.), $[\alpha]_D$ +19° (c, 0.72 in $CHCl_3$). Methanolysis of this product followed by g.l.c. examination of the mixture of methyl glycosides gave the results shown in Table 11. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g) indicated the presence of 2,6-di- and 2-O-methylgalactose in addition

to those O-methyl sugars already identified by g.l.c. examination of their methyl glycosides (Table 11).

Partial methanolysis of methylated degraded gum A.- Methylated degraded gum A (100 mg.) was refluxed with methanolic 2% hydrogen chloride (15 ml.) for 2 hours. The methanolysate was neutralised with silver carbonate, filtered and evaporated to a syrup, which was hydrolysed with 0.5N-sulphuric acid (10 ml.) for 5 hours on a boiling water bath. The cooled solution was neutralised with barium carbonate, filtered, treated with Amberlite resin IR120 (H^+), and adsorbed on to a column (33 x 3 cm.) of Duolite A-4 in the formate form. After elution of the neutral O-methyl sugars with water (3 l.), the acidic O-methyl sugars were eluted with aqueous 5% formic acid (500 ml.). Water and formic acid were removed, and paper chromatography, in solvent (d), of the resulting syrup indicated that it was free of neutral O-methyl sugars. The syrup was refluxed with methanolic 5% hydrogen chloride for 7 hours. G.l.c. examination of the products revealed peaks with retention times corresponding to the methyl glycosides of 2,3,4-tri-O-methyl-D-glucuronic acid [(i),T 2.38, (3.00); (ii),T 2.20, 2.75], and 2,3,6- [(i),T (3.00), 3.76, 4.25; (ii),T 2.45, 3.00, 3.50] and 2,3,4-tri- [(i),T 6.45; (ii),T 5.29], and 2,6- [(i),T 9.7; (ii),T 6.4, 7.3, 8.5] and 2,4-di-O-methyl-D-galactose [(i),T 14.5, 16.3; (ii),T 9.9, 11.3]. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g) confirmed these results.

Smith degradation[56,57] of degraded gum A.- Degraded gum A (1.0 g. polysaccharide) was dissolved in water (25 ml.) and 0.5M-sodium meta-periodate solution (25 ml.) was added. After 96 hours 6.8 mmoles of periodate was reduced and 4.0 mmoles of formic acid was released. After

96 hours the reaction was stopped by addition of ethylene glycol (2 ml.), and the solution was dialysed against running tap water for 48 hours. Sodium borohydride (0.5 g.) was added and the mixture kept at room temperature for 30 hours. After dialysis for a further 48 hours, the polyalcohol was hydrolysed in N-sulphuric acid at room temperature for 48 hours. The solution was neutralised with barium carbonate, filtered, treated with Amberlite resin IR120 (H^+), and dialysed against distilled water (1 l.). The syrup obtained from the dialysate (390 mg.) was shown by paper chromatography in solvents (a), (b) and (f) to contain glycerol and glycollic aldehyde as main components, together with small amounts of slower moving non-reducing glycosides. Hydrolysis of a portion of the dialysate (N-sulphuric acid for 7 hours at 100°) followed by paper chromatography in solvents (a) and (b) showed the presence of a trace of galactose. After further dialysis against running tap water for 48 hours, degraded gum B (93 mg. polysaccharide; yield, 9.3%), $[\alpha]_D$ +9° (c, 0.93), was isolated as the freeze dried product.

Mild acid hydrolysis (0.5N-sulphuric acid for 1 hour at 100°) of degraded gum B (10 mg.), followed by paper chromatography in solvent (a), showed the presence of galactose and two neutral disaccharides with the chromatographic mobilities of 3-O-β-D-galactopyranosyl-D-galactose (R_{Gal} 0.48) and 6-O-β-D-galactopyranosyl-D-galactose (R_{Gal} 0.29). Degraded gum B was exhaustively methylated. Methanolysis followed by g.l.c. examination of the mixture of methyl glycosides gave the results shown in Table 12. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g) indicated the presence of 2,6-di- and 2-O-methylgalactose in addition to those O-methyl sugars already

Table 12.

Examination of methanolysis and hydrolysis products from methylated degraded gum B.

O-Methyl sugar	Approximate relative molar proportions	Relative retention times of methyl glycosides in:- column (i)	column (ii)	R_G in solvent (d)
2,3,4,6-Me_4-D-galactose	++	1.68	1.66	0.90
2,4,6-Me_3-D-galactose	+++++	3.76; 4.25	3.00; 3.45	0.72
2,3,4-Me_3-D-galactose	++	6.45	5.29	0.72
2,4-Me_2-D-galactose	++	14.6; 16.4	9.9; 11.3	0.53
2,3,4-Me_3-D-glucuronic acid[a]	trace	2.38; 3.00	2.20; 2.75	-
2,6-Me_2-D-galactose	+	-	-	0.59
2-Me-D-galactose	+	-	-	0.34

a. As methyl ester methyl glycoside.

identified by g.l.c. examination of their methyl glycosides (Table 12).

Controlled Smith degradation[75] of degraded gum A.- Degraded gum A (1.0 g. polysaccharide) was treated in the same way as in the normal Smith degradation of degraded gum A, except that the periodate oxidation stage was carried out at 2°. Degraded gum C was isolated by freeze drying (105 mg. polysaccharide; yield, 10.5%). Hydrolysis of a portion of degraded gum C (20 mg.) followed by paper chromatography in solvent (h) indicated the presence of galactose and arabinitol, but no galactitol.

Methylation of A.drepanolobium gum Fraction A.- The purified gum (10 g. polysaccharide) was methylated by the Haworth and Purdie proceedures to give a product (5.9 g.), $[\alpha]_D$ +60° (c, 1.0 in $CHCl_3$)(Found: OMe, 39.5%, not raised on further attempted methylation). Methanolysis of a portion,

followed by g.l.c. examination of the mixture of methyl glycosides gave the same results as shown in Table 5.

The methylated gum (5 g.) was refluxed with methanolic 5% hydrogen chloride (500 ml.) for 7 hours. The methanolysate was cooled, neutralised with silver carbonate, filtered and concentrated to a syrup. The methyl glycosides were removed by hydrolysis (N-sulphuric acid for 4 hours at 100°). The cooled solution was neutralised with barium carbonate, filtered, treated with Amberlite resin IR120 (H^+) and concentrated to a syrup. This syrup was applied to a column (33 x 3 cm.) of Duolite A-4 in the formate form. Elution of the column with water (3 l.) gave the neutral O-methyl sugar fraction; elution with aqueous 5% formic acid (500 ml.) gave the acidic O-methyl sugar fraction.

The acidic O-methyl sugar fraction (165 mg.) was refluxed with methanolic 5% hydrogen chloride for 7 hours. The derived methyl ester methyl glycosides were reduced by refluxing in dry tetrahydrofuran (40 ml.) with lithium aluminium hydride (300 mg.) for 2 hours. After cooling the excess hydride was destroyed by addition of ethyl acetate and water. The mixture was evaporated to dryness, extracted exhaustively with chloroform and concentrated to a syrup. The methyl glycosides were removed by hydrolysis (N-sulphuric acid for 4 hours at 100°). The cooled solution was neutralised with barium carbonate, filtered, treated with Amberlite resin IR-120 (H^+) and concentrated to a syrup (85 mg.). Fractionation of this product on Whatman 3MM papers in solvent (d) yielded pure 2,3,4-tri-O-methyl-D-glucose (35 mg.). The derived N-phenyl-2,3,4-tri-O-methyl-D-glucosylamine, after recrystallisation from ethyl acetate, had m.p. 146° - 148° (Lit. m.p.,[62] 145° - 150°).

A portion of the neutral O-methyl sugar fraction (3.5 g.) was chromatographed on a column of cellulose (100 x 3.5 cm.) with light petroleum (b.p. 100° - 120°) - butan-1-ol (7:3, saturated with water) as eluant to give six fractions, and with light petroleum (b.p. 100° - 120°)-butan-1-ol (1:1, saturated with water) as eluant to give a further five fractions.

Fraction 1 (35 mg.) from tubes 1 - 80. Paper chromatography in solvent (d) showed the presence of 2,3,4-tri-O-methyl-L-rhamnose (R_G 1.03) and 2,3,5-tri-O-methyl-L-arabinose (R_G 0.98). Demethylation gave arabinose and rhamnose. A portion (10 mg.) was refluxed with methanolic 5% hydrogen chloride for 7 hours. G.l.c. examination of the mixture of methyl glycosides indicated the presence of 2,3,4-tri-O-methyl-L-rhamnose [(i), T 0.48; (ii), T (0.50)] and 2,3,5-tri-O-methyl-L-arabinose [(i), T 0.56, 0.73; (ii), T (0.50), 0.64].

Fraction 2 (355 mg.) from tubes 81 - 190. It had $[\alpha]_D$ -19° (c, 1.0), and the same paper chromatographic mobility [R_G 0.98 in solvent (d) and 1.00 in solvent (g)] as 2,3,5-tri-O-methyl-L-arabinose. It was characterized by conversion into 2,3,5-tri-O-methyl-L-arabonamide which, after recrystallisation from acetone, had m.p. 133° - 135° (Lit. m.p.,[62] 134° - 138°).

Fraction 3 (93 mg.) from tubes 191 - 280 had $[\alpha]_D$ +27° (c, 0.93). Demethylation gave galactose and arabinose. A portion was refluxed with methanolic 5% hydrogen chloride for 7 hours. G.l.c. examination of the mixture of methyl glycosides indicated the presence of 2,3,4,6-tetra-O-methyl-D-galactose [(i), T 1.68; (ii), T 1.66] and 2,3,5-tri-O-methyl-L-arabinose [(i), T 0.56, 0.73; (ii), T 0.50, 0.64], and 2,5- [(i), T 1.75,

3.29; (ii),T 1.24, 2.20] and 3,5-di-O-methyl-L-arabinose [(i),T 1.04, 2.40; (ii),T 0.85, 1.76].

Fraction 4 (770 mg.) from tubes 281 - 420 had $[\alpha]_D$ +26° (c, 1.00). A portion (10 mg.) was refluxed with methanolic 5% hydrogen chloride for 7 hours. G.l.c. examination of the mixture of methyl glycosides indicated that 2,5- [(i),T 1.76, 3.29; (ii),T 1.24, 2.20] and 3,5-di-O-methyl-L-arabinose [(i),T 1.04, 2.40; (ii),T 0.85, 1.76] were the main components of the mixture. A portion (600 mg.) was fractionated on Whatman 3MM papers in solvent (d) to yield a mixture (390 mg.) of 2,5- and 3,5-di-O-methyl-L-arabinose. Paper electrophoretic examination in 0.05M-borate buffer confirmed the presence of 2,5- [M_G 0.00, 10 volts/cm., 0.5 ma./cm.] and 3,5-di-O-methyl-L-arabinose [M_G 0.68, 10 volts/cm., 0.5 ma./cm., brown spot (yellow under u.v.) with aniline oxalate[76]]. These components were separated from the syrupy mixture (200 mg.) by paper electrophoresis in borate buffer[77] on a preparative scale. Zones were detected by spraying side strips. Carbohydrate material and borate buffer were eluted from each zone, sodium ions were removed with Amberlite resin IR-120 (H^+) resin and borate was removed as methyl borate by repeated evaporation with methanol. 2,5-Di-O-methyl-L-arabinose (47 mg.) was characterized by conversion into 2,5-di-O-methyl-L-arabonamide which, after recrystallisation from ethyl acetate, had m.p. 130° - 131° (Lit. m.p.,[62] 122° - 132°). 3,5-Di-O-methyl-L-arabinose (39 mg.) was characterized by conversion into 3,5-di-O-methyl-L-arabonamide which, after recrystallisation from ethyl acetate, had m.p. 139° - 141° (Lit. m.p.,[62] 144°-145°).

Fraction 5 (181 mg.) from tubes 421 - 650 had $[\alpha]_D$ +100° (c, 1.81).

Demethylation gave arabinose. Paper chromatography in solvents (d) and (g) indicated the presence of 2,3,4-tri-O-methyl-L-arabinose [R_G 0.78 in solvent (d) and 0.54 in solvent (g)] in addition to 2,5- and 3,5-di-O-methyl-L-arabinose.

Fraction 6 (123 mg.) from tubes 651 - 1200 had $[\alpha]_D$ +98° (c, 1.23). Demethylation gave galactose and a trace of arabinose. A portion (20 mg.) was refluxed with methanolic 5% hydrogen chloride. G.l.c. examination of the mixture of methyl glycosides indicated that 2,4,6- [(i),T (3.76), (4.20); (ii),T (3.05), (3.50)], 2,3,6- [(i),T 3.04, (3.76), (4.20); (ii),T 2.50, (3.05), (3.50)] and 2,3,4-tri-O-methyl-D-galactose [(i),T 6.45; (ii),T 5.25] were the main components.

Fraction 7 (56 mg.) from tubes 1201 - 1520 had $[\alpha]_D$ +97° (c, 1.12). A portion (6 mg.) was refluxed with methanolic 5% hydrogen chloride. G.l.c. examination of the mixture of methyl glycosides showed the presence of 3,4-di-O-methyl-L-arabinose [(i),T 1.90; (ii),T 1.38]. The remainder of the fraction was chromatographed on Whatman 3MM papers in solvent (d) to give a product (10 mg.). Demethylation gave arabinose. It had the same paper chromatographic mobilities [R_G 0.56 in solvent (d) and 0.20 in solvent (g)] as an authentic specimen of 3,4-di-O-methyl-L-arabinose. A portion (4 mg.) was converted into the methyl glycosides and methylated with methyl iodide (0.5 ml.) and silver oxide (0.5 g.) in N,N-dimethylformamide (0.5 ml.). G.l.c. examination of the reaction mixture showed the presence of the methyl glycoside(s) of 2,3,4-tri-O-methyl-L-arabinose [(i),T 1.00; (ii),T 0.81].

Fraction 8 (170 mg.) from tubes 1521 - 1640 had $[\alpha]_D$ +89° (c, 0.85). Paper chromatographic examination in solvent (d) showed that 2,4-di-O-

methyl-D-galactose was the main component of the mixture. After purification by thick paper chromatography, it was characterized by conversion into N-phenyl-2,4-di-O-methyl-D-galactosylamine which, after recrystallisation from acetone, had m.p. 214° - 216° (Lit. m.p.,[62] 214° - 228°).

Fraction 9 (45 mg.) from tubes 1641 - 1770 had $[\alpha]_D$ +108° (c, 0.45). Fractionation on Whatman 3MM papers in solvent (d) gave a product (15 mg.). Demethylation gave arabinose. The O-methyl sugar had the same chromatographic mobility [R_G 0.35 in solvent (d) and 0.09 in solvent (g)] as an authentic specimen of 4-O-methyl-L-arabinose. A portion (5 mg.) was converted to the methyl glycosides and methylated with methyl iodide (0.5 ml.) and silver oxide (0.5 g.) in N,N-dimethylformamide (0.5 ml.). G.l.c. examination of the reaction mixture indicated the presence of the methyl glycosides of 2,3,4-tri-O-methyl-L-arabinose [(i),T 1.00; (ii),T 0.82].

Fraction 10 (46 mg.) from tubes 1771 - 1820 had $[\alpha]_D$ +88° (c, 0.46). Paper chromatographic examination in solvents (d) and (g) indicated that 2-O-methyl-D-galactose was the main component of the mixture.

Fraction 11 (24 mg.) was obtained from tubes 1821 - 2000. Paper chromatography in solvents (a) and (b) showed the presence of galactose (major component) and arabinose (minor component).

Preparation, partial acid hydrolysis and methylation of polysaccharide A.- The gum (123 g. polysaccharide) was dissolved in water (3.1 l.), mixed with 0.25M-sodium metaperiodate solution (3.1 l.) and left for 48 hours. The amount of formic acid released in this time was 1.05 mmoles/g. The corresponding amount of periodate consumed was 3.96 mmoles/g.

The reaction was stopped by addition of ethylene glycol (50 ml.), and the solution dialysed against running tap water for 48 hours. Sodium borohydride (32 g.) was added, and the mixture kept at room temperature for 30 hours, then dialysed for a further 48 hours. The polyalcohol was hydrolysed in N-sulphuric acid at room temperature for 48 hours. After dialysis against running tap water for 48 hours, polysaccharide A was isolated as the freeze dried product (68.2 g. polysaccharide; yield, 55.5%), $[\alpha]_D$ +45° (*c*, 1.63)(Found: galactose, 43%; arabinose, 57%). Molecular-sieve chromatography of polysaccharide A (20 mg.) on a previously calibrated Bio-Gel P300 column (45 x 4.8 cm.) gave number-average molecular weight, $\overline{M_n}$, *ca.* 95,500.

Polysaccharide A (20 mg.) was hydrolysed with 0.5N-sulphuric acid (10 ml.) for 1 hour on a boiling water bath. Paper chromatographic examination of the hydrolysate indicated the presence of galactose, arabinose, two neutral disaccharides with the mobilities of 3-*O*-β-*D*-galactopyranosyl-*D*-galactose [R_{Gal} 0.49 in solvent (a) and 0.54 in solvent (f), minor component] and 6-*O*-β-*D*-galactopyranosyl-*D*-galactose [R_{Gal} 0.31 in solvent (a) and 0.38 in solvent (f), major component], and higher neutral oligosaccharides.

Polysaccharide A (200 mg.) was methylated with dimethyl sulphate and sodium hydroxide, and with methyl iodide and silver oxide, to give a product (110 mg.), $[\alpha]_D$ +39° (*c*, 0.50 in $CHCl_3$)(Found: OMe, 40.5%, not raised on further attempted methylation). Methanolysis of a sample of this product followed by g.l.c. examination of the mixture of methyl glycosides, gave the results shown in Table 13. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g)

indicated the presence of 2,6-di- and 2-O-methyl-D-galactose in addition to those O-methyl sugars already characterized by g.l.c. of their methyl glycosides (Table 13).

Preparation, partial acid hydrolysis and methylation of polysaccharide B.- Borohydride reduction of periodate oxidised polysaccharide A (67.4 g. polysaccharide), followed by controlled acid hydrolysis at room temperature for 48 hours, gave polysaccharide B (28.4 g. polysaccharide; yield, 42%), $[\alpha]_D$ +20° (c, 1.79)(Found: galactose, 46%; arabinose, 54%). The amount of formic acid released during periodate oxidation was 0.94 mmoles/g. The corresponding amount of periodate consumed was 3.38 mmoles/g. Molecular-sieve chromatography indicated that polysaccharide B had $\overline{M_n}$ ca. 9,200.

Partial acid hydrolysis of polysaccharide B (20 mg.) followed by paper chromatographic examination of the hydrolysate indicated the presence of galactose, arabinose, two neutral disaccharides with the chromatographic mobilities of 3-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.48 in solvent (a) and 0.53 in solvent (f), minor component] and 6-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.31 in solvent (a) and 0.38 in solvent (f), major component], and higher neutral oligosaccharides.

Polysaccharide B (179 mg.) was methylated to give a product (85 mg.), $[\alpha]_D$ +24° (c, 0.50 in $CHCl_3$)(Found: OMe, 41.2%, not raised on further attempted methylation). Methanolysis of a sample of this product followed by g.l.c. examination of the mixture of methyl glycosides, gave the results shown in Table 13. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g) indicated the presence of 2,6-di- and 2-O-methyl-D-galactose in addition to those O-methyl sugars

already characterized by g.l.c. of their methyl glycosides (Table 13).

Preparation, partial acid hydrolysis and methylation of polysaccharide C.- Borohydride reduction of periodate oxidised polysaccharide B (27.7 g. polysaccharide), followed by controlled acid hydrolysis at room temperature for 48 hours gave polysaccharide C (7.4 g. polysaccharide; yield, 26.7%), $[\alpha]_D$ -2° (c, 1.36)(Found: galactose, 33%; arabinose, 67%). The amount of formic acid released during periodate oxidation was 1.47 mmoles/g. The corresponding amount of periodate consumed was 4.18 mmoles/g. Molecular-sieve chromatography indicated that polysaccharide C had $\overline{M_n}$ ca. 8,000.

Partial acid hydrolysis of polysaccharide C (20 mg.) followed by paper chromatographic examination of the hydrolysate indicated the presence of galactose and arabinose, and two neutral disaccharides with the chromatographic mobilities of 3-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.48 in solvent (a) and 0.54 in solvent (f), minor component] and 6-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.30 in solvent (a) and 0.38 in solvent (f), major component], and higher neutral oligosaccharides.

Polysaccharide C (136 mg.) was methylated to give a product (60 mg.), $[\alpha]_D$ +2° (c, 0.50 in $CHCl_3$)(Found: OMe, 42.1%, not raised on further attempted methylation). Methanolysis of a sample of this product followed by g.l.c. examination of the mixture of methyl glycosides, gave the results shown in Table 13. Hydrolysis of the methyl glycosides followed by paper chromatographic examination in solvents (d) and (g) indicated the presence of 2,6-di- and 2-O-methyl-D-galactose in addition to those O-methyl sugars already characterized by g.l.c. of their methyl glycosides (Table 13).

Table 13.

Relative proportions of O-methyl sugars present in methylated polysaccharides A to E.

O-Methyl sugar	A	B	C	D	E
2,3,5-Me_3-L-arabinose	4	2	2	2	4
2,5-Me_2-L-arabinose	4	2	2	1	1
3,5-Me_2-L-arabinose	7	6	8	9	10
2,3,4,6-Me_4-D-galactose	trace	trace	1	1	1
2,4,6-Me_3-D-galactose	3	4	2	2	2
2,3,4-Me_3-D-galactose	1	2	1	1	$\frac{1}{2}$
2,4-Me2-D-galactose	3	3	1	1	1

Preparation, partial acid hydrolysis and methylation of polysaccharide D.- Borohydride reduction of periodate oxidised polysaccharide C (6.9 g. polysaccharide) was followed by controlled acid hydrolysis at room temperature for 48 hours. The hydrolysate was neutralised with barium carbonate, filtered, treated with Amberlite resin IR-120 (H^+) and dialysed against distilled water (2 l.) for 24 hours. After further dialysis against running tap water, polysaccharide D was isolated as the freeze dried product (1.40 g. polysaccharide; yield, 20.3%), $[\alpha]_D$ -21° (c, 1.24)(Found: galactose, 32%; arabinose, 68%). The amount of formic acid released during periodate oxidation was 1.15 mmoles/g. The corresponding amount of periodate consumed was 3.78 mmoles/g. Molecular-sieve chromatography indicated that polysaccharide D had $\overline{M_n}$ ca. 7,200.

The dialysate from the Smith degradation was deionised and concentrated to a syrup (1.74 g.). Paper chromatography in solvents (a)

and (b) indicated the presence of glycerol, glycolaldehyde, arabinose (a trace) and slower non-reducing glycosides. A portion of the syrup (20 mg.) was hydrolysed (N-sulphuric acid for 7 hours at 100°). Paper chromatography in solvents (a) and (b) indicated the presence of glycerol, glycolaldehyde, arabinose and galactose (a trace).

Partial acid hydrolysis of polysaccharide D (10 mg.) followed by paper chromatographic examination of the hydrolysate indicated the presence of galactose, arabinose, two neutral disaccharides with the mobilities of 3-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.47 in solvent (a) and 0.53 in solvent (f), minor component] and 6-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.31 in solvent (a) and 0.38 in solvent (f), major component], and higher neutral oligosaccharides.

Polysaccharide D (124 mg.) was methylated to give a product (53 mg.), $[\alpha]_D$ -17° (c, 0.50 in $CHCl_3$)(Found: OMe, 41.5%, not raised on further attempted methylation). Methanolysis of a sample of this product followed by g.l.c. examination of the mixture of methyl glycosides, gave the results shown in Table 13. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g) indicated the presence of 2,6-di- and 2-O-methyl-D-galactose in addition to those O-methyl sugars already characterized by g.l.c. of their methyl glycosides (Table 13).

Preparation, partial acid hydrolysis and methylation of polysaccharide E.- Borohydride reduction of periodate oxidised polysaccharide D (1.07 g. polysaccharide) was followed by controlled acid hydrolysis at room temperature for 48 hours. The hydrolysate was neutralised with barium carbonate, filtered, treated with Amberlite resin IR-120 (H^+) and dialysed against distilled water (2 l.) for 24 hours.

After further dialysis against running tap water, polysaccharide E was isolated as the freeze dried product (0.32 g. polysaccharide; yield, 29.5%), $[\alpha]_D$ -36° (*c*, 1.86)(Found: galactose, 29%; arabinose, 71%). The amount of formic acid released during periodate oxidation was 0.53 mmoles/g. The corresponding amount of periodate consumed was 1.87 mmoles/g. A portion of polysaccharide E (40 mg.) was periodate oxidised for 48 hours. The amount of formic acid released in this time was 0.35 mmoles/g. The corresponding amount of periodate consumed was 1.05 mmoles/g. Molecular-sieve chromatography indicated that polysaccharide E had $\overline{M_n}$ *ca.* 6,000.

The dialysate from the Smith degradation was deionised and concentrated to a syrup (0.35 g.). Paper chromatography in solvents (a) and (b) indicated the presence of glycerol, glycolaldehyde, arabinose (a trace) and slower non-reducing glycosides. A portion of the syrup (20 mg.) was hydrolysed (N-sulphuric acid for 7 hours at 100°). Paper chromatography in solvents (a) and (b) indicated the presence of glycerol, glycolaldehyde, arabinose and galactose (a trace).

Partial acid hydrolysis of polysaccharide E (10 mg.) followed by paper chromatographic examination of the hydrolysate indicated the presence of galactose, arabinose, equal amounts of 3-*O*-β-*D*-galactopyranosyl-*D*-galactose [R_{Gal} 0.48 in solvent (a) and 0.54 in solvent (f)] and 6-*O*-β-*D*-galactopyranosyl-*D*-galactose [R_{Gal} 0.30 in solvent (a) and 0.38 in solvent (f)] and higher neutral oligosaccharides.

Polysaccharide E (186 mg.) was methylated to give a product (65 mg.), $[\alpha]_D$ -29° (*c*, 0.50 in $CHCl_3$)(Found: OMe, 41.0%, not raised on further attempted methylation). Methanolysis of a sample of this product followed by g.l.c. examination of the mixture of methyl glycosides, gave the

results shown in Table 13. Hydrolysis of the methyl glycosides, followed by paper chromatography in solvents (d) and (g) indicated the presence of 2-O-methyl-D-galactose in addition to those O-methyl sugars already characterized by g.l.c. of their methyl glycosides (Table 13).

5.3) Discussion.

A.drepanolobium gum is not completely soluble in cold water. Extraction with water yields Fraction A (ca. 80%). Extraction of the residue with M-sodium chloride solution yields Fraction B (ca. 8%). The insoluble jelly residue, Fraction C, comprises ca. 12% of the gum. A study of the analytical data presented in Table 1 shows that there are no striking differences in the compositions of the gum Fractions A, B and C. The small difference in sugar ratios between Fractions A and B, and Fraction C is unlikely to account for the solubility differences exhibited by the Fractions. Fraction C has a lower protein content than Fractions A and B. Protein-carbohydrate interaction has been put forward as a possible explanation of gel formation; the jelly isolated from A.pycnantha gum[78] contained three times as much protein as the water soluble gum. In the case of A.drepanolobium gum Fraction C, protein-carbohydrate interaction is unlikely to be the cause of insolubility.

Fraction C is sparingly soluble in M-sodium hydroxide solution.[79] This method of dissolution is not satisfactory, since in such strongly alkaline conditions polysaccharide degradation will probably occur. Eight different solvents for Fraction C were therefore investigated (Table 2). 3M-Sodium chloride solution, 3M-magnesium chloride solution and 7M-urea solution were unsuccessful solvents. Hydrogen bonding

between polysaccharide molecules[80] is therefore unlikely to be the reason for the insolubility of Fraction C. 3% Sodium borate and 0.1M-phosphate buffer dissolve Fraction C to the extent of 7% and 10.5% respectively. Both these solvents have pH 9. The slight alkalinity of these solvents is probably the reason for the slight solubility of Fraction C. Fraction C is dissolved to some extent by 2% disodium ethylenediamine-tetra-acetate solution (17.5%) and 2% sodium hexametaphosphate solution (47%). The action of these solvents is dependent on the chelation of calcium and magnesium.[81,82] Cross-linking by heavy metal ions between polysaccharide molecules might therefore be an explanation for the insolubility of Fraction C. The better action of sodium hexametaphosphate solution is expected since its optimum conditions[83] are neutral pH and room temperature (i.e. the conditions of extraction), while the optimum conditions of disodium ethylenediamine-tetra-acetate solution[81] are pH 10 and 60° - 70°. The best solvent for Fraction C is 1% sodium borohydride solution. The fact that 1% sodium borohydride solution has pH 9 and that it reacts with water at room temperature to give borate ions, cannot account for the high solubility of Fraction C (69%).

Fraction C was extracted with 1% sodium borohydride solution in a large scale experiment to yield Soluble Fraction C as the freeze dried, electrodialysed product. Analysis showed Soluble Fraction C to have the same sugar percentages as Fraction C. On periodate oxidation it released the same amount of formic acid and consumed the same amount of periodate as Fraction C.

Fractions A and B, and Soluble Fraction C were compared using methylation analysis. The methylated products were obtained in similar

yields, and had the same specific rotation. On methanolysis and g.l.c. examination the three methylated products gave identical results. This is evidence for a close structural similarity between Fractions A and B, and Soluble Fraction C.

On Smith degradation,[56,57] Fractions A and B, and Soluble Fraction C gave similar yields of Smith degraded products. The analytical data (Table 6) for the Smith degraded polysaccharides are almost identical. On methylation analysis the three degraded polysaccharides gave identical results. These results give strong support for a close structural similarity between Fractions A and B, and Soluble Fraction C.

Soluble Fraction C has a fairly low intrinsic viscosity. Molecular-sieve chromatography on a Bio-Gel P300 column was carried out to determine if this low viscosity was the result of degradation of the polysaccharide during extraction with sodium borohydride solution. Soluble Fraction C was eluted before the void volume of the column, as determined using Blue Dextran 2000 (Pharmacia, Uppsala, Sweden). In contrast, Fractions A and B were eluted at the void volume of the column. The behaviour of Soluble Fraction C on molecular-sieve chromatography could be caused by either a much higher molecular weight than Fractions A and B, or a different molecular shape from that of Fractions A and B.[29] Differences in molecular shape imply differences in molecular structure such as frequency of branching. Smith degradation and methylation analysis show that the three Fractions are closely similar structurally. It therefore seems unlikely that the differences between Fractions A and B, and Soluble Fraction C is due to differences in molecular shape. Light-scattering measurements confirmed that Soluble Fraction C had a much higher

molecular weight (22.0×10^5) than Fraction A (9.5×10^5) and Fraction B (10.2×10^5).

These experiments show that there is a strong structural similarity between Fractions A, B and C. Soluble Fraction C, and therefore Fraction C, has a much higher molecular weight than the other two Fractions. Interaction between these very large polysaccharide molecules probably cause the water insolubility of Fraction C. Inter-polymer cross-linking by heavy metal ions seems to play some part in this insolubility, since 2% disodium ethylenediamine-tetra-acetate solution and 2% sodium hexa-metaphosphate solution dissolve Fraction C to some extent.[81,82,83] The reason why sodium borohydride solution dissolves Fraction C is not known; it must counteract some of the interactions which cause the polysaccharide molecules of Fraction C to aggregate. The interactions which cause the insolubility of Fraction C seem to be counteracted irrevocably by 1% sodium borohydride solution, since after freeze drying the product, Soluble Fraction C, is water soluble.

A.drepanolobium gum Fraction A was examined in greater detail. In preliminary experiments, samples of Fraction A were examined by zone electrophoresis and ion-exchange chromatography on D.E.A.E.-cellulose.[30] Since no sharp discontinuities in the properties of the molecular species were indicated by these experiments, it seems probable that A.drepanolobium gum Fraction A exhibits the kind of heterogeneity which has been suggested for the gums of A.senegal[1,61] and A.arabica.[4] If this is the case, then A.drepanolobium gum Fraction A may be considered to contain a continuous spectrum of related molecular species.

Fraction A is composed of residues of D-galactose (38%), L-arabinose

(52%), L-rhamnose (1%), D-glucuronic acid (7%) and 4-O-methyl-D-glucuronic acid (2%). In spite of the low rhamnose content of Fraction A, enough of this sugar was isolated to ascertain that it was present in the L-form.

Fraction A was hydrolysed with N-sulphuric acid for 7 hours at 100°. Fractionation of the hydrolysate by thick paper chromatography led to the isolation and characterization of four aldobiouronic acids, viz., 6-O-(β-D-glucopyranosyluronic acid)-D-galactose; 4-O-(α-D-glucopyranosyluronic acid)-D-galactose; 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose; and 4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose. The same four aldobiouronic acids have been characterized from the gums of A.nilotica,[55] A.seyal[84] and A.nubica;[40] they have been chromatographically detected[84] in the hydrolysates of the gums from A.arabica, A.fistula, A.karroo, A.tortilis and A.giraffae. All these Acacia gums have positive specific rotations.[4,84,85,86,87]

Autohydrolysis of Fraction A (4% polysaccharide solution) for 84 hours effected the release of all the rhamnose, most of the arabinose and some galactose. Degraded gum A isolated after dialysis, contained D-galactose (80%), L-arabinose (3%) and uronic acid (17%). Molecular-sieve chromatography gave an estimated value for the number-average molecular weight, $\overline{M}_n$, of degraded gum A of ca. 5,200. Light-scattering measurements gave a value of 950,000 for the weight-average molecular weight, $\overline{M}_w$, of A.drepanolobium gum Fraction A. Degradation on autohydrolysis is much greater than can result from the simple removal of arabinose and rhamnose from the periphery of the molecule. It does not seem to have been caused by the presence of any internal acid-labile arabinofuranose residues, since acid hydrolysis of borohydride reduced

degraded gum A yielded only galactitol. There is no evidence for any galactofuranose residues in the polysaccharide, although it must be admitted that traces of di- and tri-O-methyl-D-galactofuranoses would be difficult to detect using g.l.c. examination. It therefore seems that certain galactopyranosidic bonds must be unusually sensitive to the mild conditions of autohydrolysis.[1,61,67,68]

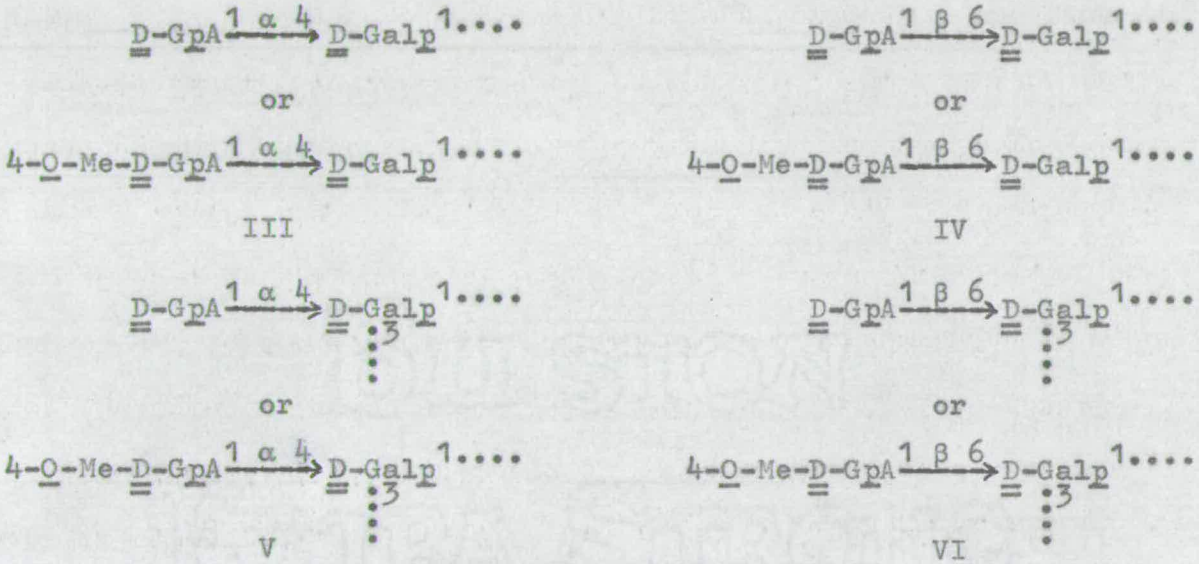
An examination of methylated degraded gum A showed the presence of 2,3,4,6-tetra- (++), 2,4,6- (++), 2,3,6-(+/2) and 2,3,4-tri- (+++), 2,4- (++++) and 2,6-di- (+), and 2-O-methyl-D-galactose (+), and 2,3,4-tri-O-methyl-D-glucuronic acid (++), and traces of 2,3,5- and 2,3,4-tri-O-methyl-L-arabinose. The presence of 2-O-methyl-D-galactose is probably due to undermethylation. This shows that I and II are structural fragments of degraded gum A. Methylation analysis has shown traces of terminal non-

L-Araf^{1}····· L-Arap^{1}·····

I II

reducing L-arabinofuranose residues to be present in the autohydrolysed gum of A.senegal,[1] and terminal non-reducing L-arabinopyranose residues to be present in the degraded gum A of A.arabica.[4] Traces of terminal non-reducing L-arabinofuranose and L-arabinopyranose residues have been detected in the autohydrolysed gum of A.laeta.[3] Lithium aluminium hydride reduction of methylated degraded gum A, followed by a Kuhn methylation,[20] yielded 2,3,4,6-tetra-O-methyl-D-glucose after methanolysis. This confirms that D-glucuronic acid and its 4-O-methyl analogue are terminal non-reducing end groups in degraded gum A. Partial methanolysis was carried out on methylated degraded gum A; after hydrolysis of the methyl glycosides the methylated aldobiouronic acids were separated from the neutral O-methyl

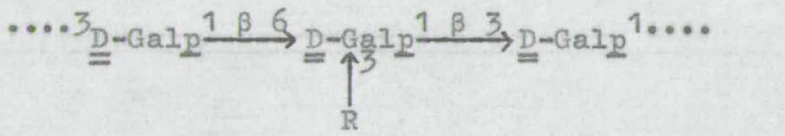
sugars by ion-exchange chromatography. Methanolysis of the methylated aldobiouronic acids followed by g.l.c. examination of the mixture of methyl glycosides showed the presence of 2,3,4-tri-O-methyl-D-glucuronic acid, and 2,3,6- and 2,3,4-tri-, and 2,4- and 2,6-di-O-methyl-D-galactose. This indicates that III - VI are structural fragments of degraded gum A. A similar experiment on A.arabica gum[4] has shown that only III, IV and VI are



structural fragments of A.arabica degraded gum A.

Smith degradation of degraded gum A yielded degraded gum B. Partial acid hydrolysis of degraded gum B gave 3-O-β-D-galactopyranosyl-D-galactose and 6-O-β-D-galactopyranosyl-D-galactose. An examination of methylated degraded gum B indicated the presence of 2,3,4,6-tetra- (++), 2,4,6- (+++++) and 2,3,4-tri- (++), 2,6- (+) and 2,4-di- (++) and 2-O-methyl-D-galactose (+), and a trace of 2,3,4-tri-O-methyl-D-glucuronic acid. The presence of a trace of glucuronic acid in degraded gum B is explained by incomplete oxidation of degraded gum A. Presence of 2,6-di- and 2-O-methyl-D-galactose is probably due to undermethylation. The proportions of the O-methyl-D-galactoses obtained from methylated degraded gum B is evidence

that degraded gum B is a galactan composed predominantly of β1,3 glycosidic linkages, with a few β1,6 branch points. The identification of some 2,3,4-tri-O-methyl-D-galactose from methylated degraded gum B indicates that VII is a possible structural unit of degraded gum A. A similar



where R = L-Araf 1....., L-Arap 1....., D-Galp 1....., ...4D-Galp 1....., or ....6D-Galp 1.....

VII

structural unit has been proposed for A.arabica degraded gum A.[4]

A controlled Smith degradation of degraded gum A yielded degraded gum C. Periodate oxidation of degraded gum A produced no formaldehyde, so it may be concluded that the reducing galactose residue is substituted at C-6. The fact that degraded gum C contains arabinitol is evidence that at least some of the reducing end-group galactose residues of degraded gum A are substituted at C-3.[57]

Mild acid hydrolysis of degraded gum A afforded two galactobioses. The first had the same paper chromatographic mobility as 3-O-β-D-galactopyranosyl-D-galactose, and was characterized as its crystalline monohydrate. Methylation analysis confirmed that a galactose unit was 3-O-substituted by a galactopyranose residue in the disaccharide. Methylation evidence for the second galactobiose indicated that a galactose unit was 6-O-substituted by a galactopyranose residue. Preparation of the phenylosazone confirmed that the disaccharide was 6-O-β-D-galactopyranosyl-D-galactose. Mild acid hydrolysis of degraded gum A also afforded two galactotrioses. Methylation analysis and polarimetry showed these to be

the β1,3-linked galactotriose and the β1,6-linked galactotriose. Characterization of these oligosaccharides shows that VIII and IX are

$$\cdots\underline{\underline{D}}\text{-Gal}\underline{p}\xrightarrow{1\ \beta\ 6}\underline{\underline{D}}\text{-Gal}\underline{p}\xrightarrow{1\ \beta\ 6}\underline{\underline{D}}\text{-Gal}\underline{p}^{1}\cdots$$

VIII

$$\cdots\underline{\underline{D}}\text{-Gal}\underline{p}\xrightarrow{1\ \beta\ 3}\underline{\underline{D}}\text{-Gal}\underline{p}\xrightarrow{1\ \beta\ 3}\underline{\underline{D}}\text{-Gal}\underline{p}^{1}\cdots$$

IX

structural fragments of degraded gum A.

Two arabinobioses were obtained from the diffusate of the autohydrolysate of Fraction A. The first had the same paper chromatographic mobility as 3-O-β-L-arabinofuranosyl-L-arabinose. Methylation analysis indicated that an arabinose unit was 3-O-substituted by an arabinofuranose residue. Preparation of the phenylosazone confirmed that the disaccharide was 3-O-β-L-arabinofuranosyl-L-arabinose. The second arabinobiose had the same paper chromatographic mobility as 3-O-β-L-arabinopyranosyl-L-arabinose. Methylation analysis indicated that an arabinose unit was 3-O-substituted by an arabinopyranose residue. Preparation of the phenylosazone confirmed that the disaccharide was 3-O-β-L-arabinopyranosyl-L-arabinose. The fact that both arabinobioses are released under such mild conditions of autohydrolysis, suggests that in each case the reducing arabinose residue was initially present in the furanose form in Fraction A.

$$\cdots\underline{\underline{L}}\text{-Ara}\underline{f}\xrightarrow{1\ \beta\ 3}\underline{\underline{L}}\text{-Ara}\underline{f}^{1}\cdots$$

X

$$\cdots\underline{\underline{L}}\text{-Ara}\underline{p}\xrightarrow{1\ \beta\ 3}\underline{\underline{L}}\text{-Ara}\underline{f}^{1}\cdots$$

XI

Thus X and XI are structural fragments of A.drepanolobium gum Fraction A.

An examination of the O-methyl derivative of Fraction A indicated the presence of 2,3,5- and 2,3,4-tri-, and 2,5-, 3,5- and 3,4-di-O-methyl-L-arabinose, 2,4-di-O-methyl-D-galactose and 2,3,4-tri-O-methyl-

D-glucuronic acid, together with small amounts of 2,3,4-tri-O-methyl-L-rhamnose, 4-O-methyl-L-arabinose, and 2,3,4,6-tetra-, 2,3,6-, 2,4,6-, and 2,3,4-tri-, and 2-O-methyl-D-galactose. The presence of small amounts of galactose and arabinose are not thought to be structurally significant. The presence of 2-O-methyl-D-galactose is ascribed to undermethylation. Smith degradation studies of Fraction A suggested that 4-O-methyl-L-arabinose probably arises from undermethylation. The presence of a high proportion of 2,4-di-O-methyl-D-galactose indicates that most of the D-galactose residues in Fraction A are 3,6-di-O-substituted. However, the identification of trace amounts of 2,3,4,6-tetra-, 2,3,6-, 2,4,6-, and 2,3,4-tri-O-methyl-D-galactose is evidence for a few terminal non-reducing D-galactose residues, and some 4-O-, 3-O-, and 6-O-substituted D-galactose units respectively. The identification of 2,3,4-tri-O-methyl-D-glucuronic acid suggests that D-glucuronic acid and its 4-O-methyl analogue occur as terminal non-reducing units in the whole gum. The identification of trace amounts of 2,3,4-tri-O-methyl-L-rhamnose indicates the presence of small amounts of non-reducing end-group L-rhamnose units in Fraction A. By analogy with A.senegal gum[46,47] it is possible that this trace of L-rhamnose is attached to D-glucuronic acid residues. The presence of 2,3,5- and 2,3,4-tri-O-methyl-L-arabinose indicates that the arabinose-containing side chains are terminated by either L-arabino-furanose (XII) or L-arabinopyranose residues (XIII). The identification

L-Araf$^{1\cdots}$ XII

L-Arap$^{1\cdots}$ XIII

of some 2,5-di-O-methyl-L-arabinose is evidence for some 3-O-substituted L-arabinofuranose residues (XIV) in the arabinose-containing side chains.

The major dimethyl ether of arabinose obtained from methylated Fraction A

....3-L-Araf-1....

XIV

was, however, the 3,5-di-O-methyl isomer. 3,5-Di-O-methyl-L-arabinose has previously been isolated from the methylated gums of A.pycnantha,[88,89] A.arabica,[4] A.karroo,[86] and A.nubica.[40] It has also been detected in the methanolysates of the methylated gums of A.cyanophylla,[85] A.decurrens,[85] A.giraffae,[85] and A.mearnsii.[85] Its presence in methylated Fraction A suggests that there is a proportion of 2-O-substituted L-arabinofuranose residues (XV) in the arabinose-containing side chains. The presence of 3,4-di-O-methyl-L-arabinose indicates that there is a small proportion of 2-O-substituted L-arabinopyranose residues (XVI) in the arabinose-containing side chains. 3,4-Di-O-methyl-L-arabinose has previously been

....2-L-Araf-1....

XV

....2-L-Arap-1....

XVI

isolated from the methylated gums of A.arabica[4] and A.karroo.[86] A trace of this O-methyl sugar has been detected in the methanolysate of the methylated gum of A.podalyriaefolia.[85] Methylation evidence for Fraction A and degraded gum A suggests that the arabinose-containing side chains are attached to the galactan framework at C-3 and C-6 positions of certain D-galactose residues.

A sample of Fraction A was subjected to five successive Smith degradations. The percentage yields of the Smith-degraded products are low compared with those obtained during successive Smith degradations of A.senegal gum[1] and A.laeta gum (Section IV). However the percentage yields, particularly of the later degradations, are greater than those

obtained during a Smith degradation study of A.arabica gum.[4] The drop in molecular weight from A.drepanolobium gum Fraction A to the first Smith-degraded product is similar to that obtained on Smith degradation of A.senegal gum.[1] Molecular-sieve chromatography indicates a pronounced degradation from polysaccharide A ($\overline{M_n}$, 95,500) to polysaccharide B ($\overline{M_n}$, 9,200). This cleavage must be due to the presence of 6-O-substituted D-galactose residues in polysaccharide A, produced by the removal of arabinose-containing side chains from the C-3 of these residues in the first degradation. The third, fourth and fifth degradations do not cause a large decrease in $\overline{M_n}$. The yields of these degradations, however, are very low. This suggests that polysaccharides B - E are structurally heterogeneous. While some of the molecules of these polysaccharides are broken down only slightly on Smith degradation, others are totally fragmented. This pattern of Smith degradation differs from that of the gums of A.senegal, A.laeta and A.arabica.

Four successive Smith degradations were required to remove all the arabinose units from A.senegal gum;[1] five successive Smith degradations were required to remove all the arabinose units from A.laeta gum (Section IV). Four successive Smith degradations of A.arabica gum[4] decreased the arabinose content, but the fourth Smith-degraded product still contained a considerable proportion of arabinose. It was concluded that the arabinose-containing side chains of A.arabica gum are at least six units long.[4] In contrast, five successive Smith degradations of A.drepanolobium gum Fraction A led to an increase in arabinose content from 52% for the whole gum to 71% for polysaccharide E.

On methanolysis, the O-methyl derivatives of polysaccharides A - E

gave the methyl glycosides of 2,3,5-tri-, 2,5- and 3,5-di-O-methyl-L-arabinose, and 2,3,4,6-tetra-, 2,4,6- and 2,3,4-tri- and 2,4-di-O-methyl-D-galactose in the proportions shown in Table 13. The absence of any evidence for arabinopyranose residues in polysaccharide A suggests that the 4-O-methyl-L-arabinose obtained from methylated Fraction A is not structurally significant. The fact that 3,5-di-O-methyl-L-arabinose is by far the most abundant O-methyl sugar in polysaccharide E, indicates that arabinose-containing side chains in A.drepanolobium gum Fraction A containing at least eight units is certain, and that arabinose-containing side chains of up to fifteen units is not impossible.

From the structural evidence obtained some suggestions can be made for the structure of A.drepanolobium gum Fraction A. Smith degradation study and methylation analysis of the whole gum and degraded gum A indicates that long chains of β1,3-linked D-galactose residues are not such an important structural feature of A.drepanolobium gum Fraction A as they are of A.senegal gum[1] and A.laeta gum (Section IV). Smith degradation and methylation analysis of the degraded gum A suggests that the galactan framework of A.drepanolobium gum Fraction A is closely similar to that of A.arabica gum.[4] If this is the case, the galactan framework of A.drepanolobium gum Fraction A is much more compact than that of A.senegal gum.[1] In this framework, blocks of periodate-resistant β1,3-linked D-galactose units must be interspersed by blocks of periodate-vulnerable β1,6-linked D-galactose units. The results of the first Smith degradation of Fraction A are very similar to those of A.senegal gum.[1] However, in this degradation groups must have been removed from the C-3 positions of 6-O-substituted D-galactose units, since the second

degradation causes a large drop in $\overline{M_n}$. In the third, fourth and fifth degradations, drastic cleavage of the polysaccharide molecules occurs, although the $\overline{M_n}$ of the periodate-resistant molecules falls only slightly. This indicates that although some of the arabinose-containing side chains are undoubtedly very long, there are also a lot of quite short side chains. The large cleavage on the second Smith degradation indicates that Fraction A contains single arabinose units attached to the galactan framework and/or arabinose-containing side chains attached by a 2-O-substituted L-arabinopyranose unit to the galactan framework. Some of these must be attached to position C-3 of 6-O-substituted D-galactose residues. Fragmentation of the galactan framework in the later degradations is to be expected as the short arabinose-containing side chains are progressively removed from the C-3 positions of 6-O-substituted D-galactose units.

Such a scheme of degradation would explain the unexpected increase in arabinose content from polysaccharide B to E. The first degradation removes from the galactan framework single arabinose residues and/or arabinose-containing side chains attached by a 2-O-substituted L-arabinopyranose unit to the galactan framework. This produces some periodate-vulnerable 6-O-substituted D-galactose residues in the core. The second degradation fragments the molecule drastically. It is to be expected that those parts of the galactan framework which resist periodate oxidation, will be those parts of the framework to which are attached a lot of arabinose-containing side chains and/or single arabinose residues. In the subsequent degradations, the molecules which survive fragmentation will be those which contain the most number of the longer arabinose-

containing side chains. Single arabinose residues and short arabinose-containing side chains attached to the galactan framework of the degraded products will be progressively removed from the C-3 positions of 6-O-substituted D-galactose units. Such polymer molecules will be rapidly fragmented on Smith degradation. On repeated Smith degradation, degraded products with comparatively long arabinose-containing side chains attached to galactan frameworks consisting mainly of β1,3-linked D-galactose residues will be favoured. ~~Figure 1 shows a possible example of a molecule from the molecular spectrum of polysaccharide D and its Smith-degraded product, polysaccharide E.~~ This shows that an increase in arabinose content on Smith degradation can be explained. It also supports the assignment of long arabinose-containing side chains (up to nine units long in polysaccharide D), since if ~~the example of~~ a molecule of polysaccharide D contained eight side chains of medium length instead of four side chains ca. eight units long, the arabinose content of polysaccharide E would be less than that of polysaccharide D.

The structural evidence suggests that A.drepanolobium gum Fraction A molecules possess a branched galactan framework to which are attached uronic acid residues and arabinose-containing side chains. The trace of rhamnose is present as terminal non-reducing end groups. The available evidence suggests that the galactan framework of Fraction A has a similar degree of branching to that of A.arabica gum, in which blocks of periodate-resistant β1,3-linked D-galactose residues are interspersed by blocks of periodate-vulnerable β1,6-linked D-galactose residues. To this framework are attached some single arabinose residues, some short arabinose-containing side chains and a few relatively long arabinose-containing

side chains. From the ratio of 2,5- to 3,5-di-O-methyl-L-arabinose obtained from the O-methyl derivatives of polysaccharides A - E (Table 13), it seems probable that the short arabinose-containing side chains are predominantly 1,3-linked and the long arabinose-containing side chains are predominantly 1,2-linked. A few arabinose-containing side chains of lengths up to fifteen units seem necessary to explain the Smith degradation results. The molecular structure of A.drepanolobium gum Fraction A is quite different from that of A.senegal gum,[1] and very similar to that of A.arabica gum.[4] Linkage analysis, methylation analysis and autohydrolysis show that A.drepanolobium gum Fraction A and A.arabica gum are very similar. The only significant difference between A.drepanolobium gum Fraction A and A.arabica gum is in the arrangement and length of the arabinose-containing side chains. The presence of long arabinose-containing side chains in A.drepanolobium gum Fraction A would be expected to cause the molecules of A.drepanolobium gum Fraction A to be less compact than molecules of A.arabica gum. This could explain differences in viscosity and behaviour on molecular-sieve chromatography between the two gums.

SECTION VI

STRUCTURAL STUDY OF THE GUM EXUDATE OF ACACIA SEYAL DEL.

6.1) Introduction.

It has already been shown that the gum exudate of Acacia seyal Del. has a positive specific rotation and a low content of L-rhamnose that does not bear a unit molar correspondance to the uronic acid content.[90] D-Galactose, L-arabinose and L-rhamnose have been recognised as constituent sugars of the gum.[90] D-Glucuronic acid and its 4-O-methyl analogue have been isolated and characterized from the gum.[84] Acid hydrolysis followed by preparative paper chromatography has led to the isolation and characterization[84] of four aldobiouronic acids, viz., 6-O-(β-D-glucopyranosyluronic acid)-D-galactose, 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose, 4-O-(α-D-glucopyranosyluronic acid)-D-galactose and 4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose. Results of a detailed analytical study of A.seyal gum has also been reported.[91] The results of a more detailed structural study of A.seyal gum are reported and discussed in this Section.

The single nodule of gum from A.seyal del. used in this study was collected by the Gum Research Officer to the Republic of Sudan, from a botanically authenticated tree at Umm Ruaba Forest Reserve, Eastern Kordofan, on 9th March 1960.

6.2) Results.

Purification of A.seyal gum.- The gum (91 g.) was dissolved in water

(2 l.), filtered and dialysed against running tap water. The polysaccharide was isolated as the freeze dried product (87.5 g.), $[\alpha]_D$ +59° (c, 0.50)(Found: moisture, 8.9%; N, 0.17%; protein, 1.1%; OMe, 0.70%; limiting viscosity number, 9.9; D-galactose, 38%; L-arabinose, 45%; L-rhamnose, 4%; D-glucuronic acid, 7%; 4-O-methyl-D-glucuronic acid, 6%).

The gum was shown to migrate as a single band on electrophoresis on cellulose acetate film in both 0.1M-ammonium carbonate buffer (pH 8.9) and 0.1M-acetate buffer (pH 4.7). The gum was chromatographed on a D.E.A.E.-cellulose column (45 x 1.3 cm.).[30] Gradient elution with sodium chloride solution (0.0M → 0.5M) in 0.02M-acetate buffer (pH 4.1) gave a single slightly asymmetric peak for its elution pattern.

Preparation of degraded gum A.- The gum (20.0 g. polysaccharide) was hydrolysed with 0.01N-sulphuric acid (800 ml.) for 100 hours on a boiling water bath. The cooled solution was neutralised with barium carbonate, filtered, treated with Amberlite resin IR-120 (H^+), and dialysed against distilled water (3 l.). Dialysis was completed against running tap water. Degraded gum A was isolated as the freeze dried product (4.4 g. polysaccharide; yield, 22%), $[\alpha]_D$ +21° (c, 0.87)(Found: uronic acid, 15%; D-galactose, 82%; L-arabinose, 3%). Hydrolysis of degraded gum A (20 mg.) gave galactose, arabinose and the four aldobiouronic acids which have been characterized from the whole gum.[84] Molecular-sieve chromatography using a previously calibrated Bio-Gel P300 column (45 x 5.0 cm.)[27,28,29] gave an estimated $\overline{M_n}$ for degraded gum A of 4,900.

The diffusate was concentrated to a syrup (11.6 g.). Chromatographic examination of the syrup showed the presence of galactose, arabinose, rhamnose, and two neutral disaccharides with the mobilities of

3-O-β-L-arabinofuranosyl-L-arabinose [R_{Gal} 1.25 in solvent (b) and 1.43 in solvent (g)] and 3-O-β-L-arabinopyranosyl-L-arabinose [R_{Gal} 0.77 in solvent (a) and 0.71 in solvent (b)].

The first arabinobiose was isolated by fractionation of a portion of the diffusate on Whatman 3MM papers in solvent (g) to give a syrup (35 mg.). This had $[\alpha]_D$ +90° (c, 0.70) and yielded arabinose on acid hydrolysis. It had the same paper chromatographic mobility [R_{Gal} 1.25 in solvent (b) and 1.44 in solvent (g)] as an authentic specimen of 3-O-β-L-arabinofuranosyl-L-arabinose. A portion (15 mg.) was methylated. G.l.c. examination of the methanolysis products revealed peaks with retention times corresponding to the methyl glycosides of 2,3,5-tri- [(i),T 0.56, 0.72; (ii),T 0.48, 0.63] and 2,5- [(i),T 1.82, 3.25; (ii),T 1.28, 2.20] and 2,4-di-O-methyl-L-arabinose [(i),T 2.07, 2.18; (ii),T 1.46, 1.56]. Hydrolysis of the methyl glycosides followed by paper chromatography in solvent (d) confirmed the presence of 2,3,5-tri- (R_G 0.98), and 2,5- (R_G 0.84) and 2,4-di-O-methyl-L-arabinose (R_G 0.65).

The second arabinobiose was isolated by fractionation of a portion of the diffusate on Whatman 3MM papers in solvent (b) to give a syrup (63 mg.). This had $[\alpha]_D$ +193° (c, 0.63), and yielded arabinose on acid hydrolysis. It had the same paper chromatographic mobility [R_{Gal} 0.77 in solvent (a) and 0.71 in solvent (b)] as an authentic specimen of 3-O-β-L-arabinopyranosyl-L-arabinose. A portion (15 mg.) was methylated. G.l.c. examination of the methanolysis products revealed peaks with retention times corresponding to the methyl glycosides of 2,3,4-tri- [(i),T 1.00; (ii),T 0.82], and 2,5- [(i),T 1.82, 3.25; (ii),T 1.28, 2.21] and 2,4-di-O-methyl-L-arabinose [(i),T 2.08, 2.17; (ii),T 1.47, 1.56].

Hydrolysis of the methyl glycosides followed by paper chromatography in solvent (d) confirmed the presence of 2,3,4-tri- (R_G 0.79), and 2,5- (R_G 0.84) and 2,4-di-O-methyl-L-arabinose (R_G 0.65). The phenylosazone of the disaccharide was prepared and, after recrystallisation from water, had m.p. 232° - 234° (decomp.)(Lit. m.p.,[74] 233° - 235°).

Partial acid hydrolysis of degraded gum A.- Degraded gum A (1.9 g. polysaccharide) was hydrolysed with 0.5N-sulphuric acid (500 ml.) on a boiling water bath for 1 hour. The cooled solution was neutralised with barium carbonate, filtered, treated with Amberlite resin IR-120 (H^+) and concentrated to a syrup (1.8 g.). The syrup was chromatographed on Whatman 3MM papers in solvent (a) to give two pure disaccharides.

Fraction 1 (30 mg.) had $[\alpha]_D$ +62° (c, 0.60) and yielded galactose on acid hydrolysis. It had the same paper chromatographic mobility [R_{Gal} 0.47 in solvent (a) and 0.53 in solvent (f)] as an authentic specimen of 3-O-β-D-galactopyranosyl-D-galactose. A portion (10 mg.) was methylated. G.l.c. examination of the methanolysis products revealed peaks with retention times corresponding to the methyl glycosides of 2,3,4,6-tetra- [(i),T 1.68; (ii),T 1.66], and 2,4,6- [(i),T 3.76, 4.25; (ii),T 3.01, 3.45] and 2,5,6-tri-O-methyl-D-galactose [(i),T 3.98; (ii),T 3.25]. Hydrolysis of the methyl glycosides followed by paper chromatography in solvent (d) confirmed the presence of 2,3,4,6-tetra- (R_G 0.90), and 2,4,6- (R_G 0.72) and 2,5,6-tri-O-methyl-D-galactose (R_G 0.88). The disaccharide was crystallised from aqueous acetone to give needles of 3-O-β-D-galacto-pyranosyl-D-galactose monohydrate, which had m.p. and mixed m.p. 157° - 159° (Lit. m.p.,[74] 159° - 160°).

Fraction 2 (44 mg.) had $[\alpha]_D$ +31° (c, 0.44) and yielded galactose on

acid hydrolysis. It had the same paper chromatographic mobility [R_{Gal} 0.30 in solvent (a) and 0.38 in solvent (f)] as an authentic specimen of 6-O-β-D-galactopyranosyl-D-galactose. A portion (15 mg.) was methylated. G.l.c. examination of the methanolysis products revealed peaks with retention times corresponding to the methyl glycosides of 2,3,4,6-tetra- [(i),T 1.68; (ii),T 1.66], and 2,3,5- [(i),T 4.05, 5.40; (ii),T 3.30, 4.38] and 2,3,4-tri-O-methyl-D-galactose [(i),T 6.45; (ii),T 5.25]. Hydrolysis of the methyl glycosides followed by paper chromatography in solvent (d) confirmed the presence of 2,3,4,6-tetra- (R_G 0.90), and 2,3,5- (R_G 0.86) and 2,3,4-tri-O-methyl-D-galactose (R_G 0.72).

Borohydride reduction of degraded gum A.- Degraded gum A (50 mg.) was dissolved in water (10 ml.), and sodium borohydride (100 mg.) was added. The solution was left for 24 hours at room temperature before further sodium borohydride (100 mg.) was added. After the solution had been stirred for 6 hours, it was dialysed against running tap water for 2 days and freeze dried to yield the product (45 mg.). A portion of the product (20 mg.) was hydrolysed (N-sulphuric acid for 7 hours at 100°) to yield galactose, arabinose, and the same four aldobiouronic acids which were detected in the hydrolysate of degraded gum A. In addition, paper chromatography in solvent (h) indicated the presence of galactitol. No arabinitol was detected. Degraded gum A gave no formaldehyde on periodate oxidation. Some formaldehyde was released on periodate oxidation of reduced degraded gum A.

Methylation of degraded gum A.- Degraded gum A (400 mg.) was methylated successively with dimethyl sulphate and sodium hydroxide, and with methyl iodide and silver oxide, to give a product (240 mg.), $[\alpha]_D$ -14° (c, 0.83

in $CHCl_3$)(Found: OMe, 41.1%, not raised on further attempted methylation). Methanolysis of a sample of this product, followed by g.l.c. examination of the mixture of methyl glycosides, gave the results shown in Table 1. Examination of the hydrolysate of the methyl glycosides by paper chromatography in solvents (d) and (g) indicated the presence of 2,6-di- and 2-O-methyl-D-galactose in addition to those O-methyl sugars already characterized by g.l.c. of their methyl glycosides.

Reduction of methylated degraded gum A.- Lithium aluminium hydride (50 mg.) was added to methylated degraded gum A (50 mg.) dissolved in tetrahydrofuran (10 ml.); the mixture was refluxed for 3 hours. After cooling, the excess hydride was destroyed by addition of ethyl acetate and water. The mixture was reduced to dryness and extracted with chloroform. The chloroform extract was concentrated to a syrup. This was methylated with methyl iodide (1 ml.) and silver oxide (1 g.) in N,N-dimethylformamide (1 ml.) to give a product (35 mg.), $[\alpha]_D$ -13° (c, 0.70 in $CHCl_3$). Methanolysis of the product, followed by g.l.c. examination of the mixture of methyl glycosides gave the results shown in Table 2. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g) indicated the presence of 2,6-di- and 2-O-methyl-D-galactose in addition to those O-methyl sugars already identified by g.l.c. examination of their methyl glycosides (Table 2).

Partial methanolysis of methylated degraded gum A.- Methylated degraded gum A (100 mg.) was refluxed with methanolic 2% hydrogen chloride (15 ml.) for 2 hours. The methanolysate was neutralised with silver carbonate, filtered and evaporated to a syrup, which was hydrolysed with 0.5N-sulphuric acid (10 ml.) for 5 hours on a boiling water bath. The

Table 1.

Examination of methanolysis and hydrolysis products from methylated degraded gum A.[a]

O-Methyl sugar	Approximate relative molar proportions	Relative retention times of methyl glycosides on:- column (i)	column (ii)	R_G in solvent (d)
2,3,5-Me_3-L-arabinose	trace	0.56; 0.73	0.50; 0.63	0.98
2,3,4-Me_3-L-arabinose	trace	1.02	0.82	0.79
2,3,4,6-Me_4-D-galactose	++	1.69	1.66	0.90
2,3,6-Me_3-D-galactose	+	(3.02); (3.80); (4.29)	2.50; (3.05); (3.51)	0.72
2,4,6-Me_3-D-galactose	++	(3.80); (4.29)	(3.05); (3.51)	0.72
2,3,4-Me_3-D-galactose	++++	6.40	5.29	0.72
2,4-Me_2-D-galactose	+++	14.4; 16.3	9.9; 11.3	0.53
2,3,4-Me_3-D-glucuronic acid[b]	++	2.40; (3.02)	2.21; 2.72	-
2,6-Me_2-D-galactose	+	-	-	0.59
2-Me-D-galactose	+	-	-	0.34

a. Figures in parenthesis indicate T values of components which are not completely resolved.
b. As methyl ester methyl glycoside.

cooled solution was neutralised with barium carbonate, filtered, treated with Amberlite resin IR-120 (H^+), and adsorbed on to a column (33 x 3 cm.) of Duolite A-4 in the formate form. After elution of the neutral O-methyl sugars with water (3 l.), the acidic O-methyl sugars were eluted with aqueous 5% formic acid (500 ml.). Water and formic acid were removed, and paper chromatography of the resulting syrup in solvent (d) indicated that it was free of neutral O-methyl sugars. The syrup was refluxed with

Table 2.

Examination of methanolysis and hydrolysis products from methylated reduced degraded gum A.[a]

O-Methyl sugar	Approximate relative molar proportions	Relative retention times of methyl glycosides on:- column (i)	column (ii)	R_G in solvent (d)
2,3,5-Me_3-L-arabinose	trace	0.56; 0.73	0.50; 0.64	0.98
2,3,4-Me_3-L-arabinose	trace	(1.00)	0.82	0.79
2,3,4,6-Me_4-D-glucose	++	(1.00); 1.40	1.00; 1.39	1.00
2,3,4,6-Me_4-D-galactose	++	1.68	1.66	0.90
2,3,6-Me_3-D-galactose	+	3.02 ; (3.79); (4.29)	2.49; (3.05) (3.51)	0.72
2,4,6-Me_3-D-galactose	++	(3.79); (4.29)	(3.05); (3.51)	0.72
2,3,4-Me_3-D-galactose	++++	6.40	5.29	0.72
2,4-Me_2-D-galactose	+++	14.4; 16.4	9.9; 11.3	0.53
2,6-Me_2-D-galactose	+	-	-	0.59
2-Me-D-galactose	+	-	-	0.34

a. Figures in parenthesis indicate T values of components which are not completely resolved.

methanolic 5% hydrogen chloride for 7 hours. G.l.c. examination of the products revealed peaks with retention times corresponding to the methyl glycosides of 2,3,4-tri-O-methyl-D-glucuronic acid [(i),T 2.36, (3.00); (ii),T 2.20, 2.76], and 2,3,6- [(i),T (3.00), 3.76, 4.26; (ii),T 2.46, 2.99, 3.49] and 2,3,4-tri- [(i),T 6.45; (ii),T 5.30], and 2,6- [(i),T 9.6; (ii),T 6.4, 7.4, 8.5] and 2,4-di-O-methyl-D-galactose [(i),T 14.6, 16.4; (ii),T 9.9, 11.4]. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g) confirmed these results.

Smith degradation[56,57] of degraded gum A.- Degraded gum A (1.0 g. polysaccharide) was dissolved in water (25 ml.) and 0.5M-sodium metaperiodate solution (25 ml.) was added. After 96 hours 7.1 mmoles of periodate was consumed and 3.2 mmoles of formic acid was released. After 96 hours the reaction was stopped by addition of ethylene glycol (2 ml.), and the solution was dialysed against running tap water for 48 hours. Sodium borohydride (0.5 g.) was added, and the mixture kept at room temperature for 30 hours. After dialysis for a further 48 hours, the polyalcohol was hydrolysed in N-sulphuric acid at room temperature for 48 hours. The solution was neutralised with barium carbonate, filtered, treated with Amberlite resin IR-120 (H^+) and dialysed against distilled water (1 l.) for 24 hours. After further dialysis against running tap water for 48 hours, degraded gum B (50 mg. polysaccharide; yield, 5%), $[\alpha]_D$ +7.5° (c, 0.50), was isolated as the freeze dried product.

The syrup obtained from the dialysate (400 mg.) was shown by paper chromatography in solvents (a), (b), and (f) to contain glycerol and glycollic aldehyde as main components, together with small amounts of slower moving non-reducing glycosides. Hydrolysis of a portion of the dialysate (N-sulphuric acid for 7 hours at 100°) followed by paper chromatography in solvents (a) and (b) showed a trace of galactose to be present.

Mild acid hydrolysis (0.5N-sulphuric acid for 1 hour at 100°) of degraded gum B (10 mg.), followed by paper chromatography in solvent (a) showed the presence of galactose and two neutral disaccharides with the chromatographic mobilities of 3-O-β-D-galactopyranosyl-D-galactose (R_{Gal} 0.48) and 6-O-β-D-galactopyranosyl-D-galactose (R_{Gal} 0.31). Degraded

Table 3.

Examination of methanolysis and hydrolysis products from methylated degraded gum B.

O-Methyl sugar	Approximate relative molar proportions	Relative retention times of methyl glycosides on:- column (i)	column (ii)	R_G in solvent (d)
2,3,4,6-Me_4-D-galactose	++	1.68	1.66	0.90
2,4,6-Me_3-D-galactose	++++	3.75; 4.25	3.00; 3.44	0.72
2,3,4-Me_3-D-galactose	+	6.45	5.30	0.72
2,4-Me_2-D-galactose	+	14.6; 16.4	9.9; 11.3	0.53
2,3,4-Me_3-D-glucuronic acid[a]	trace	2.37; 3.00	2.21; 2.74	-
2,6-Me_2-D-galactose	+/2	-	-	0.59
2-Me-D-galactose	+/2	-	-	0.33

a. As methyl ester methyl glycoside.

gum B was exhaustively methylated. Methanolysis followed by g.l.c. examination of the mixture of methyl glycosides gave the results shown in Table 3. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g) indicated the presence of 2,6-di- and 2-O-methyl-D-galactose in addition to those O-methyl sugars already identified by g.l.c. examination of their methyl glycosides (Table 3.).

Controlled Smith degradation[75] of degraded gum A.- Degraded gum A (1.0 g. polysaccharide) was treated in the same way as in the normal Smith degradation except that the periodate oxidation stage was carried out at 2°. Degraded gum C was isolated as the freeze dried product (60 mg. polysaccharide; yield, 6%). Hydrolysis of a portion of degraded gum C (20 mg.) followed by paper chromatography in solvent (h) indicated

the presence of galactose and arabinitol, but no galactitol.

Methylation of A.seyal gum.- The purified gum (300 mg. polysaccharide) was methylated by the Haworth and Purdie proceedures to give a product (255 mg.), $[\alpha]_D$ +17° (c, 1.0 in $CHCl_3$)(Found: OMe, 41.6%, not raised by further attempted methylation). Methanolysis of a portion followed by g.l.c. examination of the mixture of methyl glycosides gave the results shown in Table 4. The same O-methyl sugars were detected as were characterized from methylated A.drepanolobium gum Fraction A. However the major di-O-methyl-L-arabinose was 2,5-di-O-methyl-L-arabinose. Only a small amount of 3,4-di-O-methyl-L-arabinose was detected. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g) indicated the presence of 2-O-methyl-D-galactose and confirmed the presence of 2,3,4-tri-O-methyl-L-arabinose.

Preparation, partial acid hydrolysis and methylation of polysaccharide α.- The gum (54 g. polysaccharide) was dissolved in water (1.5 l.), mixed with 0.25M-sodium metaperiodate solution (1.5 l.) and left for 72 hours. The amount of formic acid released in this time was 0.76 mmoles/g. The corresponding amount of periodate reduced was 3.89 mmoles/g. The reaction was stopped by addition of ethylene glycol (20 ml.), and the solution dialysed against running tap water for 48 hours. Sodium borohydride (15 g.) was added and the mixture kept at room temperature for 30 hours, then dialysed for a further 48 hours. The polyalcohol was hydrolysed in N-sulphuric acid at room temperature for 48 hours. After dialysis against running tap water for 48 hours, polysaccharide α was isolated as the freeze dried product (27.5 g. polysaccharide; yield, 51%), $[\alpha]_D$ 0° (c, 0.50)(Found: galactose, 67%; arabinose, 33%).

Table 4.

Examination of methanolysis and hydrolysis products from methylated A.seyal gum.[a]

O-Methyl sugar	Relative retention times of methyl glycosides on:- column (i)	column (ii)	R_G in solvent (d)
2,3,4-Me_3-L-rhamnose	0.48	(0.50)	1.01
2,3,5-Me_3-L-arabinose	0.53; 0.73	(0.50); 0.64	0.98
2,3,4-Me_3-L-arabinose	(1.03)	(0.82)	0.79
2,5-Me_2-L-arabinose	1.81; 3.20	1.25; (2.21)	0.82
3,5-Me_2-L-arabinose	(1.03); (2.36)	(0.82); 1.75	0.82
3,4-Me_2-L-arabinose	1.99	1.44	0.56
2,3,4,6-Me_4-D-galactose	1.68	1.66	0.88
2,3,6-Me_3-D-galactose	(3.01); (3.89); (4.22)	2.45; (3.00); (3.45)	0.72
2,4,6-Me_3-D-galactose	(3.89); (4.22)	(3.00); (3.45)	0.72
2,3,4-Me_3-D-galactose	6.40	5.20	0.72
2,4-Me_2-D-galactose	14.6; 16.4	9.9; 11.3	0.52
2,3,4-Me_3-D-glucuronic acid[b]	(2.36); (3.01)	(2.21); 2.75	-
2,3-Me_2-D-glucuronic acid[b]	7.9; 9.3	5.8; 6.9	-
2-Me-D-galactose	-	-	0.32
4-Me-L-arabinose	-	-	0.36

a. Figures in parenthesis indicate T values of components which are not completely resolved.
b. As methyl ester methyl glycoside.

Polysaccharide α (20 mg.) was hydrolysed with 0.5N-sulphuric acid (10 ml.) for 1 hour on a boiling water bath. Paper chromatographic examination of the hydrolysate indicated the presence of galactose,

arabinose and two neutral disaccharides with the mobilities of 3-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.48 in solvent (a) and 0.54 in solvent (f), minor component] and 6-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.31 in solvent (a) and 0.38 in solvent (f), major component], and higher neutral oligosaccharides.

Polysaccharide α (315 mg.) was methylated with dimethyl sulphate and sodium hydroxide, and with methyl iodide and silver oxide, to give a product (274 mg.), $[\alpha]_D$ -31.7° (c, 0.89 in $CHCl_3$)(Found: OMe, 41.1%, not raised on further attempted methylation). Methanolysis of a sample of this product followed by g.l.c. examination of the mixture of methyl glycosides, gave the results shown in Table 5. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g) indicated the presence of 2,6-di- and 2-O-methyl-D-galactose in addition to those O-methyl sugars already characterized by g.l.c. examination of their methyl glycosides.

Preparation, partial acid hydrolysis and methylation of polysaccharide β.- Borohydride reduction of periodate oxidised polysaccharide α (26.6 g. polysaccharide), followed by controlled acid hydrolysis at room temperature for 48 hours, gave polysaccharide β (7.7 g. polysaccharide; yield, 29%), $[\alpha]_D$ -15° (c, 0.96)(Found: galactose, 89%; arabinose, 11%). The amount of formic acid released during periodate oxidation was 1.00 mmoles/g. The corresponding amount of periodate reduced was 3.89 mmoles/g.

Partial acid hydrolysis of polysaccharide β (20 mg.) followed by paper chromatographic examination of the hydrolysate indicated the presence of galactose, arabinose, two neutral disaccharides with the chromatographic mobilities of 3-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.47 in solvent (a)

and 0.53 in solvent (f), minor component] and 6-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.31 in solvent (a) and 0.38 in solvent (f), major component], and higher neutral oligosaccharides.

Polysaccharide β (232 mg.) was methylated to give a product (190 mg.), $[\alpha]_D$ -38.6° (c, 0.77 in $CHCl_3$)(Found: OMe, 40.8%, not raised on further attempted methylation). Methanolysis of a sample of this product followed by g.l.c. examination of the mixture of methyl glycosides, gave the results shown in Table 5. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g) indicated the presence of 2,6-di- and 2-O-methyl-D-galactose in addition to those O-methyl sugars already characterized by g.l.c. examination of their methyl glycosides.

Preparation, partial acid hydrolysis and methylation of polysaccharide γ.- Borohydride reduction of periodate oxidised polysaccharide β (7.1 g. polysaccharide), followed by controlled acid hydrolysis at room temperature for 48 hours, gave polysaccharide γ (0.93 g. polysaccharide; yield, 12.5%), $[\alpha]_D$ -24° (c, 0.85)(Found: galactose, 95%; arabinose, 5%). The amount of formic acid released on periodate oxidation was 1.99 mmoles/g. The corresponding amount of periodate reduced was 5.58 mmoles/g.

Partial acid hydrolysis of polysaccharide γ (5 mg.) followed by paper chromatographic examination of the hydrolysate indicated the presence of galactose, arabinose, two neutral disaccharides with the chromatographic mobilities of 3-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.48 in solvent (a) and 0.54 in solvent (f), minor component] and 6-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.30 in solvent (a) and 0.38 in solvent (f), major component], and higher neutral oligosaccharides.

Table 5.

Relative proportions of O-methyl sugars present in methylated polysaccharides α - δ.

O-Methyl sugar	α	β	γ	δ
2,3,5-Me_3-L-arabinose	2	2	trace	-
2,5-Me_2-L-arabinose	2	½	-	-
3,5-Me_2-L-arabinose	½	trace	-	-
2,3,4,6-Me_4-D-galactose	trace	1	1	2
2,4,6-Me_3-D-galactose	6	6	4	4
2,3,4-Me_3-D-galactose	2	3	2	2
2,4-Me_2-D-galactose	3	2	1	1

Polysaccharide γ (85 mg.) was methylated to give a product (21 mg.), $[\alpha]_D$ -39.5° (c, 0.42 in $CHCl_3$)(Found: OMe, 41.4%, not raised on further attempted methylation). Methanolysis of a sample of this product followed by g.l.c. examination of the mixture of methyl glycosides, gave the results shown in Table 5. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g) indicated the presence of 2,6-di- and 2-O-methyl-D-galactose in addition to those O-methyl sugars already identified by g.l.c. examination of their methyl glycosides.

Preparation, partial acid hydrolysis and methylation of polysaccharide δ.- Borohydride reduction of periodate oxidised polysaccharide γ (700 mg. polysaccharide), followed by controlled acid hydrolysis at room temperature for 48 hours gave the galactan polysaccharide δ (50 mg. polysaccharide; yield, 7%), $[\alpha]_D$ -25° (c, 0.30). The amount of formic acid released on periodate oxidation was 1.56 mmoles/g.

The corresponding amount of periodate reduced was 4.89 mmoles/g.

Partial acid hydrolysis of polysaccharide δ (5 mg.) followed by paper chromatographic examination of the hydrolysate indicated the presence of galactose, arabinose, two neutral disaccharides with the chromatographic mobilities of 3-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.48 in solvent (a) and 0.53 in solvent (f), minor component] and 6-O-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.30 in solvent (a) and 0.38 in solvent (f), major component], and higher neutral oligosaccharides.

Polysaccharide δ (30 mg.) was methylated to give a product (7 mg.). Methanolysis followed by g.l.c. examination of the mixture of methyl glycosides, gave the results shown in Table 5. Hydrolysis of the methyl glycosides followed by paper chromatography in solvents (d) and (g) indicated the presence of 2,6-di- and 2-O-methyl-D-galactose in addition to those O-methyl sugars already characterized by g.l.c. examination of their methyl glycosides.

Molecular-sieve chromatography of A.seyal gum and the Smith degraded polysaccharides.- On molecular-sieve chromatography using a previously calibrated Bio-Gel P300 column (45 x 5.0 cm.)[27,28,29] both A.seyal gum and polysaccharide α were eluted before the elution volume of Blue Dextran 2000. This indicates that A.seyal gum and polysaccharide α have very high number-average molecular weights (perhaps as high as 500,000). Light-scattering measurements indicated that the weight-average molecular weight, $\overline{M_w}$, of A.seyal gum was 8.5×10^5. Molecular-sieve chromatography indicated that polysaccharides β and γ had number-average molecular weights, $\overline{M_n}$, of ca. 8,000, and less than 3,000 respectively. This indicates large scale cleavage during the later Smith degradations.

6.3) Discussion.

A.seyal gum is composed of residues of D-galactose (38%), L-arabinose (45%), L-rhamnose (4%), D-glucuronic acid (7%) and 4-O-methyl-D-glucuronic acid (6%). The gum was examined by zone electrophoresis and ion-exchange chromatography on D.E.A.E.-cellulose.[30] Since no sharp discontinuities in the properties of the molecular species were indicated by these experiments, it seems probable that A.seyal gum exhibits the same kind of heterogeneity which has been suggested for the gums of A.senegal,[1,61] A.arabica,[4] A.laeta (Section IV) and A.drepanolobium Fraction A (Section V). If this is the case, then A.seyal gum may be considered to contain a continuous spectrum of related molecular species.

Mild acid hydrolysis of the gum (0.01N-sulphuric acid for 100 hours at 100°) effected the release of all the rhamnose, most of the arabinose and some galactose. Degraded gum A, isolated after dialysis, contained D-galactose (82%), L-arabinose (3%) and uronic acid (15%). Molecular-sieve chromatography gave an estimated value for the number-average molecular weight, $\overline{M_n}$, of degraded gum A of ca. 4,900. Light-scattering measurements gave a value of ca. 8.5×10^5 for the weight-average molecular weight, $\overline{M_w}$, of A.seyal gum. Degradation on mild acid hydrolysis is much greater than can result from the simple removal of arabinose and rhamnose from the periphery of the molecule. It does not seem to have been caused by the presence of any internal acid-labile arabinofuranose residues, since acid hydrolysis of borohydride reduced degraded gum A yielded galactitol but no arabinitol. There is no evidence of any galactofuranose residues in the polysaccharide, although it must be admitted that traces of di- and tri-O-methyl-D-galactofuranoses would be

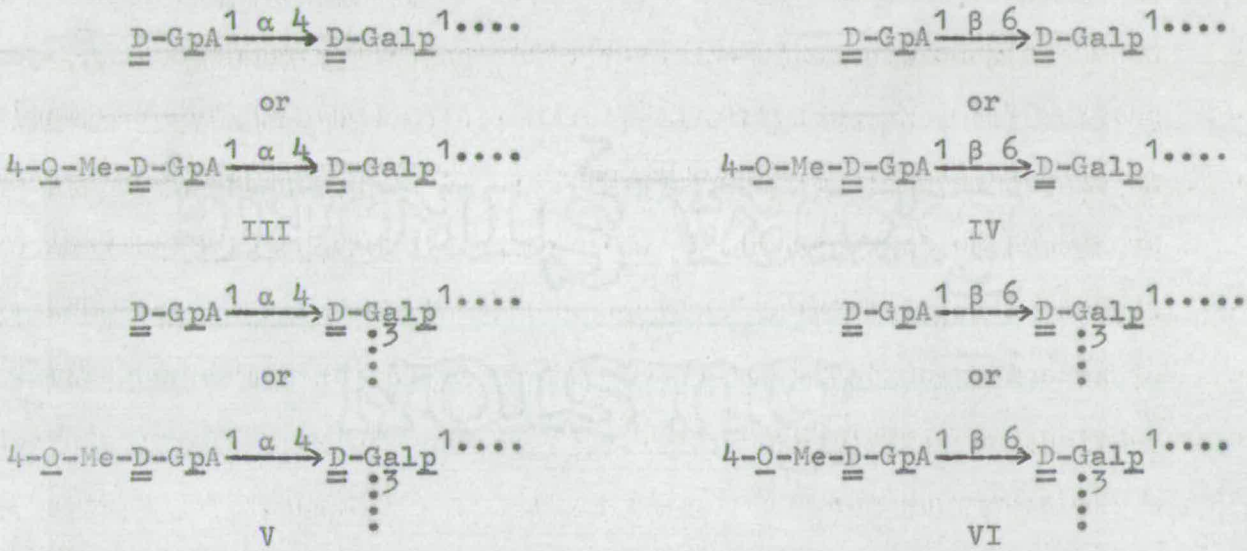
difficult to detect using g.l.c. examination. Bouveng and Lindberg have observed that certain galactopyranosidic bonds in highly branched arabinogalactans are unusually sensitive to mild acid hydrolysis.[92 - 96] It therefore seems probable that certain galactopyranosidic bonds in A.seyal gum are unusually sensitive to the conditions of mild acid hydrolysis.

An examination of methylated degraded gum A showed the presence of 2,3,4,6-tetra- (++), 2,4,6- (++), 2,3,6- (+), and 2,3,4-tri- (++++), 2,4- (+++) and 2,6-di- (+) and 2-O-methyl-D-galactose (+), and 2,3,4-tri-O-methyl-D-glucuronic acid (++), and traces of 2,3,5- and 2,3,4-tri-O-methyl-L-arabinose. The presence of 2-O-methyl-D-galactose is probably due to undermethylation. This shows that I and II are structural fragments of degraded gum A. Methylation analysis has shown traces of terminal

L-Araf$^{1\cdots\cdot}$ (I) L-Arap$^{1\cdots\cdot}$ (II)

non-reducing L-arabinofuranose residues to be present in the autohydrolysed gum of A.senegal,[1] and terminal non-reducing L-arabinopyranose residues to be present in A.arabica degraded gum A.[4] Traces of terminal non-reducing L-arabinofuranose and L-arabinopyranose residues have been detected in the degraded gum A of A.laeta[97] and A.drepanolobium Fraction A.[98] Lithium aluminium hydride reduction of methylated degraded gum A, followed by a Kuhn methylation,[20] yielded 2,3,4,6-tetra-O-methyl-D-glucose after methanolysis. This confirms that D-glucuronic acid and its 4-O-methyl analogue are terminal non-reducing end groups in degraded gum A; similar results have been obtained in studies of A.arabica gum[4] and A.drepanolobium gum Fraction A.[98] Partial methanolysis was carried out on methylated degraded gum A; after hydrolysis of the methyl glycosides, the methylated

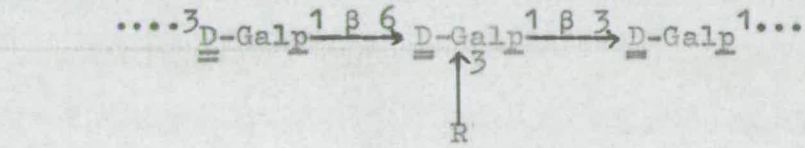
aldobiouronic acids were separated from the neutral O-methyl sugars by ion-exchange chromatography. Methanolysis of the methylated aldobiouronic acids followed by g.l.c. examination of the mixture of methyl glycosides showed the presence of 2,3,4-tri-O-methyl-D-glucuronic acid, and 2,3,6- and 2,3,4-tri-, and 2,4- and 2,6-di-O-methyl-D-galactose. This indicates that III - VI are structural fragments of degraded gum A. Similar



experiments on the methylated degraded gum A of A.arabica[4] and A.drepanolobium Fraction A[98] has shown the presence of structural fragments III, IV and VI, and III - VI respectively.

Smith degradation of degraded gum A yielded degraded gum B. Partial acid hydrolysis of degraded gum B gave 3-O-β-D-galactopyranosyl-D-galactose and 6-O-β-D-galactopyranosyl-D-galactose. An examination of methylated degraded gum B indicated the presence of 2,3,4,6-tetra- (++), 2,4,6- (++++) and 2,3,4-tri- (+), 2,4- (+) and 2,6-di- (+/2), and 2-O-methyl-D-galactose (+/2) and a trace of 2,3,4-tri-O-methyl-D-glucuronic acid. The presence of a trace of glucuronic acid in degraded gum B is explained by incomplete oxidation of degraded gum A. Presence of 2,6-di- and

2-O-methyl-D-galactose is probably due to undermethylation. The proportion of the O-methyl-D-galactoses obtained from methylated degraded gum B is evidence that degraded gum B is a galactan composed predominantly of β1,3-glycosidic linkages, with a few β1,6 branch points. The identification of some 2,3,4-tri-O-methyl-D-galactose from methylated degraded gum B indicates that VII is a possible structural unit of degraded gum A. A



where R = L-Araf$^{1\cdots}$, L-Arap$^{1\cdots}$, D-Galp$^{1\cdots}$, $\cdots^{4}$D-Galp$^{1\cdots}$, $\cdots^{6}$D-Galp$^{1\cdots}$.

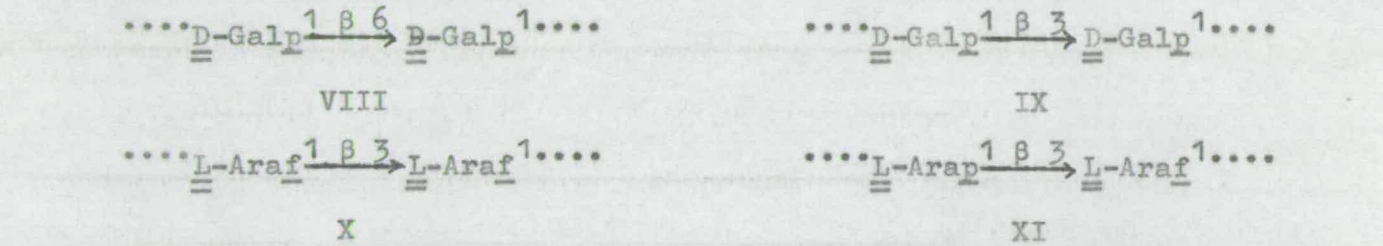
VII

similar structural unit has been proposed for the degraded gum A of *A.arabica*[4] and *A.drepanolobium* Fraction A.[98]

A controlled Smith degradation of degraded gum A yielded degraded gum C. Periodate oxidation of degraded gum A produced no formaldehyde so it may be concluded that the reducing galactose unit is substituted at C-6. The fact that degraded gum C contains arabinitol is evidence that at least some of the reducing end group galactose residues of degraded gum A are substituted at C-3.[57]

Mild acid hydrolysis of degraded gum A afforded two galactobioses which were characterized as 3-O-β-D-galactopyranosyl-D-galactose and 6-O-β-D-galactopyranosyl-D-galactose. Two arabinobioses were obtained from the diffusate of the mild acid hydrolysate of *A.seyal* gum. These were characterized as 3-O-β-L-arabinofuranosyl-L-arabinose and 3-O-β-L-arabinopyranosyl-L-arabinose. The fact that both arabinobioses

are released under such mild conditions of acid hydrolysis suggests that in each case the reducing arabinose residue was initially present in the furanose form in A.seyal gum. Thus VIII, IX, X and XI are structural



VIII IX X XI

fragments of A.seyal gum.

An examination of the O-methyl derivative of A.seyal gum indicated the presence of 2,3,4-tri-O-methyl-L-rhamnose, 2,3,5- and 2,3,4-tri-, and 2,5-, 3,5- and 3,4-di-O-methyl-L-arabinose, 2,4-di-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-glucuronic acid, together with small amounts of 4-O-methyl-L-arabinose, and 2,3,4,6-tetra-, 2,3,6-, 2,4,6- and 2,3,4-tri-, and 2-O-methyl-D-galactose, and 2,3-di-O-methyl-D-glucuronic acid. The presence of 2-O-methyl-D-galactose is ascribed to undermethylation. Smith degradation study of A.seyal suggested that 4-O-methyl-L-arabinose probably arises from undermethylation. The presence of a high proportion of 2,4-di-O-methyl-D-galactose indicates that most of the D-galactose residues in A.seyal gum are 3,6-di-O-substituted. However, the identification of trace amounts of 2,3,4,6-tetra-, 2,3,6-, 2,4,6- and 2,3,4-tri-O-methyl-D-galactose is evidence for a few terminal non-reducing D-galactose residues, and some 4-O-, 3-O- and 6-O-substituted D-galactose units respectively. The identification of 2,3,4-tri-O-methyl-D-glucuronic acid and 2,3,4-tri-O-methyl-L-rhamnose suggests that D-glucuronic acid, 4-O-methyl-D-glucuronic acid and L-rhamnose occur as terminal non-reducing units in the whole gum. The presence of some 2,3-di-O-methyl-D-glucuronic

acid suggests that terminal non-reducing L-rhamnose units are attached to D-glucuronic acid residues.[46,47] The presence of 2,3,5- and 2,3,4-tri-O-methyl-L-arabinose indicates that the arabinose-containing side chains are terminated by either L-arabinofuranose (XII) or L-arabinopyranose (XIII).

L-Araf^{1}····

XII

L-Arap^{1}····

XIII

In contrast to A.arabica gum[4] and A.drepanolobium gum Fraction A (Section V), 2,5-di-O-methyl-L-arabinose is the major dimethyl-L-arabinose obtained from methylated A.seyal gum. This shows that many L-arabinofuranose residues are 3-O-substituted (XIV) in the arabinose-containing side chains.

····^{3}L-Araf^{1}····

XIV

A much smaller proportion of 3,5-di-O-methyl-L-arabinose was detected in the methanolysate of methylated A.seyal gum. This suggests that some 2-O-substituted-L-arabinofuranose residues (XV) are present in the arabinose-containing side chains. The presence of 3,4-di-O-methyl-L-arabinose indicates that there is a small proportion of 2-O-substituted-L-arabinopyranose residues (XVI) in the arabinose-containing side chains.

····^{2}L-Araf^{1}····

XV

····^{2}L-Arap^{1}····

XVI

Methylation analysis of A.seyal gum and degraded gum A suggests that the arabinose-containing side chains are attached to the galactan framework at C-3 and C-6 positions of certain D-galactose residues.

A sample of A.seyal gum was submitted to four successive Smith degradations. The percentage yields of the Smith-degraded products are low compared with those obtained during successive Smith degradations on

A.senegal gum[1], A.laeta gum[97] and A.drepanolobium gum Fraction A (Section V), and are very similar to those obtained during successive Smith degradations on A.arabica gum.[4] Molecular-sieve chromatography indicates that there is a pronounced cleavage of the macromolecule during Smith degradations; this cleavage of internal D-galactose residues is particularly drastic during the second and subsequent Smith degradations. This complete fragmentation of the macromolecule during successive Smith degradations shows that long chains of periodate-resistant β1,3-linked D-galactose residues are not such an important structural feature of A.seyal gum as they are of A.senegal gum[1] and A.laeta gum.[97] A.seyal gum must contain blocks of periodate-resistent β1,3-linked D-galactose residues interspersed by blocks of periodate-vulnerable β1,6-linked D-galactose residues in a highly branched galactan framework. Fragmentation of this galactan core is to be expected as the arabinose-containing side chains are progressively removed from the C-3 positions of 6-O-substituted-D-galactose units.

Arabinose residues were not eliminated from the product obtained from A.arabica gum[4] after four successive Smith degradations or from the product obtained from A.drepanolobium gum Fraction A after five successive Smith degradations (Section V); in contrast four successive Smith degradations removed all the arabinose residues from A.seyal gum. This indicates that a proportion of the arabinose-containing side chains in A.seyal gum contain up to four arabinose residues. Methylation analysis of A.seyal gum indicated the presence of some 2-O-substituted-L-arabinopyranose residues in the arabinose-containing side chains. This indicates that a proportion of the arabinose-containing side chains could be much longer

than four units. Periodate oxidation of these 2-O-substituted-L-arabinopyranose residues in A.seyal gum would yield arabinose-containing side chains of up to three units long in polysaccharide α (Figure 1). The observation that A.seyal gum contains shorter arabinose-containing side chains than A.arabica gum[4] and A.drepanolobium gum Fraction A (Section V) is predictable from the fact that the arabinose content of A.seyal gum is lower than that of A.arabica gum and A.drepanolobium gum Fraction A. As in the case of A.drepanolobium gum Fraction A (Section V), the specific rotation of the Smith-degraded products decreases to a negative value after four successive Smith degradations. During successive Smith degradations on A.arabica gum[4] the specific rotation decreases but remains positive.

On methanolysis, the O-methyl derivatives of polysaccharides α, β, γ and δ gave the results shown in Table 5. 2,3,5-Tri-O-methyl-L-arabinose was the only O-methyl-L-arabinose to be detected in the methanolysate of methylated polysaccharide γ. The absence of any evidence for L-arabinopyranose residues in polysaccharide α suggests that the 4-O-methyl-L-arabinose identified in the hydrolysate of methylated A.seyal gum is not structurally significant.

The structural evidence indicates that A.seyal gum is very similar to A.arabica gum.[4] It is suggested that gum molecules of A.seyal possess a highly branched galactan framework to which are attached uronic acid residues and arabinose-containing side chains. Successive Smith degradations suggest that the arabinose-containing side chains of A.seyal gum are considerably shorter than those of A.arabica gum[4] and A.drepanolobium gum Fraction A (Section V). As in the case of A.arabica

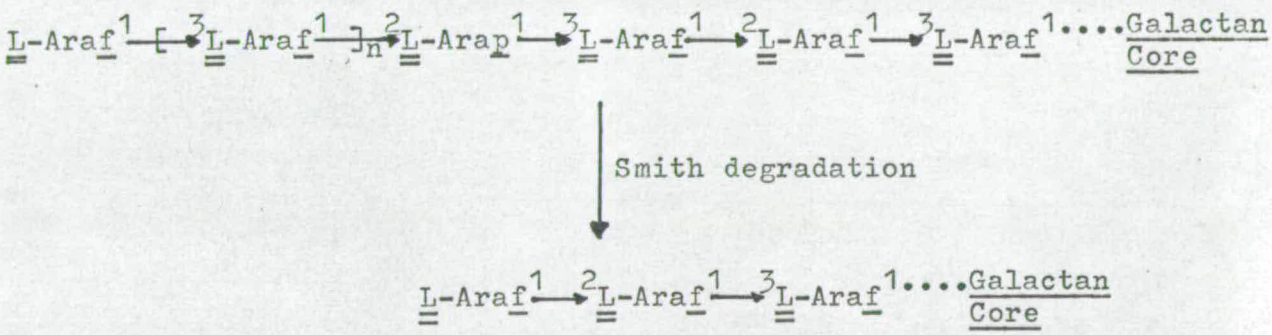

L-Araf 1 → [3 L-Araf 1] n 2 L-Arap 1 → 3 L-Araf 1 → 2 L-Araf 1 → 3 L-Araf 1 .... Galactan Core
Smith degradation
L-Araf 1 → 2 L-Araf 1 → 3 L-Araf 1 .... Galactan Core

Figure 1.

gum[4] the galactan framework is more highly branched than those of A.senegal gum[1] and A.laeta gum.[97] Viscosity measurements of A.seyal gum, and the behaviour of A.seyal gum on molecular-sieve chromatography using Bio-Gel P300 indicates that molecules of A.seyal gum are similar to molecules of A.arabica gum[4] in having a much more compact structure than molecules of A.senegal gum[1] and A.laeta gum.[97]

SECTION VII

GENERAL DISCUSSION OF GUM EXUDATES OF THE GENUS ACACIA.

> The camel is eating a twig of acacia, and he has just finished saying "humph" once too often (the Djinn told him he would), and so the Humph is coming.
>
> Rudyard Kipling.

The genus Acacia is one of the largest in the Plant Kingdom and contains over 900 species.[99] Acacia twigs are more varied and complex than either the camel without a "humph" or Rudyard Kipling realised. It is probable that the chemical components of the twigs of different Acacia species and the gum which exudes from these twigs will mirror this variation and complexity.

Despite the size of the genus Acacia, the gum exudates of only 27 Acacia species have been studied chemically, viz., A.adansonii, Guill. et Perr.;[84] A.arabica, Willd.;[4,70,84,100] A.campylacantha, Hochst.;[70,84,100,101] A.catechu, Benth.;[102,103] A.cyanophylla, Lindl.;[85,104] A.dealbata, Link.;[70,84,100] A.drepanolobium, Harms ex Sjösted;[70,84,98,100] A.elata, Cunn.;[85] A.fistula, Schweinf.;[70,84,100] A.giraffae, Willd.;[84,85,100] A.harpophylla, F. Muell.;[105] A.karroo, Hayne;[84,86,100,106] A.laeta, R. Br. ex Benth.;[2,84,97,100] A.mearnsii, De Wild.;[84,85,100,107] A.mellifera, (Vahl.) Benth.;[84,100] A.microbotrya, Benth.;[105] A.mollissima, Willd.;[108,109] A.nilotica, (L) Willd. ex Del.;[70,84,100] A.nubica, Benth.;[40,70,84,100] A.penninervis, Sieb.;[105] A.podalyriaefolia, Cunn.;[85] A.pycnantha, Benth.;[78,84,89] A.senegal, Willd.;[1,34-54,61] A.seyal, Del.;[84,91,100]

A.sundra, Spreng.;[110-113] and A.tortilis, Hayne.[84,100] Some of these gums have been studied only superficially. Some of these names are now thought to be synonymous. For example, A.decurrens, A.mearnsii and A.mollissima are now not thought to be distinct species.[114]

The genus Acacia has been classified into Series and Sub-series by Bentham,[5] and into Sections and Sub-sections by Taubert.[6] Bentham divided the Acacia species into six Series, viz., Series 1, Phyllodinae; Series 2, Botryocephalae; Series 3, Pulchellae; Series 4, Gummiferae; Series 5, Vulgares; Series 6, Filicinae. Bentham's classification (1875) was based upon habit inflorescence and geographical distribution. Species from Series 1 - 3 are native to Australia, while species from Series 6 are native to South America. Species from Series 4 and 5 are found throughout tropical and semitropical parts of the world. The 27 Acacia species whose gums have been studied chemically are divided between these Series as shown in Table 1. A.drepanolobium was named in 1914, and does not appear in Bentham's classification (1875). However it has been placed in Series Gummiferae, Sub-series Medibracteatae, by Harms.[115] Studies of gum exudates from Acacia species from Series 3 and 6 have not been reported. Gum exudates from species from Series 4 and 5 have been most widely studied.

Results of structural studies on gum exudates of species from Series 4 and 5 support the botanical classification of these species into Series 4 and 5. The gum exudates of six species in Series 5 have been studied. All have negative specific rotations, and a unit molar ratio of rhamnose content to uronic acid content. On acid hydrolysis all yield two aldobiouronic acids, viz., 6-O-(β-D-glucopyranosyluronic acid)-D-galactose and

Table 1.

Classification of the Acacia species whose gum exudates have studied.

Series 1.	Phyllodinae.	
Sub-series.	Alatae	
	Continuae	
	Pungentes	
	Calamiformes	
	Brunioideae	
	Uninerves	A.cyanophylla; A.penninervis; A.podalyriaefolia; A.pycnantha; A.microbotrya.
	Plurinerves	A.harpophylla.
	Juliflorae	
Series 2.	Botryocephalae.	A.dealbata; A.decurrens; A.elata; A.mearnsii; A.mollissima.
Series 3.	Pulchellae.	
Series 4.	Gummiferae.	
Sub-series.	Summibracteatae	A.giraffae.
	Medibracteatae	A.adansonii; A.arabica; A.drepanolobium; A.fistula; A.karroo; A.nilotica; A.nubica; A.seyal; A.tortilis.
	Basibracteatae	
Series 5.	Vulgares.	
Sub-series.	Gerontogeae Spiciflorae	A.campylacantha; A.catechu; A.laeta; A.mellifera; A.senegal; A.sundra.
	Americanae Spiciflorae	
	Americanae Capitulatae	
	Gerontogeae Capitulatae	
Series 6.	Filicinae.	

6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose. In Series 5 the gum exudates of A.campylacantha,[101] A.laeta,[97] and A.senegal[1,61] have been most systematically studied. On partial acid hydrolysis all give five disaccharides, viz., 3-O-β-D-galactopyranosyl-D-galactose; 6-O-β-D-galactopyranosyl-D-galactose; 3-O-α-D-galactopyranosyl-L-arabinose; 3-O-β-L-arabinofuranosyl-L-arabinose and 3-O-β-L-arabinopyranosyl-L-arabinose. Methylation of these gums followed by methanolysis yield 2,3,5- and 2,3,4-tri- and 2,5-di-O-methyl-L-arabinose as the only O-methyl-L-arabinoses. On successive Smith degradation of these gums the specific rotation of the products rises and eventually becomes positive. Successive Smith degradations show that the molecular structures of these gums are based on a branched β1,3-linked galactan, to which are attached short arabinose-containing side chains (up to five units long in A.laeta gum). Molecular-sieve chromatography suggests that these gums have similar molecular weights; the weight-average molecular weight of A.senegal gum is ca. 500,000[1] while the weight-average molecular weight of A.campylacantha is less than 400,000.[101]

The gum exudates of ten species in Series 4 have been studied. All have positive specific rotations, and do not have a unit molar ratio of rhamnose content to uronic acid content. On acid hydrolysis all yield four aldobiouronic acids, viz., 6-O-(β-D-glucopyranosyluronic acid)-D-galactose; 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose; 4-O-(α-D-glucopyranosyluronic acid)-D-galactose and 4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose. On methylation and methanolysis the gums of A.arabica,[4] A.drepanolobium (Section V), A.nilotica,[116] and A.seyal (Section VI) yielded 2,3,5- and 2,3,4-tri-, and 2,5-, 3,5- and

3,4-di-O-methyl-L-arabinose. Methylation and methanolysis of the gums of A.nubica[40] and A.giraffae[85] yielded similar results except that A.nubica gum did not yield 3,4-di-O-methyl-L-arabinose, and A.giraffae gum did not yield 2,3,4-tri- and 3,4-di-O-methyl-L-arabinose. Partial acid hydrolysis of the gums of A.arabica, A.drepanolobium, A.nubica and A.seyal yielded 3-O-β-D-galactopyranosyl-D-galactose, 6-O-β-D-galactopyranosyl-D-galactose, 3-O-β-L-arabinofuranosyl-L-arabinose and 3-O-β-L-arabinopyranosyl-L-arabinose. On successive Smith degradation of the gums of A.arabica, A.drepanolobium and A.seyal the specific rotation of the products fell; on successive Smith degradation of A.nubica gum the specific rotation remained highly positive. Successive Smith degradations show that molecules of the gums of A.arabica, A.drepanolobium, A.nubica and A.seyal contain blocks of periodate-resistant β1,3-linked D-galactose residues interspersed by blocks of periodate-vulnerable β1,6-linked D-galactose residues in a branched galactan framework; some of the arabinose-containing side chains are at least six units long. Light-scattering measurements have shown the weight-average molecular weights of A.arabica, A.seyal, A.nubica and A.drepanolobium gums to be 23×10^5, 8.5×10^5, 8.6×10^5 and 9.5×10^5 respectively. A comparison of the chemical properties of the gum exudates from species in Series 4 and 5 is listed in Table 2.

The situation of the gum exudates from species in Series 1 and 2 is not so clear. Only the gums of A.cyanophylla, A.podalyriaefolia and A.pycnantha from Series 1, and the gums of A.elata and A.mearnsii (synonymous with A.decurrens and A.mollissima) from Series 2, have been studied in any detail. None of these gums have been submitted to successive Smith degradations. Charlson, Nunn and Stephen[104] realised

Table 2.

Comparison of gum exudates from species in Series 4 and 5.

Properties	Series 4	Series 5
1 Sign of specific rotation	positive	negative
2 Ratio of rhamnose content to uronic acid content	greater than unity	unity
3 Aldobiouronic acids yielded on acid hydrolysis	6-O-(β-D-GA)-D-Gal 6-O-(4-Me-β-D-GA)-D-Gal 4-O-(α-D-GA)-D-Gal 4-O-(4-Me-α-D-GA)-D-Gal	6-O-(β-D-GA)-D-Gal 6-O-(4-Me-β-D-GA)-D-Gal
4 Neutral disaccharides obtained on mild acid hydrolysis	3-O-β-D-Galp-D-Gal 6-O-β-D-Galp-D-Gal 3-O-β-L-Araf-L-Ara 3-O-β-L-Arap-L-Ara	3-O-β-D-Galp-D-Gal 6-O-β-D-Galp-D-Gal 3-O-β-L-Araf-L-Ara 3-O-β-L-Arap-L-Ara 3-O-α-D-Galp-L-Ara
5 O-Methyl-L-arabinoses obtained from the methylated gums	2,3,5- and 2,3,4-tri-, 2,5-, 3,5- and 3,4-di-O-methyl-L-arabinose. [A.nubica gum gave no 3,4-di-O-methyl-L-arabinose].	2,3,5- and 2,3,4-tri- and 2,5-di-O-methyl-L-arabinose.
6 Length of arabinose-containing side chains	Some are at least six units long.	Longest observed is five units long.
7 Change in specific rotation of products on successive Smith degradations.	falls	rises
8 Basis of molecular structure.	Blocks of β1,3-linked-D-galactoses interspersed by blocks of β1,6-linked-D-galactoses in a branched galactan framework.	Branched β1,3-linked galactan.
9 Approximate weight-average molecular weights	All have $\overline{M}_w$ greater than 800,000.	Have $\overline{M}_w$ of 600,000 or less.

that A.cyanophylla and A.pycnantha were closely related botanically, and expressed surprise that the gums of these species were so different. The sugar composition of these gums differ significantly; the uronic acid contents of A.cyanophylla and A.pycnantha gums are 20% and 5% respectively. However the gums of A.seyal (Section VI) and A.nubica,[40] both of which occur in Series 4, Sub-series 2, have similar molecular structures although their uronic acid contents are 13% and 7% respectively. Joubert has found an unusually low molecular weight ($\overline{M_w}$ of 189,000) for A.cyanophylla gum.[117] Recent experiments have shown that A.pycnantha gum also has an unusually low molecular weight.[118] More work must be done on gums from species in Series 1 before their relationships can be assessed. Series 2 is much smaller than Series 1. The gums of only three species in Series 2 have been examined chemically (A.decurrens, A.mollissima and A.mearnsii are synonymous). Their specific rotations range from $+4^{\circ}$ to -46°. As in the case of Series 1, more work must be carried out before the relationships of the gums from species in Series 2 can be assessed.

At present the indication is that the gums from species in Series 1 are quite varied. This is not surprising. Many native Australian genera exhibit considerable taxonomic variation; this is thought to be due to the isolation of Australia from the other land masses.[119] Variation in the chemical components of Australian species might therefore be expected.

In the past it has sometimes been assumed that all Acacia gums are very similar, and structural studies have been interpreted to support this.[107] It has been claimed that the gum exudates of A.pycnantha (Series 1), A.mearnsii (Series 2) and A.senegal (Series 5) show distinct similarity to one another.[107] All three gums have negative specific

rotations. The ratios of uronic acid content to rhamnose content for the gums of A.pycnantha, A.mearnsii and A.senegal are 1:2.5, 1:1.3 and 1:1.1 respectively. On acid hydrolysis they all yield two aldobiouronic acids, viz., 6-O-(β-D-glucopyranosyluronic acid)-D-galactose and 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose. On partial acid hydrolysis A.pycnantha gum[89] yielded 3-O-β-L-arabinofuranosyl-L-arabinose as the only arabinose-containing disaccharide, while A.mearnsii gum[107] yielded only 3-O-β-L-arabinopyranosyl-L-arabinose. Partial acid hydrolysis of A.senegal gum has yielded 3-O-α-D-galactopyranosyl-L-arabinose, 3-O-β-L-arabinofuranosyl-L-arabinose and 3-O-β-L-arabinopyranosyl-L-arabinose. Methylation of A.pycnantha gum[89] followed by methanolysis yielded 2,3,5-tri-, and 2,5- and 3,5-di-O-methyl-L-arabinose as the only O-methyl-L-arabinoses, while methylation and methanolysis of A.senegal gum[1] yielded 2,3,5- and 2,3,4-tri- and 2,5-di-O-methyl-L-arabinose. Methylation of A.mearnsii gum[107] followed by methanolysis yielded 2,3,5- and 2,3,4-tri-, and 2,5- and 2,3-di-O-methyl-L-arabinose; 2,3-di-O-methyl-L-arabinose was present to approximately the same extent as 2,5-di-O-methyl-L-arabinose. The weight-average molecular weights of A.senegal gum[1] and A.pycnantha gum[118] are ca. 500,000 and ca. 200,000 respectively; the molecular weight of A.mearnsii gum has not been determined. Successive Smith degradations have shown that A.senegal gum is based on a branched β1,3-linked galactan. No analogous experiments have been performed on the gums of A.pycnantha and A.mearnsii.

A comparison of the gums of A.pycnantha, A.mearnsii and A.senegal is listed in Table 3. Despite the fact that the three gums have negative specific rotations, and yield the same aldobiouronic acids on acid

Table 3.

Comparison of the gums from A.pycnantha (Series 1), A.mearnsii (Series 2) and A.senegal (Series 5).

Properties	A.pycnantha	A.mearnsii	A.senegal
1 Sign of specific rotation	negative	negative	negative
2 Ratio of L-rhamnose content to uronic acid content	1:2.5	1:1.3	1:1.1
3 Aldobiouronic acids yielded on acid hydrolysis	All three gums yielded 6-O-(β-D-glucopyranosyluronic acid)-D-galactose and its 4-O-methyl analogue.		
4 Arabinose-containing disaccharides obtained on mild acid hydrolysis	L-Araf $^{1\beta3}$ L-Ara	L-Arap $^{1\beta3}$ L-Ara	D-Galp $^{1\alpha3}$ L-Ara L-Arap $^{1\beta3}$ L-Ara L-Araf $^{1\beta3}$ L-Ara
5 O-Methyl-L-arabinoses obtained from methylated gums	2,3,5-tri- and 2,5- and 3,5-di-O-methyl-L-arabinose	2,3,5- & 2,3,4-tri-, & 2,5- & 2,3-di-O-methyl-L-arabinose	2,3,5- and 2,3,4-tri- and 2,5-di-O-methyl-L-arabinose
6 Approximate weight-average molecular weight	ca. 200,000	n.d.	ca. 500,000

hydrolysis, it can be seen that they have differing properties. More gums of species from Series 1 and 2 need to be studied in greater detail before any firm conclusion can be made on their relationship to the gums of species in other Series. However, the limited data on gums from species in Series 1 and 2 available to date, suggests that it might be possible to construct a chemical classification of Acacia gums which mirrors the botanical classification of the Acacia genus.

Studies on the flavonoid compositions of the heartwood, leaves and flowers of various Acacia species have been carried out. The results for 33 species have been summarised by Clark-Lewis and Dainis.[120] Of these species, 26 were from Series 1, and four were from Series 2; one species from each of Series 4, 5 and 6 were studied. From the results quoted by Clark-Lewis and Dainis, it is seen that the flavonoid content of an Acacia species varies according to which Series the species belongs. In addition, the heartwood flavonoids of an Acacia species in Series 1 varies according to which Sub-series the species belongs. These results show that the phenolic hydroxylation patterns of the heartwood flavonoids isolated from Acacia species correlate closely with the botanical system of classification. The correlation of heartwood flavonoid content and molecular structure of gum exudates of Acacia species with taxonomy, shows that botanical differences in the Acacia genus are accompanied by biochemical differences.

Interest in the correlation of chemical composition with botanical classification (Chemical Taxonomy) is growing quickly. Bentham has classified the Eucalyptus genus using the shape of the anthers.[121] It was suggested as long ago as last century that the chemical nature of the exuded kino might aid the classification of the Eucalyptus genus.[122-124] More recently it has been shown that Eucalyptus species can be differentiated on the basis of the chemical composition of their leaf oils.[125] The genus Prunus is divided into five sections; these can be identified, and the taxonomy confirmed, on the basis of their flavonoid contents.[126]

The time required to determine the molecular structure of a gum exudate makes it unlikely that the molecular structure of gums will become

useful in chemical taxonomical studies of the genus Acacia. Studies of heartwood flavonoids and other small molecular weight compounds of Acacia species are more likely to give useful phytochemical results. However, the correlation of molecular structure of gum exudates and taxonomy should be borne in mind when any further work on Acacia gums is planned. To date most of the structural work on Acacia gums has been concentrated on species in Series Gummiferae Sub-series Medibracteatae, and Series Vulgares Sub-series Gerontogeae Spiciflorae. Some work has been done on gums from species in Series Phyllodinae Sub-series Uninerves, and Series Botryocephalae, while no work has been done on gums from species in Series Pulchellae and Filicinae. In the future gum exudates from Acacia species should be studied with the aim of obtaining a more comprehensive survey of the Acacia gums. Much previous work on the genus Acacia, in such disciplines as cytology and physiology, has proved subsequently to be of limited scientific value owing to the fact that the identity of the plants originally worked on has proved to be doubtful, and that because no specimens were preserved the doubts remain permanently. For this reason good voucher specimens of the material worked on, especially where the results are likely to be of permanent significance, should be deposited in a herbarium. A resolution to this effect was passed at the International Botanical Congress in 1964.

Although gums of species from Series 4 Sub-series 2 have been studied fairly well, it would be interesting to see how they compare with gums from species in Series 4 Sub-series 1 and 3. Similarly it would be interesting to compare the molecular structures of the gums from species in Series 5 Sub-series 1 with those of gums from species in Series 5 Sub-

series 2, 3 and 4. More work needs to be done on gums from species in Series 1 and 2 to determine whether there are major structural differences between the gums from species in Series 1, 2, 4 and 5. In addition, it would be useful to study gums from species in the different Sub-series of Series 1; differences in heartwood flavonoids between the Sub-series of Series 1 suggest that species in these Sub-series may differ biochemically.

A chemical taxonomic approach might be applicable to the study of gums from other genera. The genus Albizia is closely related to the genus Acacia, and before Bentham's classification of the Mimoseae[5] the Albizias were often confused with the Acacias. All the five Albizia gums studied to date contain mannose.[127-132] Since no Acacia gum has been found to contain mannose, the analysis of the gum exudates of the two genera support their taxonomic separation. The genus Albizia has been divided into three Sections.[5] Of the five species whose gums have been studied chemically, four are from Section 2 (Albizia glaberrima,[128,132] Albizia lebbek,[129] Albizia procera[130,131] and Albizia sericocephela[132]) and one is from Section 3 (Albizia zygia[127]). In any future work on Albizia gums a knowledge of the taxonomy of the genus might help in assessing the results.

Another interesting problem about Acacia gums is the reason for their formation and the method of their synthesis. It has been suggested that gum exudates may arise from a pathological condition of the tree or from invasion by micro-organisms.[57,133] However, the simplest and most plausable theory to date is that gum formation is a normal metabolic function of the plant, and that gum formation serves merely to prevent

excessive loss of moisture from the tissues of the tree.[57,133] Gum formation is greatest in unusually hot, dry weather following plentiful rain, and the optimum time for tapping is at leaf-fall when desiccation is setting in.[134] Strongly dehydrating conditions are necessary climatically for gum formation to be extensive; Acacia trees grown in Britain rarely exude gum.

Results from studies of different types of A.senegal gum (Section III) suggest that gum exudates are synthesised near the site of exudation; it is unlikely that gums would be translocated in the tree.[59] It is interesting that the highly branched arabinogalactans obtained from the heartwood of many species of Larch, Spruce and Pine bear a strong resemblance to Acacia gums. The arabinogalactans from Larix and from other conifer genera possess many structural characteristics in common, including a β1,3-linked galactan framework to which are attached chains of β1,6-linked D-galactose residues, L-arabinose residues and D-glucuronic acid residues.[135] Some workers have interpreted their results from arabinogalactans by proposing a main chain of β1,3-linked D-galactose residues.[136] However, Husemann concluded that the arabinogalactan from Larix decidua must contain a highly branched network since its viscosity in water was less than one-twentieth of that of a (4-O-methylglucurono)-xylan of the same molecular weight.[137] Most, if not all, Larch species contain, not one, but two closely related types of arabinogalactan, usually referred to as A and B.[135] Mosimann and Svedberg found that a Larch arabinogalactan contained two components having molecular weights of 100,000 and 16,000 respectively.[138]

A.senegal wood water extracts have been studied.[139] Wood shavings

from an A.senegal tree prior to tapping were extracted with water to give 0.75% polysaccharide (Extract I). Wood shavings from an A.senegal tree after it had been tapped were extracted with water to give 0.04% polysaccharide (Extract II). On molecular-sieve chromatography, Extract I was shown to contain two components of number-average molecular weights ca. 100,000 and 7,600. This heterogeneity is very similar to what is found for the water-soluble Larch arabinogalactans.[135,138] The two fractions of Extract I were separated by molecular-sieve chromatography, dialysed against tap water and isolated as freeze dried products. Hydrolysis followed by paper chromatography showed that both fractions contained approximately similar amounts of the same sugars, viz., D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid. The sugar composition of Extract I (i.e. the mixture of the two fractions) was D-galactose (60%), L-arabinose (29%), L-rhamnose (3%) and D-glucuronic acid (8%), compared with sugar compositions of D-galactose (37%), L-arabinose (32%), L-rhamnose (14%) and uronic acid (17%) for A.senegal gum. The heterogeneity of Extract I makes a comparison with A.senegal difficult; sugar compositions of the higher molecular weight component of Extract I might be similar to those of A.senegal gum. Methylation data for Extract I, the autohydrolysed Extract I and the Smith-degraded Extract I, together with the isolation after partial acid hydrolysis of two neutral disaccharides obtained from A.senegal gum (3-O-α-D-galacto-pyranosyl-L-arabinose and 3-O-β-L-arabinopyranosyl-L-arabinose) indicates a structural similarity between the two polysaccharides.

It is interesting to note that the water-soluble polysaccharide content of A.senegal wood decreases twenty-fold after tapping. It appears

that tapping deprives the tree of the water-soluble polysaccharide present in its interior. On water extraction of A.penninervis heartwood, Stewart obtained a polysaccharide identical to deionised A.penninervis gum.[140] It is thought that the free gum acid occurs in the heartwood owing to the presence of free acetic acid in this region.[140,141] Stewart considers that the gum might be stored in ray parenchyma cells and perhaps in vessels.[140] It has been shown that arabinogalactans of Larch species are located in the lumen of tracheids and of parenchyma and epithelial cells. It was also shown that the acetic acid which is gradually formed as the wood ages causes a progressive degradation of the arabinogalactan within Larch trees.[135]

The water extract of A.senegal wood could be an example of a hardwood arabinogalactan. On the other hand it could be gum stored in the wood, which has been degraded by acetic acid. This could explain the low molecular weight ($\overline{M_n}$ of 100,000 compared with ca. 250,000 for A.senegal gum) and the quite different sugar composition of the water extract. Whatever the identity of the water extract, it seems there is some connection between the exuded gum and the water-soluble wood polysaccharide. An understanding of the biosynthesis of the water-soluble wood polysaccharide and the conifer wood arabinogalactans would undoubtedly lead to a better understanding of Acacia gum formation.

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STUDIES ON URONIC ACID MATERIALS

PART XXII*. THE COMPOSITION OF THE GUM FROM *Acacia drepanolobium* HARMS EX SJÖSTED

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INTRODUCTION

A preliminary study[2] showed that *Acacia drepanolobium* is a further example (*cf. A. nilotica*[3], *A. nubica*[4], and *A. arabica*[1]) of an *Acacia* gum having a positive specific rotation and a low content of L-rhamnose that does not bear a unit molar correspondence to the uronic acid content. It was also noted that *A. drepanolobium* gum does not dissolve completely in cold water, giving about 20% of a water-insoluble gel that can be dispersed in cold, dilute, alkaline solution.

The solubility difficulty associated with some commercial specimens of gum arabic (*A. senegal* syn. *verek*) has received comment recently[5]. The reason for the occasional formation of mucilaginous gels by some *Acacia* species is not known. We have examined many specimens of *A. senegal* gum, but they have not given sufficient quantities of the insoluble gel to facilitate its study. In contrast, *A. drepanolobium* conveniently provides considerable proportions of water-insoluble material, and we now present the results of an analytical study of the differences in composition between the water-soluble gum, a fraction soluble in dilute sodium chloride solution, and the insoluble gel.

EXPERIMENTAL

Origin of specimens. — Nodules of the gum from *Acacia drepanolobium* Harms ex Sjősted were collected by Mr. W. M. C. Bagshawe, Provincial Forest Officer, at Tabora, Western Province, Tanganyika, in July, 1961.

Analytical methods. — The standard methods involved have been described previously[6].

Fractionation procedure. — Two portions of crushed, bark-free, *A. drepanolobium* gum (*ca.* 20 g) were each stirred with distilled water (500 ml) for 12 h; the soluble fraction (*A*) was collected by filtration (muslin, and then paper), dialysed against tap water, and then electrodialysed. The residue was stirred with M sodium chloride solution (500 ml) for 12 h; the salt-soluble fraction (*B*) was collected by filtration, dialysed against tap water, and then electrodialysed. The residual insoluble gel

*For Part XXI, see Ref. 1.

(Fraction *C*) was dialysed against tap water. The three fractions were freeze-dried from aqueous solutions or dispersions. The percentage yields of the three fractions are shown in Table I.

Viscosity measurements. — Determinations on Fractions *A* and *B* were made in M sodium chloride solution in an Ubbelohde dilution viscometer at 25° (solvent flow-time, 193.6 sec). Fraction *C* was studied in M sodium hydroxide solution at 25° (solvent flow-time, 215.2 sec).

Specific rotations. — Fractions *A* and *B* were studied in distilled water and in M sodium hydroxide; Fraction *C* was studied in M sodium hydroxide. Specific rotations of sugars were measured in aqueous solutions. All measurements were made at $19 \pm 1°$.

Periodate oxidations. — These were performed at room temperature in darkness by mixing equal volumes of 4% polysaccharide solutions and 0.25M sodium metaperiodate. The formic acid released was titrated (Methyl Red) with standard sodium hydroxide. The reduction of periodate was determined with standard arsenite solution. Periodate oxidation of oligosaccharides was performed at room temperature in darkness, using excess of sodium metaperiodate. Formaldehyde was estimated colorimetrically with chromotropic acid[7]. Periodate oxidations of *O*-methyl sugars were carried out by the method of Lemieux and Bauer[8].

Paper chromatography. — Whatman No. 1 and 3MM papers were used with the following solvent systems (v/v):(*a*) benzene–butyl alcohol–pyridine–water (1:5:3:3, upper layer); (*b*) ethyl acetate–acetic acid–formic acid–water (18:3:1:4); (*c*) ethyl acetate–acetic acid–formic acid–water (18:8:3:9); (*d*) butyl alcohol–ethanol–water (4:1:5, upper layer); (*e*) ethyl acetate–pyridine–water (10:4:3); (*f*) butanone–acetic acid–water (9:1:1, saturated with boric acid); (*g*) butanone–water–ammonia (sp. gr. 0.880) (200:17:1). Chromatograms were developed as described previously[6], except that D-glucose was detected by the following enzymic method. Chromatograms were sprayed, in succession, with 0.5M phosphate buffer (pH 7.0), 0.05% peroxidase, 0.05% glucose oxidase, and 0.1% *o*-dianisidine in 90% aqueous ethanol. Glucose oxidase specifically oxidises D-glucose, giving hydrogen peroxide, which is coupled with *o*-dianisidine by peroxidase, giving a purple spot. R_{Gal}, R_G, and R_{Rha} values refer to distances moved relative to D-galactose, 2,3,4,6-tetra-*O*-methyl-D-glucose, and L-rhamnose, respectively.

Gas–liquid chromatography. — G.l.c. of mixtures of *O*-methyl sugars was performed on a chromatograph (Type S3A, Gas Chromatography Ltd.) fitted with flame-ionisation detectors, at nitrogen flow-rates of *ca.* 100 ml/min on columns of (*i*) 15% by weight of poly(butane-1,4-diol succinate) on Celite (120×0.5 cm) at 175°, (*ii*) 15% by weight of poly(ethylene glycol adipate) on Celite (75×0.5 cm) at 160°. Retention times (*T*) are quoted relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside.

Conversion of 4-O-methyl-D-glucuronic acid into 4-O-methyl-D-glucose. — 4-*O*-Methyl-D-glucuronic acid (29 mg) was converted into the methyl ester methyl glycoside by treatment for 8 h with boiling, 5% methanolic hydrogen chloride. The resulting solution was neutralised with silver carbonate and filtered, and the residue was washed

TABLE I

ANALYTICAL DATA[a] FOR *Acacia drepanolobium*, FRACTIONS *A*, *B*, AND *C*

	First series of extractions			*Second series of extractions*		
	A	B	C	A	B	C
Recovery, %	79	8	13	80	8	12
Ash, %	0.00	0.00	2.78	0.00	0.00	2.56
Nitrogen, %	1.11	1.21	0.58	1.18	1.24	0.61
$[\eta]$, cm^3g^{-1}	17.8	19.5	22.8	15.0	17.2	20.2
Methoxyl, %	0.43	0.43	0.41	0.42	0.42	0.42
$[\alpha]_D$ (*c* 1.0, water)[b]	+77.8°	+77.2°	—	+82.2°	+82.6	—
$[\alpha]_D$ (*c* 1.0, N sodium hydroxide)[b]	+77.2°	+78.2°	+78.0°	+81.5°	+81.9°	+80.0°
Equivalent weight [b,c]	1976	1997	—	1985	1980	—
Uronic anhydride, %[b,d]	8.9	8.8	—	8.8	8.8	—
Uronic anhydride, %[b,e]	9.6	9.9	9.7	9.5	9.8	9.8
Formic acid released on periodate oxidation (moles/g × 10^3)[b]	1.07	1.05	1.02	1.06	1.08	1.05
Periodate reduced (moles/g × 10^3)[b]	3.96	3.97	3.93	3.95	3.98	3.96
L-Arabinose, %[b]	52	51	49	53	53	51
D-Galactose, %[b]	38	39	41	37	37	39
L-Rhamnose, %[b,f]	1	1	1	1	1	1
D-Glucuronic acid, %[b,g]	7	7	7	7	7	7
4-*O*-Methyl-D-glucuronic acid, %[b,g]	2	2	2	2	2	2

[a]All data corrected to a dry-weight basis; [b]data corrected for all non-carbohydrate material; [c]direct titration (potentiometric) with 0.02N sodium hydroxide; [d]assuming that all acidity arises from uronic acid groups; [e]by acid decarboxylation (Ref. 14); [f]by acetaldehyde released on periodate oxidation (Ref. 15); [g]assuming that all the methoxyl content arises from 4-*O*-methyl-D-glucuronic acid.

with warm methanol. The extract was evaporated to dryness, and dissolved in distilled water (10 ml). This solution was mixed with sodium borohydride (0.25 g in 25 ml of water) and kept overnight. Excess of borohydride was destroyed by the addition of Amberlite IR-120 (H^+) resin. The resin and the solvent were removed, and the borate was removed as methyl borate. The product (4-*O*-methyl-D-glucose) was purified on sheets of filter paper with solvent (*a*).

Borohydride reduction of aldobiouronic acids. — Sodium borohydride (25 mg in 5 ml of water) was added dropwise to the aldobiouronic acid (5–10 mg), and the mixture was kept overnight. Excess of borohydride was destroyed by the addition of Amberlite IR-120 (H^+). The resin and the solvent were removed, and the borate was volatilised as methyl borate. The product was boiled with 5% methanolic hydrogen chloride for 4 h, the solution was neutralised (silver carbonate) and filtered, and the residue was washed with warm methanol. The extract was evaporated to dryness, the residue was dissolved in distilled water (10 ml), and sodium borohydride (25 mg in 5 ml of water) was added. After storage overnight, excess of borohydride was destroyed, and borate was removed as before; the reduced product, (glucopyranosylgalactitol and/or 4-*O*-methylglucopyranosylgalactitol) was isolated as a syrup.

Methylation of aldobiouronic acids. — The micro Kuhn method developed by Perila and Bishop[9] was used. The aldobiouronic acid (10 mg) was shaken with methyl iodide (1 ml), *N*,*N*-dimethylformamide (1 ml), and silver oxide (1 g) at room temperature, in darkness, for 18 h. The mixture was filtered, the residue washed with chloroform, and the filtrate evaporated to dryness. The product was boiled with 5% methanolic hydrogen chloride for 8 h. The resulting methyl glycosides were analysed by g.l.c. A portion of the methanolysate was evaporated to dryness, and reduced with sodium borohydride (25 mg in 5 ml of water) for 12 h. The methyl glycosides were hydrolysed with N sulphuric acid for 4 h at 100°, and the methylated reducing sugars were examined by paper chromatography in solvents (*d*) and (*g*).

RESULTS

Comparative study of Fractions A, B, *and* C. — Hydrolysis (N sulphuric acid, 7 h) of each fraction, followed by paper-chromatographic examination, showed the presence of galactose, arabinose, and acidic disaccharides. Paper chromatograms, examined in ultraviolet light after spraying with aniline hydrogen oxalate, revealed that a trace of rhamnose was present in each fraction. After more drastic hydrolysis (2N sulphuric acid, 7 h), examination in solvent (*b*) showed that D-glucuronic acid, D-glucurono-6,3-lactone, and 4-*O*-methyl-D-glucuronic acid were present in each fraction.

Analytical data for the fractions are shown in Table I. Methylated sugars were not detected in the gum hydrolysates; if the methoxyl content of the gum is assumed to be located solely in residues of 4-*O*-methyl-D-glucuronic acid, calculation shows that this acid is present in each fraction to the extent of 2%.

Periodate oxidation of each fraction was followed by simultaneous determination

(2-ml aliquots) of formic acid and sodium metaperiodate. Oxidation of all fractions was complete after 48 h. For Fraction *C*, the oxidation mixtures did not become homogeneous until after 18 h.

The intrinsic viscosities shown in Table I were measured within a few days after isolation of the fractions. When the measurements were repeated after 2 months, it was found that the intrinsic viscosity of each fraction had increased, and a further increase was apparent after storage for 6 months in the free-acid form. This phenomenon is under investigation.

Study of Fraction A. — *A. drepanolobium* gum (160 g) was extracted with cold water, as described above, to yield Fraction *A* (130 g), which contained 10% moisture and 7% protein.

The neutral sugars of Fraction A. — The polysaccharide (0.75 g) was hydrolysed (N sulphuric acid, 7 h). The hydrolysate was fractionated on Whatman 3MM sheets for 36 h with solvent (*a*); crystalline D-galactose and L-arabinose were isolated.

D-Galactose (114 mg) had $[\alpha]_D^{19}$ +81° (equil., *c* 1.0) and, after recrystallisation from ethanol, m.p. and mixed m.p. 168°. The X-ray diffractogram was identical with that of an authentic specimen.

L-Arabinose (180 mg) had $[\alpha]_D^{19}$ +105° (equil., *c* 1.0) and, after recrystallisation from aqueous ethanol, m.p. and mixed m.p. 159°. The X-ray diffractogram was identical with that of an authentic specimen. After recrystallisation from water, the phenylosazone had m.p. and mixed m.p. 165°.

Fraction *A* (3 g) was hydrolysed (N sulphuric acid, 7 h). The hydrolysate was fractionated on Whatman 3MM sheets for 16 h with solvent (*a*). The position of rhamnose was located by spraying side-strips on which L-rhamnose had been run as a marker. Elution of the appropriate zone with water gave L-rhamnose (21 mg) having $[\alpha]_D^{19}$ +6° (*c* 0.21). After recrystallisation from aqueous ethanol, the L-rhamnose had m.p. and mixed m.p. 93–94°.

The acidic sugars of Fraction A. — The polysaccharide (10 g) was hydrolysed for 7 h with N sulphuric acid. Paper chromatography of the hydrolysate in solvent (*b*) indicated the presence of galactose, arabinose, and two acidic fractions (I) and (II) having R_{Gal} values of 0.26 and 0.59: these acidic fractions were isolated as syrups from filter sheets developed in solvent (*b*).

Fraction I (147 mg) was examined by paper chromatography in solvent (*b*) for 72 h; two acidic components were observed, having R_{Gal} 0.23 and 0.29, respectively. Similarly, paper chromatography in solvent (*c*) for 24 h gave two acidic components (aldobiouronic acids *D* and *E*) having R_{Gal} 0.56 and 0.61. Fraction I was hydrolysed (2N sulphuric acid, 7 h); paper chromatography of the hydrolysate in solvent (*b*) showed *ca.* equal amounts of galactose and glucurono-6,3-lactone, accompanied by traces of glucuronic acid and unhydrolysed aldobiouronic acids *D* and *E*. Crystalline D-galactose and D-glucurono-6,3-lactone were isolated from Whatman 3MM sheets developed in solvent (*b*). D-Galactose (50 mg) had $[\alpha]_D^{19}$ +80° (equil., *c* 1.0) and m.p. and mixed m.p. 167°, after recrystallisation from ethanol. D-Glucurono-6,3-lactone (45 mg) had $[\alpha]_D^{19}$ +19° (equil., *c* 0.9); after recrystallisation from

aqueous ethanol, it had m.p. and mixed m.p. 176°, with an X-ray diffractogram identical with that of authentic material.

Fraction II (78 mg) was examined by paper chromatography in solvent (*b*) for 72 h; two acidic components were observed, having R_{Gal} 0.56 and 0.66. Similarly, paper chromatography in solvent (*c*) for 24 h gave two acidic components (aldobiouronic acids *F* and *G*) having R_{Gal} 0.90 and 0.94. Fraction II was hydrolysed (2N sulphuric acid, 7 h); paper chromatography of the hydrolysate in solvent (*b*) showed *ca.* equal amounts of galactose and 4-*O*-methylglucuronic acid, accompanied by traces of unhydrolysed aldobiouronic acids *F* and *G*. Crystalline D-galactose and pure 4-*O*-methylglucuronic acid were isolated from 3MM sheets developed in solvent (*b*). D-Galactose (25 mg) had $[\alpha]_D^{19}$ +80° (equil., *c* 0.5) and m.p. and mixed m.p. 168°. 4-*O*-Methylglucuronic acid (31 mg) had $[\alpha]_D^{18}$ +36° (*c* 0.62); borohydride reduction of the methyl ester gave 4-*O*-methylglucose, which was purified on filter sheets with solvent (*a*) and isolated as a syrup. 4-*O*-Methylglucose had R_{Gal} 1.92 and R_{Rha} 0.90 in solvent (*a*), R_{Gal} 2.19 and R_{Rha} 0.84 in solvent (*b*), and R_{Gal} 1.74 and R_{Rha} 0.93 in solvent (*d*). After recrystallisation from aqueous acetone, the phenylosazone had m.p. 156–158° (lit[10], 158–159°).

The aldobiouronic acids of Fraction A. — The hydrolysate from Fraction *A* was fractionated [solvent (*b*), 96 h] on 3MM filter sheets; four aldobiouronic acids *D*,*E*,*F*, and *G* were isolated as syrups, and identified as follows.

Acid D (102 mg) had R_{Gal} 0.23 [solvent (*b*)] and R_{Gal} 0.56 [solvent (*c*)], and was chromatographically identical with 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose in these solvents. The syrup had $[\alpha]_D^{19}$ −3° (*c* 1.02), and this suggests the presence of a β-D-linkage. The syrup (5 mg) was hydrolysed (2N sulphuric acid), and paper chromatography [solvent (*b*)] revealed *ca.* equal amounts of D-galactose and D-glucurono-6,3-lactone. Acid *D* (10 mg) was reduced, using borohydride, as described above. The resulting hydrolysate was examined chromatographically [solvent (*f*)]; *ca.* equal amounts of glucose and galactitol were detected. The oxidation of known weights (3–5 mg) of acid *D* with excess of sodium metaperiodate did not give formaldehyde. Acid *D* (10 mg) was methylated by the Kuhn method, and the methyl glycosides produced on methanolysis of the product were examined by g.l.c.; the products were 2,3,4-tri-*O*-methyl-D-glucuronic acid [column (*i*), *T* 2.33, 2.96; column (*ii*), *T* 2.08, 2.70]; 2,3,4-tri-*O*-methyl-D-galactose [column (*i*), *T* 6.59; column (*ii*) *T* 5.25]; and a trace of 2,3,5-tri-*O*-methyl-D-galactose [column (*i*), *T* 4.09, 4,50; column (*ii*), *T* 3.44, 4.14]. A portion of the methanolysate was reduced with sodium borohydride; after removal of methyl glycosides by mild hydrolysis with acid, the products were examined chromatographically in solvent (*d*). This revealed 2,3,4-tri-*O*-methyl-D-galactose (red spot, R_G 0.72) and a red–black spot (R_G 0.84) corresponding to 2,3,4-tri-*O*-methyl-D-glucose and 2,3,5-tri-*O*-methyl-D-galactose. Double development (solvent (*g*)] separated the 2,3,4-tri-*O*-methyl-D-glucose (red spot) from 2,3,5-tri-*O*-methyl-D-galactose (black spot). These experiments led to the identification of acid *D* as 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose.

Acid E (91 mg) had R_{Gal} 0.29 [solvent (*b*)] and R_{Gal} 0.61 [solvent (*c*)], and

was chromatographically identical with 4-*O*-(α-D-glucopyranosyluronic acid)-D-galactose. The syrup had $[\alpha]_D^{20}$ +106° (*c* 0.91) and this suggests an α-D linkage. The syrup (5 mg) was hydrolyzed (2N sulphuric acid), and paper chromatography [solvent (*b*)] showed *ca.* equal amounts of D-galactose and glucurono-6,3-lactone. Acid *E* (5–10 mg) was reduced, using borohydride, as described above. The resulting glucosylgalactitol was hydrolysed (N sulphuric acid), and the hydrolysate was examined chromatographically [solvent (*f*)]; *ca.* equal amounts of glucose and galactitol were detected. The oxidation of known weights (3–5 mg) of acid *B* with excess of sodium metaperiodate gave 1.01 moles of formaldehyde per mole of acid *E*. Acid *E* (10 mg) was methylated by the Kuhn method, and the methyl glycosides produced on methanolysis of the product were examined by g.l.c.; the products were 2,3,4-tri-*O*-methyl-D-glucuronic acid [column (*i*), *T* 2.40, (2.99); column (*ii*), *T* 2.04, 2.70] and 2,3,6-tri-*O*-methyl-D-galactose [column (*i*), *T* (2.99), 3.88, and 4.23; column (*ii*), *T* 2.42, 3.07, and 3.41]. A portion of the methanolysate was reduced with sodium borohydride; after removal of methyl glycosides by mild hydrolysis with acid, the products were examined chromatographically in solvent (*d*). This revealed 2,3,6-tri-*O*-methyl-D-galactose (R_G 0.76) and 2,3,4-tri-*O*-methyl-D-glucose (R_G 0.84). Double development [solvent (*g*)] revealed the same *O*-methyl sugars. These experiments led to the identification of acid *E* as 4-*O*-(α-D-glucopyranosyluronic acid)-D-galactose.

Acid F (42 mg) had R_{Gal} 0.56 [solvent (*b*)] and R_{Gal} 0.90 [solvent (*c*)], and was chromatographically identical with 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose. The syrup had $[\alpha]_D^{19}$ +3° (*c* 0.42) (for lit. values, see Ref. 5); this suggests a β-D linkage. The syrup (5 mg) was hydrolysed; paper chromatography [solvent (*b*)] showed *ca.* equal amounts of D-galactose and 4-*O*-methylglucuronic acid. Acid *F* (5–10 mg) was reduced with borohydride to give a 4-*O*-methylglucosylgalactitol, and the hydrolysate was examined chromatographically [solvents (*a*) and (*f*)]; *ca.* equal amounts of galactitol and 4-*O*-methylglucose were detected. The remainder of the hydrolysate was oxidised by the method of Lemieux and Bauer[8], and the products were examined in solvent (*a*). Spraying with aniline hydrogen phthalate revealed 2-*O*-methylerythrose, a characteristic product of 4-*O*-methylglucose, as a yellow-green spot (R_F 0.55). Known weights of acid *F* (3–5 mg) did not give formaldehyde when oxidised with excess of metaperiodate. On methylation, acid *F* gave the same methylated sugars as acid *D*. These experiments led to the identification of acid *F* as 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose.

Acid G (31 mg) had R_{Gal} 0.66 [solvent (*b*)] and R_{Gal} 0.94 [solvent (*c*)], and was chromatographically identical with 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose. The syrup (5 mg) was hydrolysed; paper chromatography [solvent (*b*)] showed *ca.* equal amounts of D-galactose and 4-*O*-methyl-D-glucuronic acid. Acid *G* (5–10 mg) was reduced with borohydride to a 4-*O*-methylglucosylgalactitol; the hydrolysate in solvents (*a*) and (*f*) showed *ca.* equal amounts of 4-*O*-methylglucose and galactitol. The hydrolysate was periodate oxidised[8], and the aniline hydrogen phthalate spray revealed the presence of a 2-*O*-methylerythrose. Periodate oxidation led to the production of 0.96 moles of formaldehyde per mole of acid *G*. On methylation,

acid *G* gave the same methylated sugars as acid *E*. These experiments led to the identification of acid *G* as 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose.

The aldobiouronic acids of fractions B *and* C. — After hydrolysis under the same conditions as for Fraction *A*, fractions *B* and *C* were examined chromatographically and were found to contain similar proportions of the four aldobiouronic acids identified in Fraction *A*.

DISCUSSION

Acacia drepanolobium gum has been shown to contain the same sugars, and the same four aldobiouronic acids, as other gums of the genus *Acacia* having high, positive rotations (*cf.*, *A. nilotica*[3], *A. arabica*[1], *A. nubica*[4], and *A. seyal*[4]).

A study of the analytical data presented in Table I shows that there are no striking differences in the composition of the gum fractions *A* (water-soluble), *B* (salt-soluble) and *C* (water- and salt-insoluble, but soluble to the extent of 1% in molar sodium hydroxide). Fractions *A* and *B* have an arabinose–galactose ratio of $52\pm1:38\pm1$, and this must be compared with the ratio of $50\pm1:40\pm1$ for Fraction *C*; although this may not be analytically significant, some small differences may be sufficient to account for the solubility differences exhibited by Fractions *A*, *B*, and *C*. It is possible that the differences may involve some variation in fine structure or degree of branching, as in the case with soluble and insoluble laminaran[11]. Although the sugar composition and periodate oxidation data for Fractions *A*, *B*, and *C* are in good agreement, structural differences between these fractions cannot be discounted until a full structural study has been made.

The most significant analytical differences between Fractions *A*, *B*, and *C* lie in the ash and protein contents of the purified fractions. The high ash content of Fraction *C* may reflect the difficulty encountered in purifying an aqueous dispersion, as opposed to a true solution. It is possible, however, that the gel formation shown by Fraction *C* is a consequence of crosslinking effects arising from the presence of polyvalent cations, although no foundation for this theory was found in experiments with *A. senegal* gum[12].

It is of interest that the nitrogen content of Fraction *C* is only *ca.* 50% of that present in Fractions *A* and *B*. Protein–carbohydrate interaction has been offered in the past as a possible explanation of gel formation; the jelly isolated from *Acacia pycnantha*[13] contained three times as much nitrogen as the water-soluble gum.

A further explanation of the physical differences between Fractions *A*, *B*, and *C* may involve the molecular size and/or shape of the polysaccharides involved: Table I shows that the intrinsic viscosity of Fractions *A*, *B*, and *C* increases in the same order as the increase in insolubility. Physico-chemical studies of Fractions *A*, *B*, and *C* will be carried out in due course.

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SUMMARY

The gum polysaccharide from *Acacia drepanolobium* is not completely water-soluble, and has been subjected to graded extractions giving Fractions *A* (cold-water soluble, 80%), *B* (salt-soluble, 8%), and *C* (a gel soluble in dilute alkali, 12%). Fraction *A* contains L-arabinose (52%), D-galactose (38%), L-rhamnose (1%), D-glucuronic acid (7%), and 4-*O*-methyl-D-glucuronic acid (2%). Fractions *A*, *B*, and *C* each contain four aldobiouronic acids, *viz.*, 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose; 4-*O*-(α-D-glucopyranosyluronic acid)-D-galactose; 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose; and 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose.

The analytical differences between fractions *A*, *B*, and *C* have been investigated, and the factors that may account for their physical differences are discussed.

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Studies on uronic acid materials
Part XXIII. Possible degradations with the sodium hydride–methyl iodide–methyl sulphoxide methylation system

Anderson and Cree[2] have described the potential use of the sodium hydride–methyl iodide–methyl sulphoxide system for methylating acidic mono- and di-saccharides, and also for some acidic polysaccharides. The method appeared to offer advantages when compared with the classical Haworth and Purdie methods, but it was suggested[2] that the method should be evaluated with a wider range of polysaccharides before general conclusions could be reached.

Following a communication[3] that the sodium hydride system caused undesirable degradation of dextrans with the formation of abnormally high yields of 2,3,4,6-tetra-*O*-methyl-D-glucose, we have now compared the methylation products, obtained with sodium hydride, of a potato starch and of three different *Acacia* gum polysaccharides, with their respective methylated products obtained by treatments with the Haworth and Purdie reagents.

EXPERIMENTAL

Methylations with the sodium hydride–methyl iodide–methyl sulphoxide system, and the analyses by paper and gas chromatography, were carried out by the procedures and apparatus described previously[2].

RESULTS

Experiments with *Acacia nubica* gum showed that two additions of sodium hydride and methyl iodide were required to give complete methylation (OMe, 40.3%); the methoxyl content after 1 addition was only 16.3%. Lithium hydride was less effective (OMe after 1 treatment, 11.5%) than sodium hydride. Calcium hydride was ineffective when used to replace the sodium hydride, and stable emulsions were formed (*cf.* the effect of barium salts[2]).

Potato starch. — A laboratory sample of potato starch (var. Kerr's Pink) was methylated exhaustively with (*a*) the sodium hydride–methyl iodide–methyl sulphoxide system, and (*b*) several additions of the Haworth and the Purdie reagents. After methanolysis, the methyl glycosides of products (*a*) and (*b*) were examined by g.l.c.; the chromatograms were identical, except that product (*a*) showed a small peak caused by a trace of residual methyl sulphoxide.

Acacia drepanolobium *gum.* — This gum polysaccharide contains[1] D-galactose (38%), L-arabinose (52%), L-rhamnose (1%), D-glucuronic acid (7%), and 4-*O*-methyl-D-glucuronic acid (2%). The gum was methylated by methods (*a*) and (*b*), as above. After methanolysis of each product, the chromatograms for products (*a*) and (*b*) were identical, except for the presence in (*a*) of a trace of methyl sulphoxide.

Acacia campylacantha *gum.* — This gum polysaccharide contains[4] D-galactose (56%), L-arabinose (28%), L-rhamnose (7%), D-glucuronic acid (7%), and 4-*O*-methyl-D-glucuronic acid (2%). The chromatograms of the methanolysed, fully methylated gum obtained by methods (*a*) and (*b*) were similar in all respects other than that the proportion of 2,3,4-tri-*O*-methyl-L-rhamnose in product (*a*) was slightly less than in product (*b*).

Acacia senegal *gum.* — This gum polysaccharide contains[5] D-galactose (39%), L-arabinose (28%), L-rhamnose (14%), D-glucuronic acid (17.5%), and 4-*O*-methyl-D-glucuronic acid (1.5%). The gum was methylated by methods (*a*) and (*b*), as above. When the chromatograms of the methanolysate were compared, the proportion of 2,3,4 tri-*O*-methyl-L-rhamnose in product (*a*) was only about 33% of that in product (*b*). The proportion of 2,3-di-*O*-methyl-D-glucuronic acid also appeared to be less in product (*a*) than in (*b*).

DISCUSSION

Base-catalysed reactions proceed at greatly increased rates in methyl sulphoxide, in which the strongly basic methylsulphinyl carbanion is readily formed[6]; the methyl sulphoxide is a reactant, as well as being the solvent. There is no doubt that the sodium hydride–methyl iodide–methyl sulphoxide system is much more strongly alkaline in reaction[7] than a well-conducted Haworth methylation.

Hamilton and Thompson[8] have shown that alkaline hydrolysis of gum arabic (*Acacia senegal*) leads to the loss of residues of L-rhamnose and D-glucuronic acid. At least some of the L-rhamnopyranose residues in gum arabic were shown[9] to be

(1→4)-linked to D-glucuronic acid residues; subsequent structural studies[5,10] have confirmed earlier indications[11] that all of the L-rhamnopyranose residues are present as non-reducing end-groups.

It appears that those *Acacia* gum exudates (*e.g.*, *A. nubica* and *A. drepanolobium*) having low L-rhamnose and low methoxyl contents (and therefore having the majority of their D-glucuronic acid residues as non-reducing end-groups) are reasonably stable under the conditions described[2] for methylation by the sodium hydride–methyl sulphoxide–methyl iodide system. In contrast, those *Acacia* gum polysaccharides having significant proportions of L-rhamnopyranose residues as end groups attached to position 4 of D-glucuronic acid residues (*e.g.*, *A. senegal*) appear to be much less stable; loss of 2,3,4-tri-*O*-methyl-L-rhamnose, and also probably 2,3-di-*O*-methyl-D-glucuronic acid, occurs. This may be explained in terms of a β-elimination at C-4 of the glucuronic acid; such an elimination did not occur[2] with *A. nubica* gum.

Although our results show that the sodium hydride–methyl sulphoxide methylation system does offer certain advantages for some polysaccharides, we must repeat here the suggestion[2] that its performance should be assessed with care on a wider range of polysaccharides. All too frequently, structural studies are based on the product from only one methylation system; methylation by more than one method should be attempted, so that the results can be compared.

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STUDIES ON URONIC ACID MATERIALS

PART XXIV*. AN ANALYTICAL STUDY OF DIFFERENT FORMS OF THE GUM FROM *Acacia senegal* WILLD.

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ABSTRACT

Some of the analytical parameters for *Acacia senegal* gum have been determined for (*a*) twelve "bulk" samples of tapped gum from three different districts of the Sudan, and (*b*) thirteen, different, single-nodule specimens of "natural exudate" gum. These samples show the same range of analytical variation that has been noted previously for samples of gum from other *Acacia* species. In addition, the following atypical variants of *Acacia senegal* gum have been studied: (*a*) three specimens of "non-nodular "gum, (*b*) a sample from a tree infested with wood-boring beetles, and (*c*) a sample of "Hennawi" gum, a dark-colored, sweet-tasting gum from the main stem of trees.

The viscosity data obtained indicate that values of $\overline{M}_w$ for the samples fall in the range 0.26×10^6 to 1.16×10^6; this explains the lack of agreement for $\overline{M}_w$ of *A. senegal* gum samples studied by earlier investigators.

The atypical, non-nodular and beetle-borer specimens of gum do not show any striking analytical differences from the mean values obtained for the typical samples. The "Hennawi" variant differs from typical, tapped samples in two important respects: the uronic acid residues present in the free acid form are fewer in number, and the rhamnose content is considerably less.

INTRODUCTION

Earlier investigators[2,3] considered that different samples of gum arabic (*Acacia senegal* Willd., syn. *verek*) did not vary in properties to any great extent, but this is not the current industrial view. Botanically, *A. senegal* is very variable[4], and the Sudanese consider that the gum viscosity of a sample is dependent on the age of the tree involved. A sa result of empirical, technological observations, some manufacturers restrict their purchases to gum originating from a certain district; others decline a current season's crop ("green gum"), preferring gum stored in the Sudan since the previous season.

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It is now clearly established that distinct internodule variations occur in the gums from *A. seyal*[5], *A. nilotica*[6], and *A. laeta*[7], but there are no modern analytical values for specimens of *A. senegal* gum. Widely differing values for the molecular weight (both for $\overline{M}_n$ and $\overline{M}_w$) of *A. senegal* gum have been reported, and these have been summarised[8]; the implication that different samples of *A. senegal* gum may vary widely in $\overline{M}_n$ or $\overline{M}_w$ must therefore be considered, and the possible range of variation requires evaluation. Information on the viscosity behaviour of a wide selection of specimens would therefore indicate those likely to display the widest extremes when studied more fundamentally by the light-scattering technique.

Accordingly, authentic specimens of *A. senegal* gum from different geographical locations and different types of soil, from different seasons, and from different times of collection within a season, have been investigated. In addition, the variation from tree to tree has been investigated for normal specimens of the gum obtained (*i*) by "tapping" and (*ii*) by natural exudation. We have also examined three unusual forms of natural-exudate gum from *A. senegal*: (*a*) non-nodular forms of the gum, exuded as thin, spiral filaments or ribbons, rather than as oval-shaped nodules; (*b*) "Hennawi" gum, which is a very dark-colored, sweet-tasting form of *A. senegal* gum exuded from the main stems of trees, as opposed to the upper branches usually tapped; (*c*) the gum exuded by trees infested with wood-boring beetles. This exudate was in the form of hard, dark-brown masses of wood borings and debris, bound together with a gum exudate comprising about 40% of the masses by weight.

EXPERIMENTAL

Analytical methods. — The standard methods involved have been described[9] previously.

Origins of specimens. — All of the specimens, from *A. senegal* trees that could be identified botanically beyond all doubt, were collected by (the late) Mr. M. P. Vidal-Hall, formerly Gum Research Officer to the Republic of the Sudan. Details of their collection are as follows:

1. "*QN*" *samples.* These were bulk samples from many trees, growing on heavy clay soil at Qala en Nahal, Kassala Province, Eastern Sudan: 1960 crop, first (QN1), second (QN2), third (QN3), and final collection (QN4); 1962 crop, first collection (QN5).

2. "*UR*" samples. These were bulk samples from many trees, growing on sandy soil at Umm Ruaba, Kordofan Province, Central Sudan: 1960 crop, representative sample (UR1), final collection (UR2).

3. "*GG*" samples. These were bulk samples from many trees, growing on sandy soil at Goz el Ganzara, Kordofan Province, Central Sudan: 1960 crop, representative sample (GG1); 1962 crop, first (19 January, GG2), second (4 February, GG3), third (19 February, GG4), and final collection (5 March, GG5).

All of the QN, UR, and GG samples were normal samples of top commercial-quality gum from *A. senegal*, pale straw in colour, and virtually free from sand

and bark. Exudation was in response to tapping. Each sample (*ca.* 1500 g) was crushed and sieved to give material representative of the complete sample.

4. Single-nodule samples of natural exudate gum from individual trees of *A. senegal*, collected at Umm Ruaba (sandy soil) on 9 March, 1960:
Tree *A* (age 25 years) gave 3 nodules (samples A1, A2, and A3); *B* (12 years), 2 nodules (B1 and B2); *C* (8 years), 2 nodules (C1 and C2); *D* (15 years), 1 nodule (D); *E* (12 years 1 nodule (E); *F* (17 years), 1 nodule (F); *G* (20 years), 1 nodule (G); *H* (10 years), 1 nodule (H); *I* (15 years), 1 nodule (I). Each of these samples (15–30 g) was crushed to a fine powder and investigated individually.

5. "*Hennawi*" *sample*. This very dark brown variant of *A. senegal* gum has a sweet taste and is exuded naturally at wounds to the lower, main stem of the tree. Such gum is not usually tapped. It is known in the Sudan as "*Hennawi*" gum, eagerly sought by native children as a sweetmeat. The specimen (HW) of *Hennawi* gum studied was collected at Goz el Ganzara on 13 March 1963.

6. "*NN*" *samples*. Occasionally, *A. senegal* trees exude gum that differs from the customary oval nodules in forming long filaments, spirals, and ribbons. Three non-nodular (NN) forms of the gum were recognised: broad, opaque, flat ribbons (NN1); thin, circular, opaque filaments (NN2); and thin, circular, strongly refractive filaments (NN3). These samples were collected at Goz el Ganzara on 11 February 1961.

7. "*BB*" *samples*. Trees infested with wood-boring longhorn (*Cerambycidae*) or jewel (*Buprestidae*) beetles exude hard, dark-brown masses of a gum mixed with wood borings and other debris. The sample (180 g) of beetle-borer gum was collected at Goz el Ganzara in January 1965. One half-portion of the exudate (90 g) was stirred with cold water (750 ml) for 48 h, filtered, dialysed against tap-water, and then exhaustively electrodialysed. The gum polysaccharide, sample BB(*a*), was isolated as the freeze-dried product (33.4 g, 37%). The remainder of the gum exudate (90 g) was stirred with cold water (750 ml) for 24 h, and this extraction was processed in the same way as for BB(*a*) to give sample BB(*b*) (27.7 g, 31%). The residue from the extraction yielding BB(*b*) was then further exhaustively extracted with cold water (500 ml) and, after processing as before, this extraction gave sample BB(*c*) (8.9 g, 10%).

Purification of samples. — All of the QN, UR, GG, HW, and NN samples, and, the single-nodule samples A-I, were purified by dissolution in cold water, filtration dialysis, and exhaustive electrodialysis.

RESULTS

Table I gives the analytical data obtained for the various bulk samples QN, UR, GG, NN, and HW. Table II gives the analytical data for the single-nodule samples from individual trees. Table III gives more-detailed analytical information for samples QN1, HW, BB(*a*), BB(*b*), and BB(*c*). Figure 1 gives the viscosity plots from which the limiting-viscosity numbers, $[\eta]$, recorded in Tables II and III were found.

Since the modified Staudinger constants K' and a are now known[10] to have

TABLE I

ANALYTICAL DATA FOR BULK SAMPLES OF *Acacia senegal* GUM

Sample	*Moisture* %	*Ash* %	*Nitrogen* %	*Insoluble*[a] %	*Flow-time number* ($cm^3\ g^{-1}$) *Crude gum*[b]	*Purified gum*[c,d]
QN1	12.8	3.64	0.33	0.4	57	25 (19.9)
QN2	13.4	3.37	0.34	0.3	47	18
QN3	13.3	3.07	0.36	0.4	43	20
QN4	13.1	2.91	0.35	0.2	37	17
QN5	12.7	3.62	0.34	0.6	62	30
UR1	13.2	3.05	0.39	0.4	35	21
UR2	13.5	3.52	0.39	1.0	34	22
GG1	12.4	3.68	0.39	1.5	54	36
GG2	12.4	4.16	0.42	1.6	64	43 (24.5)
GG3	12.9	3.98	0.41	1.3	67	36
GG4	13.0	3.93	0.38	0.5	79	38
GG5	13.2	3.81	0.38	0.2	71	32
NN1	13.4	3.88	0.40	0.4	83	36
NN2	13.2	3.70	0.34	0.2	62	36
NN3	13.1	3.97	0.39	0.3	70	35
HW	13.6	5.67	0.37	0.5	39	35 (20.6)
Average	13.1	3.75	0.37	0.6	56	30
Range	12.4–13.6	2.91–5.67	0.33–0.42	0.2–1.6	34–83	17–43
Standard deviation	0.36	0.63	0.028	0.47		

[a]Insoluble after 24 h in cold water; [b]0.03 g/ml in water at 25.0°; [c]0.03 g/ml in 4% sodium chloride solution at 25.0°; [d]values in brackets are the corresponding limiting (C→O) flow-time numbers.

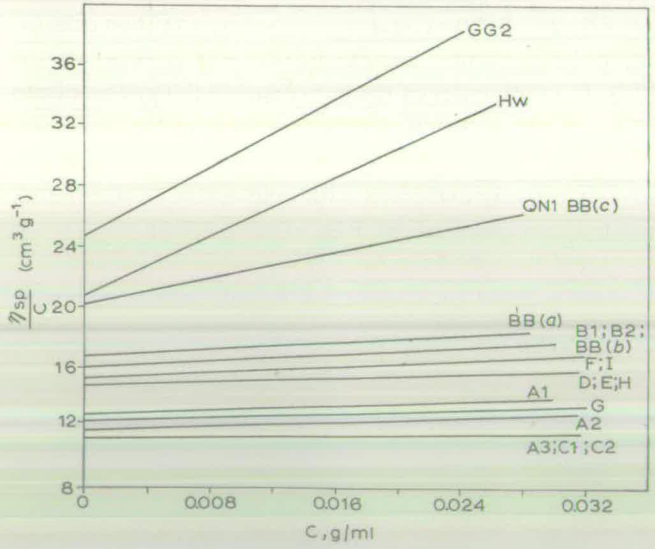


Fig. 1. The viscosity-concentration relationship for different specimens of *Acacia senegal* gum. The following limiting-viscosity numbers and M_w values (from $[\eta]=K'M_w^a$, where[10] $K'=1.3\times10^{-2}$, and $a=0.54$) were obtained for samples GG2, 24.6, 1.16×10^6; HW, 20.6, 0.84×10^6; QN1 and BB(*c*), 19.9, 0.79×10^6; BB(*a*), 16.5, 0.55×10^6; A1, 12.4, 0.33×10^6; A3, C1, C2, 11.0, 0.26×10^6.

TABLE II

ANALYTICAL DATA FOR SINGLE-NODULE NATURAL EXUDATE *Acacia senegal* GUM

Sample	*Age of tree (years)*	*Crude material*		*Electrodialysed material*			$\overline{M}w^a$
		Moisture %	*Ash* %	*Nitrogen* %	$[\alpha]_D$ *(degrees)*	*Limiting-viscosity number (cm $^3g^{-1}$)*	
A1	25	12.6	3.72	0.24	−29	12.5	334,000
A2		11.6	3.78	0.23	−31	11.6	291,000
A3		11.1	3.80	0.24	−32	11.0	264,000
B1	12	11.7	4.43	0.23	−28	15.6	504,000
B2		12.9	4.44	0.23	−29	15.8	516,000
C1	8	10.4	3.40	0.27	−29	11.0	264,000
C2		9.8	3.66	0.26	−31	11.0	264,000
D	15	15.6	3.88	0.32	−30	14.1	418,000
E	12	12.8	4.64	0.34	−32	14.5	440,000
F	17	13.3	4.01	0.35	−29	15.1	474,000
G	20	15.4	4.15	0.23	−31	12.2	320,000
H	10	14.7	3.97	0.45	−29	14.4	434,000
I	15	12.1	3.27	0.33	−27	15.0	468,000
Average		12.6	3.93	0.29	−30	13.4	
Range		9.8–15.6	3.27–4.64	0.23–0.45	−27–−32	11.0–15.8	
Standard deviation		1.8	0.40	0.06	1.5	1.9	

[a] From $[\eta] = K'M_w^a$, where $K' = 1.3 \times 10^{-2}$ and $a = 0.54$ (see Ref. 10).

values of 1.30×10^{-2} and 0.54, respectively, values for $\overline{M}_w$ can be calculated from the viscosity data by the relationship $[\eta] = K'M_w^a$, and these values are shown in Fig. 1.

DISCUSSION

From Fig. 1 and the viscosity data in Tables I, II, and III, it is clear that $\overline{M}_w$ for *Acacia senegal* gum varies widely from sample to sample; the variation, for the samples studied, is from 1.16×10^6 (for GG2) to 0.26×10^6 (for nodules A3, C1, and C2). This explains the lack of agreement[8] between the values for $\overline{M}_w$ reported by earlier investigators, *viz.* 0.58×10^6 (Deb and Mukherjee[11]) and 1.00×10^6 (Veis and Eggenberger[12]). From our experiments, a value of 0.58×10^6 (Ref. 8) appears to be a more representative value than 1.00×10^6, although values higher than the latter may yet be found. *A. senegal* gum has a broad, skew, molecular-weight distribution, and fractions having $\overline{M}_w$ ranging from 1.18×10^6 to 0.1×10^6 have been obtained[10] for sample QN1. It should be emphasised that the values deduced for $\overline{M}_w$ in this paper are based on the modified Staudinger constants obtained from fractionations[10] of only one *A. senegal* gum sample: these values for K' and a do not hold for other *Acacia* species[10], and, indeed, they may not be generally applicable to all specimens of such a complex polymer system as *A. senegal* gum.

TABLE III

COMPARATIVE ANALYTICAL DATA[a] FOR SOME ELECTRODIALYSED TYPICAL (QN1) AND ATYPICAL (HW, BB) SPECIMENS OF *Acacia senegal* GUM

	QN1	*HW*	*BB(a)*	*BB(b)*	*BB(c)*
Ash, %	0.07	0.01	0.02	0.01	0.02
Nitrogen, %	0.33	0.35	0.25	0.21	0.28
$[\eta]$, cm^3g^{-1}	19.9	20.6	16.4	15.8	20.1
$[\alpha]_D$ (*c* 1.0, water)[b]	−31.0°	−27.6°	−30.5°	−31.3°	−31.5°
Methoxyl[b,c], %	0.25	0.25	0.25	0.25	0.25
Equivalent weight[b]	1085	1047	1066	1065	1060
Hence, uronic anhydride[b,d], %	16.2	16.8	16.5	16.5	16.5
Uronic anhydride [b,c], %	17.2	17.5	17.4	17.3	17.5
Formic acid released on periodate oxidation[b] (mole/g) × 10^3	1.62	1.69	1.76	1.85	1.90
Periodate consumed[b] (mole/g) × 10^3	5.07	5.18	5.23	5.34	5.42
Hence, $\frac{\text{periodate consumption}}{\text{formic acid released}}$	3.13	3.06	2.97	2.89	2.85
Rhamnose[b,c], %	14.0	6.2	13.0	12.6	12.8
Galactose[b], %	40	46	43	43	44
Arabinose[b], %	28	30	27	27	26
Glucuronic acid[b], %	16.0	16.5	15.5	15.5	15.5
4-*O*-methylglucuronic acid, %	1.5	1.5	1.5	1.5	1.5

[a]All data corrected to a dry-weight basis; [b]corrected for all non-carbohydrate material; [c]determined by vapour-phase, infrared methods; [d]assuming that all acidity arises from uronic acid groups; [e]assuming that all methoxyl content arises from 4-*O*-methylglucuronic acid.

Many factors influence[13] the viscosity of *Acacia* gum solutions. We have observed that electrodialysed specimens, after storage in the freeze-dried state for several months, give increased limiting-viscosity numbers when solutions are made up in a standardised way to facilitate comparison; for sample QN1, $[\eta]$ increased from 19.9 to 29.0, and for sample GG2, $[\eta]$ increased from 24.5 to 35.1. Other *Acacia* species give the same effect, and this is under investigation. The viscosity data presented in Tables I and II were obtained for 3% solutions immediately after dissolution. Table II gives some support for the belief that gum viscosity is dependent on the age of the tree; the oldest and youngest trees studied gave gum of low viscosity.

The "bulk" samples, representative of many nodules, show distinct analytical differences, although they must be expected to show "averaged" values. The differences shown in Table II for the single-nodule specimens are perhaps more fundamental in significance. Table I shows that the GG samples are more viscous than the QN or UR samples; it is interesting (*cf.* Ref. 5) that the most-viscous samples have higher than average nitrogen contents, although a high nitrogen content does not necessarily imply high viscosity, as shown by the UR samples. Insufficient data are available for any conclusions regarding seasonal effects to be reached; there is a suggestion, however, that the viscosity of the QN samples decreases as the season continues, whereas, in contrast, the viscosity of the GG samples increases.

The results for trees *A*, *B*, and *C* in Table II indicate that each tree tends to

produce a characteristic form of the gum, the different nodules from a particular tree being strikingly similar to each other in comparison to those from other trees.

The "Hennawi" variant of the gum has two distinctive features. It contains only 6% of rhamnose, *i.e.*, less than 50% of that present normally. A solution of "Hennawi" gum has pH 6.25; all of the other samples studied fell within the range pH 4.49 to 4.66, and the electrodialysed specimens fell within the range pH 2.47 to 2.60. The comparatively sweet taste of "Hennawi" gum is not due to the presence of free sugars; its reducing power is closely similar to that of the other samples. The explanation appears to involve the fact that "Hennawi" gum has very few, if any, free uronic carboxyl groups, with many more than usual being involved in salt formation with cations. The fact that the ash content of clean "Hennawi" gum is much higher than usual [5.67%, compared with an average (Table I) of 3.75%] lends support to this explanation.

When the data given in Tables I and II were obtained, it became apparent that a structural study of the HW and BB samples offered an opportunity to acquire information on the biosynthesis of *A. senegal* gum. The effect of different external stimuli to gum production (*e.g.*, natural exudation, tapping wounds, beetle infestation) is not yet known. Accordingly, it was decided to compare the structural features of samples HW, BB(*a*), BB(*b*), and BB(*c*) with those of a typical tapped-gum sample QN1. Table III shows the analytical differences between these samples; their structural differences will be discussed in a subsequent paper.

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STUDIES ON URONIC ACID MATERIALS

PART XXV*. SOME UNUSUAL FORMS OF THE GUM FROM *Acacia senegal* WILLD.

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ABSTRACT

A comparative study, involving classical methylation and Smith-degradation techniques, has been carried out on samples of normal "tapped" gum from *Acacia senegal*, natural exudate gum ("Hennawi" gum) from the main trunk of the tree, and the gum exuded by a tree infested with wood-boring beetles. The studies have shown that the beetle-borer specimen was virtually identical to the specimen of normal exudate gum; the specimen of "Hennawi" gum was basically similar, but differed in two important aspects concerning some of the peripheral end-group positions involving rhamnose and glucuronic acid residues. Theories of the origin, function, precursors, and mode of biosynthesis of plant gums are examined critically in terms of the results obtained.

INTRODUCTION

In the preceding part of this series, we reported the results of an analytical study of some different forms of the gum exudate from *Acacia senegal* Willd. Two of the specimens were of particular interest; these were (*a*) the gum exuded by a tree infested with wood-boring beetles, and (*b*) "Hennawi" gum, exuded from the lower, main trunk of the tree, and found[1] to contain less than 50% of the customary proportion of rhamnose. There is, at present, a lack of knowledge regarding the biosynthetic mechanism of gum production, and the nature of the external injury or physiological stimulus necessary to cause the formation of gum. It was therefore decided to carry out structural studies of the "Hennawi" and beetle-borer variants of *Acacia senegal* gum, and, for comparison, of a normal specimen of "tapped" gum.

EXPERIMENTAL

Origins of specimens. — These have been described[1]. The sample of "Hennawi" gum is designated HW; the beetle-borer sample is designated BB [extraction into three

*For Part XXIV, see Ref. 1.

fractions BB(*a*), BB(*b*), and BB(*c*) has been described[1]]. These atypical specimens of gum have been compared, in this study, with specimen QN1, which is representative[1] of the best quality, tapped gum from the first collection of the 1960 gum season at Qala en Nahal, Kassala Province, Eastern Sudan.

Analytical methods. — The standard methods involved have been described previously[2]. Paper chromatography was carried out on Whatman No. 1 paper with the following solvent systems (v/v): (*a*) benzene–butyl alcohol–pyridine–water (1:5:3:3, upper layer); (*b*) ethyl acetate–pyridine–water (10:4:3); (*c*) ethyl acetate–acetic acid–formic acid–water (18:3:1:4); (*d*) butyl alcohol–ethanol–water (4:1:5, upper layer); (*e*) butyl alcohol–acetic acid–water (4:1:5, upper layer); (*f*) butanone–water–ammonia (sp. gr. 0.880) (200:17:1). R_G values of methylated sugars refer to distances moved relative to that of 2,3,4,6-tetra-*O*-methyl-D-glucose in solvent (*d*). Chromatograms were developed with aniline hydrogen oxalate, alkaline silver nitrate, or the periodate–permanganate reagent.

Gas–liquid partition chromatography (chromatograph Type S3A, fitted with flame-ionisation detectors, Gas Chromatography Ltd.) was carried out at nitrogen flow-rates of *ca.* 100 ml/min. on columns of (*i*) 15% of poly(butane-1,4-diol succinate) on Celite (120 × 0.5 cm) at 175°, (*ii*) 15% of poly(ethylene glycol adipate) on Celite (75 × 0.5 cm) at 160°. Retention times (T) are quoted relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside as standard.

Polysaccharides were methylated successively with methyl sulphate and sodium hydroxide solution, and with methyl iodide and silver oxide. Methanolyses were carried out under reflux for 7 h with 5% methanolic hydrogen chloride.

Periodate oxidations were carried out at room temperature in darkness by mixing equal volumes of 4% polysaccharide solutions and 0.25M periodate. The formic acid released was determined by titration with standard sodium hydroxide solution. Periodate consumption was determined by titration with standard arsenite solution[3].

Attempted fractionations. — Samples HW, BB(*a*), BB(*b*), BB(*c*), and QN1 each migrated as a single band on electrophoresis on cellulose acetate film in (*a*) 0.1M ammonium carbonate buffer (pH 8.9), or (*b*) 0.1M acetate buffer (pH 4.7), and all the samples had the same mobility when run under comparable conditions at 18.8 volts/cm.

Samples HW, BB(*a*), BB(*b*), BB(*c*), and QN1 were examined chromatographically on a DEAE-cellulose column (45 × 1.5 cm). On gradient elution with sodium chloride solutions (0.0 → 0.3M) in 0.02M acetate buffer (pH 4.1), each sample gave a slightly asymmetric peak.

RESULTS

The analytical data for samples HW, BB(*a*), BB(*b*), BB(*c*), and QN1 have been reported[1].

Methylation study of the samples. — Each (300 mg) of the five samples HW, BB(*a*), BB(*b*), BB(*c*), and QN1 was methylated with the classical Haworth and Purdie reagents. Yields, specific rotations, and methoxyl contents of the methylated products

are shown in Table I; methoxyl contents were not raised on further attempted methylation. On methanolysis, and examination of the mixture of methyl glycosides by g.l.c., the methylated product of each sample gave identical chromatograms, except

TABLE I

DATA FOR *O*-METHYL DERIVATIVES OF SAMPLES QN1, HW, BB(*a*), BB(*b*), AND BB(*c*)

	QN1	*HW*	*BB*(a)	*BB*(b)	*BB*(c)
Yield (mg)[a]	250	240	250	260	240
$[\alpha]_D$ (*c* 1.0, chloroform)	−46°	−42°	−45°	−46°	−46°
Methoxyl, %	41.5	42.0	42.1	41.6	41.5

[a]Weight of sample methylated = 300 mg.

that the methylated product from HW gave less 2,3,4-tri-*O*-methyl-L-rhamnose and more 2,3,4-tri-*O*-methyl-D-glucuronic acid than did the other methylated products. Table II gives the results of the chromatographic examinations; retention times on columns (*i*) and (*ii*) were comparable with those for methyl glycosides from the authentic *O*-methyl sugars. A portion of each methanolysate was hydrolysed (N sulphuric acid, 4 h) on a boiling water-bath. The cooled solutions were neutralised (barium carbonate), filtered, treated with Amberlite resin IR-120 (H^+), and concentrated. Paper chromatography of each hydrolysate [solvents (*d*) and (*f*)] revealed the presence of some 2-*O*-methylgalactose in addition to those *O*-methyl sugars already identified by g.l.c. of the methyl glycosides (Table II).

TABLE II

CHROMATOGRAPHIC EXAMINATIONS OF METHANOLYSIS PRODUCTS FROM SAMPLES QN1, HW, BB(*a*), BB(*b*), AND BB(*c*)

Relative retention times (T) *on g.l.c.*[a] *Column* (i)	*Column* (ii)	*Paper chromatography*, R_G *in solvent* (d)	O-*Methyl sugars identified*
0.48	(0.51)	1.01	2,3,4-Tri-*O*-methyl-L-rhamnose
0.58; 0.72	(0.51); 0.64	0.97	2,3,5-Tri-*O*-methyl-L-arabinose
1.00	0.85	0.79	2,3,4-Tri-*O*-methyl-L-arabinose
1.79; 3.16	1.29; (2.21)	0.82	2,5-Di-*O*-methyl-L-arabinose
1.68	1.64	0.88	2,3,4,6-Tetra-*O*-methyl-D-galactose
3.77; 4.22	3.01; 3.51	0.74	2,4,6-Tri-*O*-methyl-D-galactose
6.35	5.14	0.74	2,3,4-Tri-*O*-methyl-D-galactose
14.6; 16.4	9.7; 11.0	0.53	2,4-Di-*O*-methyl-D-galactose
2.35; 2.94	(2.21); 2.65	—	2,3,4-Tri-*O*-methyl-D-glucuronic acid[b]
7.9; 9.3	5.8; 6.9	—	2,3-Di-*O*-methyl-D-glucuronic acid[b]
		0.34	2-*O*-Methyl-D-galactose
1.18; 1.44	0.98; 1.13; 2.50	—	Unknown sugars

[a]Incompletely resolved components shown in brackets. [b]As methyl ester methyl glycoside.

Smith degradations[4] *of samples.* — Each of the samples HW, BB(*a*), BB(*b*), BB(*c*), and QN1 was subjected to the Smith degradation process as follows. The sample (2.0 g) was dissolved in water, and 0.25M sodium metaperiodate (50 ml) was added. The oxidation mixtures were left in darkness for 48 h at room temperature. The reactions were stopped by the addition of ethylene glycol (2 ml), and the solutions were dialysed against tap water for 48 h. Sodium borohydride (0.5 g) was added to the solution, and the solutions were kept for 30 h at room temperature and were then dialysed for 48 h. The resulting polyalcohol was hydrolysed at room temperature (N sulphuric acid; 48 h), followed by dialysis against tap water for 48 h to remove sulphuric acid and small carbohydrate fragments. The Smith-degraded polysaccharides were then isolated as the freeze-dried products (yields and analytical data shown in Table III), and the values of $\bar{M}_n$ were determined by molecular-sieve chromatography[2] on a previously calibrated "Bio-Gel P300" column.

TABLE III

ANALYTICAL DATA[a] FOR THE SMITH-DEGRADED POLYSACCHARIDES

	Sample				
	QN1	*HW*	*BB*(a)	*BB*(b)	*BB*(c)
Yield, %	67	63	67	66	65
Nitrogen, %	0	0	0	0	0
$[\alpha]_D$ (*c* 1.0, water)	−28.2°	−28.0°	−29.1°	−28.0°	−28.5°
M_n[b]	98,000	96,000	93,000	96,000	93,000
Uronic acid[c], %	3.7	1.6	4.4	3.8	4.3
Galactose, %	70	71	70	70	69
Arabinose, %	26	27	26	26	27
Formic acid released on periodate oxidation (moles/g) × 10^3	1.85	1.80	1.83	1.78	1.75
Periodate consumed (moles/g) × 10^3	5.53	5.45	5.31	5.29	5.42

[a]All data corrected to dry-weight basis. [b]Estimated by molecular-sieve chromatography. [c]By acidic decarboxylation.

Hydrolysis (N sulphuric acid) of the Smith-degraded product from sample HW, followed by paper chromatography of the hydrolysate, showed the presence of galactose and arabinose. Hydrolyses of the Smith-degraded products from samples BB(*a*), BB(*b*), BB(*c*), and QN1, followed by paper chromatography, showed the presence of galactose, arabinose, and a trace of an aldobiouronic acid having the mobility of 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose [R_{Gal} 0.23, solvent (*c*)]. Mild hydrolysis of the Smith-degraded polysaccharides (0.5N sulphuric acid, 1 h, on a boiling water-bath), followed by paper chromatography, showed the presence of galactose, arabinose, and two neutral disaccharides having the chromatographic mobilities of 3-*O*-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.49, solvent (*a*); R_{Gal} 0.53, solvent (*b*)] and 6-*O*-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.29, solvent (*a*); R_{Gal} 0.38, solvent (*b*)].

Methylation of the Smith-degraded polysaccharides. — Each of the Smith-

degraded polysaccharides obtained respectively from samples HW, QN1, BB(*a*), BB(*b*), and BB(*c*) was methylated successively with the Haworth and Purdie reagents. Yields, specific rotations, and methoxyl contents of the methylated products are shown in Table IV; methoxyl contents were not raised on further attempted methylation. On methanolysis and g.l.c. of the resulting mixtures of methyl glycosides, the methylated products from each of the Smith-degraded polysaccharides gave identical traces, with the exception that the methylated product from Smith-degraded sample HW gave no detectable amounts of 2,3,4-tri-*O*-methyl-D-glucuronic acid. The results of the g.l.c. analyses are shown in Table V. A portion of each methanolysate was hydrolysed (N sulphuric acid, 4 h, boiling water-bath). The cooled solutions were neutralised with barium carbonate, filtered, treated with Amberlite resin IR-120 (H^+), and concentrated. Paper-chromatographic examination of the hydrolysates [solvents (*d*) and (*f*)] showed the presence of 2-*O*-methylgalactose, in addition to those *O*-methyl sugars already identified by g.l.c. of their methyl glycosides (Table V).

TABLE IV

DATA FOR *O*-METHYL DERIVATIVES OF THE SMITH-DEGRADED POLYSACCHARIDES FROM SAMPLES QN1 HW, BB(*a*), BB(*b*), AND BB(*c*)

	QN1	*HW*	*BB*(a)	*BB*(b)	*BB*(c)
Yield (mg)[a]	220	212	220	215	225
$[\alpha]_D$ (*c* 1.0, chloroform)	−41°	−40°	−41°	−41°	−42°
Methoxyl, %	40.5	40.9	41.1	40.9	40.5

[a]Weight of sample taken = 300 mg.

TABLE V

CHROMATOGRAPHIC EXAMINATIONS OF METHANOLYSIS PRODUCTS FROM METHYLATED SMITH-DEGRADED POLYSACCHARIDES FROM SAMPLES QN1, BB(*a*), BB(*b*), AND BB(*c*)[a]

Relative retention times (T) *on g.l.c.*[b]		*Paper chromatography*, R_G *in solvent* (d)	O-*Methyl sugars identified*
Column (i)	*Column* (ii)		
0.57; 0.73	0.50; 0.64	0.97	2,3,5-Tri-*O*-methyl-L-arabinose
1.79; 3.15	1.30; (2.21)	0.82	2,5-Di-*O*-methyl-L-arabinose
1.68	1.65	0.88	2,3,4,6-Tetra-*O*-methyl-D-galactose
3.78; 4.22	2.99; 3.50	0.74	2,4,6-Tri-*O*-methyl-D-galactose
6.34	5.14	0.74	2,3,4-Tri-*O*-methyl-D-galactose
9.1	6.5; 6.9; 7.7	0.59	2,6-Di-*O*-methyl-D-galactose
14.6; 16.4	9.7; 11.0	0.53	2,4-Di-*O*-methyl-D-galactose
2.35; 2.95	(2.21); 2.66	—	2,3,4-Tri-*O*-methyl-D-glucuronic acid[c]
—	—	0.34	2-*O*-Methyl-D-galactose
1.08; 1.52	0.86; 2.48	—	Unknown sugars

[a]Identical results for sample HW, except that 2,3,4-tri-*O*-methyl-D-glucuronic acid was absent. [b]Incompletely resolved peaks shown in brackets. [c]As methyl ester methyl glycoside.

DISCUSSION

The results presented in this paper indicate that, structurally, the three fractions from the beetle-borer gum exudate are very similar to normal, tapped gum from *Acacia senegal*. Although the sample of "Hennawi" gum is basically similar as far as the interior structure is concerned, it differs in two important aspects that concern some of the peripheral end-group positions of the gum molecule.

It is now some time since theories[5-9] concerning the origin and function of plant gums were considered, and there has been little agreement on either of these points to date. It has also been debated whether gum exudates are formed at the site of a wound, or whether they are generated elsewhere in the plant and then transported to the site of exudation. It has been suggested that gum exudates may be a product of normal plant metabolism[6,7], that they may arise from a pathological condition of the tree[6], or that they may arise from some infection or invasion[7] by micro-organisms which may be fungal[6] or bacterial [7,10,11] in nature. These theories were advanced much earlier this century; and there has been no modern work to substantiate or refute them. Some of these theories are discussed in non-critical reviews that contain factual errors *e.g. A. verek* is not a variety of *A. arabica*[11,12], and gum arabic does not contain galacturonic acid[11,12]. Misprints and such errors in reviews tend to be perpetuated (*cf.* Ref. 13).

It has been suggested[5,9] that starch may undergo transformation into gum, but the enzyme systems necessary to transform a polyglucan into a highly branched polysaccharide containing galactose, arabinose, rhamnose, glucuronic acid, and 4-*O*-methylglucuronic acid appear to be impossibly complex; starch was not found[14] in the wood tissues of excised branches from *A. senegal* trees. It seems more reasonable that the gum acid has, as its precursor, some highly branched arabino-galactan of a hemi-cellulosic type, to which is added rhamnose-, glucuronic acid-, and 4-*O*-methylglucuronic acid-terminated side-chains in the final stages of gum production. The enzyme systems probably differ at different parts of the tree (*cf.* "Hennawi" gum).

Although modern commercial samples of good quality, Sudanese gum arabic originate almost entirely from *A. senegal*, contamination or adulteration with other genera[15] and with other *Acacia* species is quite possible, since the various nodules cannot be distinguished by colour, shape, or size. Commercial samples should never be used for fundamental studies. Early specimens of gum arabic were undoubtedly mixtures from various *Acacia* species[8,16], which are now known (*cf.* earlier parts of this series) to differ considerably in sugar composition and structure. The reason why no rhamnose was detected in certain samples of gum arabic[17] is now quite clear, if the term "gum arabic" was taken to apply to the exudate from any *Acacia* species[8]; some *Acacia* species contain 12–14% of rhamnose, others 6–7% of rhamnose, and some less than 1% of rhamnose[18]. In addition to this variation between species, our results now show that authentic nodules from *A. senegal* can have different amounts of rhamnose. Much of the evidence to date regarding the chemical heterogeneity of gum arabic was based on the depleted rhamnose content of certain fractions from initially hetero-

geneous, commercial samples; the point was made some time ago[7] that the classical work of Heidelberger and his colleagues should be repeated on specimens from a single *Acacia* tree or at least from one variety of *Acacia*.

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The Use of "Biogel-P" in the Gel Filtration of Polysaccharides

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LATHE and RUTHVEN[1] suggested that columns of swollen starch might provide a simple chromatographic method for determining the molecular size of proteins and polysaccharides. Gel filtration on cross-linked dextrans ("Sephadex") has, however, been much more successful, particularly for proteins,[2-5] and molecular weights of up to 225,000 can now be determined.[6] Cross-linked polyacrylamide gels have been used for gel-filtration studies on proteins.[7] These are now available commercially ("Bio-Gel P") and they offer the opportunity of studying the gel-filtration behaviour of polysaccharides on noncarbohydrate material.

Accordingly, we have examined different grades of "Biogel-P" for their possible application to the estimation of the molecular size of polysaccharides. For "Bio-Gel P 300" the empirical relationship between log $\overline{M}_n$ and elution volume[2,8] is linear for values of $\overline{M}_n$ between 5000 and 125,000; although the useful working range extends slightly beyond these values (cf. ref. 9) the exclusion limit of "Biogel-P 300" for polysaccharides appears to fall considerably below the value of 300,000 quoted commercially and found, presumably, for proteins. We have used columns measuring 2·5 × 50 cm. and 5·0 × 50 cm. at a loading of 2—10 mg. of polysaccharide. Calibration can be effected with dextran fractions having known values of $\overline{M}_n$ and, when molar sodium chloride is used as eluant, we have found such a calibration to be valid for acidic polysaccharides. Thus two gum fractions (obtained[10] from *Acacia senegal* gum), for which osmotic-pressure measurement had indicated $\overline{M}_n$ 105,000 and 37,000, respectively, gave $\overline{M}_n$ = 99,000 ± 10,000 and $\overline{M}_n$ = 35,000 ± 3,000 by gel filtration. A sample of the degraded (autohydrolysis) gum, having $\overline{M}_n$ = 4400 (periodate end-group analysis, as formaldehyde) gave $\overline{M}_n$ = 4800 ± 500 by gel filtration. The elution pattern of a de-ionised sample of the whole gum indicated that the molecular-weight distribution extended over a very wide range.

More experiments with "Bio-Gel P" materials are necessary to assess the importance of their application in fractionation and degradative studies and to establish their validity, applicability, and useful working range for molecular-weight estimations of polysaccharides.

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