ANALYTICAL   AND   STRUCTURAL   STUDIES   ON

PLANT   GUM   EXUDATES

by

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TO MY PARENTS AND MAUREEN

ABSTRACT

An analytical study of six gum specimens from the Series
Phyllodineae of the genus Acacia showed them to be chemically similar
to the other species from this series studied to date, except for
A. cyanophylla which has anomalous properties for a gum exudate of
this Series. Methylation studies showed a highly-branched 1,3 -
linked galactose framework.

An analytical study of two samples of gum from Anacardium
Occidentale showed glucose to be one of the neutral sugars present,
galactose being the major component. The glucose was isolated and
characterised. Hydrolysis, Smith degradation and methylation studies
on one of the samples revealed a highly-branched β - 1,3 - linked
galactose framework with a small amount of β - 1,6 - linkages.
Glucuronic acid was present as end-groups, linked 1,6 - to galactose.
Glucose and rhamnose were also end-group and arabinose was present
in 1,2 - linked chains, some five units long.

An analytical study of fifteen Combretum samples and two
Terminalia samples showed a wide variation in properties. The
intrinsic viscosities were all high. The samples varied in their
aldobiuronic acid content: although all the gums contained 6 - $\underline{O}$ -
(β- $\underline{\underline{D}}$ - glucopyranosyluronic acid) - $\underline{\underline{D}}$ - galactose, most of the
Combretum samples contained 2 - $\underline{O}$ - (α- $\underline{\underline{D}}$ - galactopyranosyluronic
acid) - $\underline{\underline{L}}$ - rhamnose, whereas the other Combretum samples and the
Terminalia samples contained a mannose - galacturonic acid. The
broad structural features of one example of each of the two types
were examined. The two types had widely differing structures although

both contained a β - 1,6 - linked galactose backbone. In one type, most of the uronic acid was intra-chain whereas in the other it was end-group.

Analysis of specimens from three of the 5 known subspecies of Acacia tortilis showed each subspecies to be analytically distinct. Homogeneity studies showed subsp. heteracantha to be the most heterogeneous Acacia gum exudate studied to date.

CONTENTS

SECTION I

GENERAL INTRODUCTION

Plant gums are complex acidic polysaccharides exuded from the stems of certain tropical and sub-tropical trees found in Africa, Australia, India, South America and parts of Asia, after mechanical injury or bacterial infection[1]. The precise mechanism of gum formation is still a matter of conjecture. The viscous mass hardens in the sun to form nodules, which normally dissolve in water to form neutral or slightly acidic solutions since the carbohydrate gum acid in the natural state is wholly or partly neutralised (salt-formation) by calcium, magnesium, sodium and potassium; iron, copper, strontium, zinc and manganese are also present in smaller amounts, as shown by a recent atomic absorption study[2].

Some gum species give highly viscous solutions, and this property makes them very useful commercially[3]. The Egyptians used gums as paint thickeners and for embalming purposes, and for many centuries natives of the countries where they are found have considered plant gums to be of therapeutic value. Nowadays they are extensively used commercially as thickening agents, emulsifiers, and adhesives, particularly in the dairy, confectionery, and pharmaceutical industries.

The molecular weights of plant gums are normally within the range 2×10^5 to 2×10^6, although the work on <u>Combretum</u> species reported in this thesis (see Section VA) shows that some gums have molecular weights of over 8×10^6.

All the plant gum polysaccharides studied so far are complex and highly branched, containing more than one type of monosaccharide

unit. The neutral sugars present most frequently are $\underline{\underline{D}}$ - galactose, $\underline{\underline{L}}$ - arabinose, and $\underline{\underline{L}}$ - rhamnose, with $\underline{\underline{D}}$ - xylose and $\underline{\underline{D}}$ - mannose found in certain species. This thesis contains the first report of the occurrence of $\underline{\underline{D}}$ - glucose in a gum polysaccharide. The acidity of plant gums most frequently arises from the presence of $\underline{\underline{D}}$ - glucuronic acid and its 4 - $\underline{\underline{O}}$ - methyl derivative, but certain gums also contain $\underline{\underline{D}}$ - galacturonic acid. The uronic acid content varies from gum to gum, and geographical and seasonal variations occur for each species; the uronic acid content may well be analytically different in different nodules of gum obtained from one tree[4]. Acacia pycnantha[5, 6] has the lowest uronic acid content (3.3%) found so far in any genus, and typical values for the uronic acid content of gums fall in the range of 10-15%, but some Combretum species have been found in the present work to contain over 30% uronic acid.

Gums also contain proteinaceous material. In many species the percentage is small (1-2%), but Neem gum (Azadirachta indica)[7] has been found to contain 30-40% protein and an amino sugar; the evidence available to date seems to imply that there is chemical bonding between protein and polysaccharide.

Gum exudates are found in hot tropical climates and the fact that gum formation is maximal in very hot dry weather after heavy rainfall tends to suggest that their formation is to prevent excessive loss of moisture through an injured part of the tree stem[8]. The high viscosity of gum solutions makes their translocation within plants appear to be less likely than their synthesis near

the site of injury.

Recently a great deal of analytical work has been done to obtain data[9-11] to support chemical plant taxonomy[12], especially in the genus Acacia, the most studied of all gum-bearing genera and the most important commercially. Bentham has classified the genus Acacia into six Series[13]. Most chemical structural studies have centred round his Series 4 and 5, but Section III of this thesis consists of a detailed analytical study of six previously unstudied Acacias from Bentham's Series 1 (Phyllodineae) together with an examination of the methylated products of some of these to obtain a certain amount of information concerning their structural features.

Section IV of this thesis reports work on the gum from Anacardium occidentale; this has been found to contain glucose, which has not been found previously in a plant gum. Sub-section A gives analytical data for two specimens of this gum, and sub-section B gives structural details of one of the specimens with special reference to the location of the glucose in the polysaccharide molecule.

Very little work has been done previously on the family Combretaceae. Section VA of this thesis contains a detailed analytical study of the gums from fourteen Combretum species, and two botanically related Terminalia species. These all differ widely analytically. Section VB reports the structural features of two of these Combretum samples, which structurally appear to differ widely from other genera.

The final section of the thesis reports the analytical differences shown by gum specimens from Acacia tortilis and its closely related but readily distinguishable subspecies which were, until recently, classified botanically as different, distinct species. During this study it was found unexpectedly that these specimens gave the clearest evidence obtained to date for the occurrence of heterogeneity in Acacia gum exudates.

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SECTION II

EXPERIMENTAL METHODS

II.1. GENERAL METHODS

Weighings. All accurate weighings were made within the range of the graticule scale (range, 0-100 mg) of a Stanton Unimatic Model C.L.I. single-pan balance, having an accuracy of ± 0.1 mg.

Dialyses of polysaccharides, to remove low molecular weight material, were carried out in cellophane tubing (Kalle Aktiengesellschaft, Wiesbaden) against running tap-water for 48-72 hours unless otherwise stated.

Electrodialyses of polysaccharides were carried out in a three-compartment perspex cell fitted with cellophane membranes. The water in the outer electrode compartments was changed regularly to prevent overheating. Electrodialysis was continued until a current (applied voltage = 300V) ceased to flow.

Reductions in volume were carried out on a rotary evaporator at temperatures below $40^{\circ}C$.

Moisture contents were determined by heating to constant weight at $105^{\circ}C$.

Ash contents were determined by heating to constant weight in a muffle furnace at $550^{\circ}C$.

Nitrogen contents were determined by a semi-micro Kjeldahl method.

Carbon, hydrogen and nitrogen contents were determined using a Perkin-Elmer 240 Elemental Analyser.

Methoxyl contents were determined by a vapour-phase infrared method[1,2]; a calibration curve was based on known weights of methyl iodide.

Acetyl contents were determined by alkaline hydrolysis
followed by distillation of the acetic acid liberated and titration
with 0.01N - sodium hydroxide[3].

Equivalent weight determinations on exhaustively electro-
dialysed polysaccharides (test: % ash $\neq$ 0.1%) were carried out
by direct titration with standard sodium hydroxide solution
(ca. 0.01N).

Uronic acid contents were calculated from the equivalent
weights as (17600/E.W.) ie:- Values for uronic acid are expressed
as the anhydride.

Quantitative estimate of sugars. Sugars were separated by
chromatography on previously washed Whatman No. 3MM papers. After
elution from the paper, sugars were estimated colorimetrically by
the phenol-sulphuric acid method[4]. The optical density was read cn
a Unicam SP 1300 spectrophotometer using filter 2. Calibration
curves were drawn for known weights of sugars.

Melting points were observed on a Kofler hot stage
microscope.

II.2. PHYSICAL METHODS

Specific rotations of aqueous, chloroform, and urea[5] solutions
were measured using the sodium D-line with a Perkin-Elmer Model 141
polarimeter at $20 \pm 2^{\circ}C$.

Viscosity determinations were carried out in M-sodium chloride
solution in an Ubbelohde suspended-level dilution viscometer at
$25.0 \pm 0.1^{\circ}C$. Solutions were filtered carefully before additions
were made to the viscometer. Flow times were measured to within

0.1 sec by means of a stop watch. The isoionic dilution technique was used; a solution of the gum (6 ml, 2-4%) was placed in the viscometer and the flow time measured. Flow times were also obtained for successive dilutions with M-sodium chloride solutions (four additions of 2 ml each). Since preliminary experiments had indicated that any loss of gum from M-sodium chloride solution on filtering was negligible, concentration values were estimated from the dry weight of gum dissolved in a known volume.

Assuming the densities of M-sodium chloride and gum solutions to be equal for low concentrations of gum, the viscosity number, $[\eta]$ is given by

$$[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c} = \lim_{c \to 0} \frac{t-t_0}{ct_0}$$

where c is the concentration of gum (gm/cm^3) and t_0 and t are the flow times (sec) for solvent and solution respectively. Extrapolation of the linear plot of $\frac{t-t_0}{ct_0}$ against c to c=0 gives $[\eta]$.

Light-scattering measurements were carried out at 27.0 ± 0.5°C using a SOFICA photogoniodiffusometer Model 4200. Unpolarised green light (546 nm) was selected from a mercury lamp spectrum by use of a Wratten Kodak N61 filter.

Using the limiting viscosity number as a guideline to the desirable concentration and using N-sodium chloride solution as solvent, gum solutions were accurately prepared (0.1-0.3 g in 20 ml). Dilutions of this solution were made; the molecular weight was calculated as an average of three of these solutions. Solutions

were clarified and made dust-free by passage through filters of average pore size 0.45 μm (Millipore Ltd., Bedford, Mass., U.S.A.) using a stainless-steel filter holder attached to a 20 ml syringe. Concentrations of gum solutions were assumed to be unaltered by ultrafiltration[6].

For each concentration, the intensity of scattered light at various angles between 30° and 150° was recorded, and corrected scale readings, I_{θ}, for angle θ were calculated[7] from the equation

$$I_{\theta} = \frac{(I \text{ soln.} - I \text{ sol.}) \sin \theta}{1 + \cos^2 \theta},$$

where I soln. and I sol. are the scale readings for polymer solution and solvent respectively. The reciprocal corrected scale reading, $1/I_{\theta}$, is plotted against $\sin^2 \theta/2$. Extrapolation of the linear portion of this graph to $\theta = 0$ gives a value for $[\,1/I_{\theta}\,]_{\theta = 0}$. The downward curvature of these graphs at low angles is thought to be caused by scatter from dust particles suspended in solution[8]. Molecular weights are found from the equation

$$M = \frac{R}{\frac{2\pi^2 n_0^2}{\lambda^4 N} \cdot [dn/dc]^2 \cdot I_B \cdot c \cdot [1/I_{\theta}]_{\theta=0}},$$

where n_0 = refractive index of solvent (1.340)

   n = refractive index of solution

   N = Avogadro's Number (6.023×10^{23})

   λ = wavelength of incident light (546 nm = 5.46×10^{-5} cm)

   R = Rayleigh constant of standard benzene (16.3×10^{-6} for
      = 546 nm)

   C = concentration in gm/ml

I_B = intensity diffused, selected for standard benzene (0.5)

dn/dc = refractive index increment.

Using the dn/dc value of 0.146, which is the average value found[9]

for a series of <u>Acacia</u> gums, then the equation is simplified to

$$M = \frac{2.309 \times 10^2}{C \cdot [1/I_\theta]_{\theta=0}}$$

<u>Infrared spectroscopy</u> was carried out using a Hilger-Watts
H.1200 double-beam grating spectrophotometer.

<u>Ultracentrifugation</u> was carried out using a Beckman-Spinco
Model E Analytical Ultracentrifuge. Polysaccharide solutions
(o.5% in 0.5M-sodium chloride solution) were examined at 44,000
r.p.m. After the ultracentrifuge had attained this speed, the
boundary patterns, obtained by Schlieren optical system, were
photographed at 16, 8 or 4 min intervals.

II.3. CHEMICAL METHODS

<u>Small-scale polysaccharide hydrolyses</u> were carried out with
N-sulphuric acid for 7.5 hours on a boiling water bath, unless
otherwise stated. Preliminary tests showed that such conditions
do not cause hydrolysis of uronosyl linkages in the materials
studied in this investigation: this was taken into account when
determining sugar ratios. Hydrolysates were neutralised with
barium carbonate, filtered, deionised with Amberlite IR-120(H)
resin, and concentrated on a rotary evaporator.

<u>Small-scale polysaccharide methylations.</u>

(a) The Haworth[10] method - Methylations were carried out in an
atmosphere of nitrogen at room temperature. Dimethyl sulphate

(2 ml) and sodium hydroxide $[(2\ ml),\ 30\%\ (w/v)]$ 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 added
to the reaction mixture, and six further additions of dimethyl
sulphate (12 ml) and sodium hydroxide (17 ml) were made, allowing
3 hours for each addition. After stirring for 12 hours, the
reaction mixture was heated at $60^{\circ}C$ for 30 min, with nitrogen
bubbling vigorously through the solution. After cooling, the
reaction mixture was neutralised with 4N-sulphuric acid and made
slightly acid (pH 4.0); a white precipitate was normally observed
at this stage. The methylated product was extracted into
chloroform (4 x 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 and
concentrated on a rotary evaporator. The concentrated syrup was
poured into light petroleum (bp $60^{\circ}-80^{\circ}$, ca 400 ml) with stirring;
the precipitated methylated polysaccharide was isolated, after
filtration and drying, as a white amorphous powder.

(b) The Purdie and Irvine method - The partially methylated
polysaccharide (100-400 mg) was dissolved in methanol (5 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 the dark in a dry flask fitted with a water condenser and a
calcium chloride tube. The mixture was cooled and filtered through
sintered glass and the residue extracted six times with hot
chloroform (ca 50 ml). The combined filtrate and extracts were

reduced in volume and any dissolved silver ions removed by passing hydrogen sulphide through the solution and refiltering. After concentration to a small volume, the syrup was poured into light petroleum (bp 60°-80°, ca 400 ml) with stirring. After filtration and drying, the precipitated, methylated polysaccharide was isolated as a white amorphous powder.

Small-scale oligosaccharide methylations[12, 13]. 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 concentrated to a syrup on a rotary evaporator.

Methanolyses were carried out under reflux for 6 hours with methanolic 5% hydrogen chloride. Solutions were cooled, neutralised with silver carbonate and filtered. The residue was washed with methanol and any dissolved silver ions were removed by passing hydrogen sulphide through the solution. After refiltration, the solution was taken to dryness on a rotary evaporator, taken up in chloroform and concentrated to small volume.

Periodate oxidations of polysaccharides were carried out in the dark at room temperature.

(a) Consumption of periodate. - The amount of periodate consumed by a polysaccharide was estimated by back-titration of excess periodate. Excess potassium iodide was added to a portion (1 ml) of the periodate solution, and the iodine liberated was titrated, after the addition of sodium bicarbonate (ca 200 mg), with

standard sodium arsenite solution (ca 0.05N) using Thyodene as indicator[14].

(b) The formic acid released was estimated titrimetrically[15] with standard sodium hydroxide (ca 0.1N) for portions (1 ml) of the solution. Methyl red was used as indicator.

Molecular weight of polysaccharides by end-group analysis was obtained by periodate oxidation of the polysaccharide followed by colorimetric estimation of the formaldehyde released using chromotropic acid[16]. The polysaccharide (30-50 mg) was dissolved in p-hydroxybenzaldehyde solution [10 ml, 0.1% (w/v)]. This solvent prevents recombination of formaldehyde with oxypolysaccharide[17]. To the solution was added a portion (1 ml) of sodium metaperiodate solution such that a slight excess of sodium metaperiodate was present. At suitable time intervals, samples (1 ml) were transferred to centrifuge tubes, treated with 0.5M-sodium sulphite solution (1 ml) to destroy excess periodate, and with ethanol (4 ml) to precipitate the oxypolysaccharide. The tubes were stored for 2 days at 2°C then centrifuged. Portions (1 ml) of the supernatant were treated with 9 ml chromotropic acid reagent [2 g of the sodium salt of chromotropic acid (B.D.H., "for formaldehyde determinations") dissolved in a solution of Analar sulphuric acid (566 ml) and water (320 ml)[18]] on a boiling water bath for 30 min. After cooling, thiourea solution [2 ml 4.6% (w/v)] was added and the optical density measured on a Unicam SP 1300 spectrophotometer using filter 4. A calibration curve for formaldehyde was constructed by periodate oxidation of solutions of Analar glucose, 0.5M with respect to sodium bicarbonate.

Assuming production of one molecule of formaldehyde per average polymer unit, a value for the number - average molecular weight, $\bar{M}_n$, of the polysaccharide may be calculated.

II.4.   CHROMATOGRAPHIC AND ELECTROPHORETIC SEPARATIONS

Ion-exchange chromatography on diethylaminoethylcellulose[19].
DEAE - cellulose powder (Whatman DE 32, microgranular form, 10 g) was treated with 0.5N-hydrochloric acid (250 ml) for 30 min. After filtration and washing until the effluent pH was ca.4, the exchanger was treated with 0.5N-sodium hydroxide solution (250 ml) for 30 min. After further filtration and washing until the effluent was neutral, the exchanger was equilibrated with 0.02M acetate buffer (pH 4.1). Columns (45 x 1.3 cm) were packed by continuous addition of a slurry of the exchanger (the column outlet being opened after the first 2 cm of exchanger had been packed) and allowed to settle. Samples (5-10 mg) of polysaccharides in buffer (1 ml) were washed into the columns with excess of buffer; elution of the acidic polysaccharides was performed by application of a sodium chloride concentration gradient (0 - 0.5M) in 0.02M-acetate buffer (pH 4.1) with total elution volume of 250 ml. Fractions (2.1 ml), collected using an automatic collector, were screened by the phenol-sulphuric acid method[4]. The optical density of each fraction was read on a Unicam SP 1300 spectrophotometer using filter 2.

Molecular-sieve chromatography (M-SC)[20,21,22] was carried out on columns of:-

Bio-Gel A-5m, Bio-Gel A-15m, Bio-Gel A-150m (Bio-Rad Laboratories,

Richmond, California)

Sephadex G-25 (Pharmacia Ltd., Uppsala, Sweden).

M-sodium chloride solution, containing thymol (0.0005%) as a bacteriostatic agent, was used as eluant. To prevent deformations by "wall-effects", columns were pretreated with dichlorodimethyl-silane (1% in benzene) at $60^{o}C$ and oven-dried.

Gel bed materials were fully swollen by gentle stirring in M-sodium chloride solution for one day. A thin layer of glass beads or fine sand was used to support the gel, and the gel slurry was added continuously to the column from a large filter funnel; the gel slurry in the funnel was stirred while excess eluant was allowed to percolate through the growing gel bed by regulating the flow of liquid from the tap at the bottom of the column. The top surfaces of columns of gel were stabilised by a thin layer of fine sand. Eluant was allowed to flow through the columns for 2 days before use.

For the Bio-Gel columns - polysaccharides (5-10 mgs) in M-sodium chloride (1 ml) was applied to the column by careful layering on top of the bed material. Elution diagrams were obtained by

(a)   fractions (2.1ml) collected by an automatic collector and screened by the phenol-sulphuric acid method[4]. The optical density was read on a Unicam SP 1300 spectrophotometer using filter 2. Collection of fractions was begun as soon as the polysaccharide solution had been applied to the top of the column.

(b) automatic recording of dyed polysaccharides[23,24] with a Unicam SP 1300 using filter 3 connected to a recorder.

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

(a) ethyl acetate, pyridine, water (10:4:3)

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

(c) ethyl acetate, acetic acid, formic acid, water (18:3:1:4)

(d) ethyl acetate, acetic acid, formic acid, water (18:8:3:9)

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

(f) butan -2- one, water, ammonia (d. 0.88) (200:17:1)

(g) ethanol, hydrochloric acid (0.1N), butan -1- ol (10:5:1)[25]

Before using solvent (g), papers were dipped in 0.3M-sodium dihydrogen ortho phosphate solution and air-dried.

The following spray reagents were used:-

(1) Aniline oxalate. Reducing sugars were detected by spraying chromatograms with a saturated solution of aniline oxalate in ethanol, water [1:1 (v/v)], then heating at $150^{\circ}C$ for ca 3 min.

(2) Silver nitrate. Reducing sugars and sugar alcohols were detected by dipping chromatograms in a saturated solution of silver nitrate in acetone, prepared by adding saturated aqueous silver nitrate (1 ml) to acetone (100 ml), and sufficient water to redissolve the precipitate which formed. After drying, chromatograms were sprayed with aqueous ethanolic N-sodium hydroxide [water:ethanol, 1:9 (v/v)]. Chromatograms were preserved by treatment with 10% (w/v) sodium thiosulphate solution and washing with water.

(3) Periodate-Permanganate. Reducing sugars and sugar alcohols were detected by spraying chromatograms with a mixture of 4 parts of 2% (w/v) sodium metaperiodate solution to 1 part (by volume) of 1% (w/v) potassium permanganate in 2% (w/v) sodium carbonate solution. After 10 mins, excess permanganate was removed by washing with water.

(4) Glucose was detected by an enzymic method. Chromatograms were sprayed in succession with (a) 0.05% peroxidase in 0.5M-phosphate buffer pH 7.0, (b) 0.05% glucose oxidase in 0.5M-phosphate buffer pH 7.0 and (c) 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 greeny 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 D-galactose. 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 (g.l.c.) of mixtures of O-methyl sugars[26,27] was carried out using a Pye Argon Chromatograph, at argon flow rates of ca 100 ml/min on columns (120 x 0.5 cm) of:-

(1) 15% by weight of ethylene glycol adipate polyester on 45-60 mesh Gas-Chrom Z at 176°C.

(2) 15% by weight of butan -1,4- diol succinate polyester on 80-100 mesh Gas-Chrom P at 176°C.

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

Zone electrophoresis of polysaccharides was carried out on strips (18 x 5 cm) of cellulose acetate film (Schleicher and Schüll, Dassel), using a Shandon Universal Electrophoresis Apparatus, Mark II, fed by a Vokam Power Unit, Model 2541, capable of providing constant voltage or constant current. 0.1M-ammonium carbonate buffer (pH 8.9) or 0.1M-acetate buffer (pH 4.7) was used as electrolyte. Electrophoresis was carried out at a field strength of 17.2 volts/cm for 2 hours. Polysaccharide bands were located by a modification of the periodate-rosaniline hydrochloride method[28]:-

(1) strips were immersed in ethanol for 5 mins

(2) strips were immersed in a solution of sodium metaperiodate [2% in water-ethanol, 1.5:10.0 (v/v)] for 10 mins

(3) strips were immersed in a solution of reduced rosaniline hydrochloride (2%)[28], until staining was complete (ca 15 mins). Iodine, from reduced periodate, normally appeared on the strips at this stage but dissolved in the rosaniline hydrochloride solution

(4) strips were washed four times in a solution of potassium metabisulphite (1 g) in N-hydrochloric acid (100 ml).

(5) strips were washed in ethanol and dried between sheets of glass.

Polysaccharides were located as a dark mauve band on a pale pink background.

Thin-layer electrophoresis of polysaccharides was carried out on "Phoroslides" (Millipore Ltd.) using 0.05M-ammonium carbonate buffer (pH 8.9) and 0.05M-borate buffer (pH 9.2) as electrolyte. The following procedure was adopted:-

(1) slides (7.5 x 2.5 cm) were immersed in buffer, blotted to remove excess moisture and placed in a "Phoroslide" cell connected to a Vokam Power Unit.

(2) polysaccharide solutions (1% in buffer) were applied from a micropipette, as thin bands 2 cm from the cathode end of the strip.

(3) electrophoresis was carried out, at field strengths of 26 volts/cm along the strip, for 10 mins.

(4) polysaccharide bands were located by the periodate rosaniline hydrochloride technique[28] described for cellulose acetate electrophoresis; After electrophoresis of dyed polysaccharides, strips were dried in a current of air.

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SECTION III

A STUDY OF ACACIA GUM EXUDATES OF THE SERIES PHYLLODINEAE

III.1. INTRODUCTION

Although Bentham[1] placed 277 Australian species in his Series 1 (Phyllodineae) of the genus _Acacia_, Tindale[2] now believes the correct number to be at least 570. Apart from some studies of the distribution of amino acids in the seeds[3] and of the flavonoid content[4] of the heartwoods and barks, relatively few species of this Series have been studied in any respect so far. There have been studies of the gum exudates from only six of the Phyllodineae, namely _A. cyanophylla_[5,6], _A. harpophylla_[7], _A. microbotrya_[7], _A. penninervis_[7], _A. podalyriifolia_[6,8], and _A. pycnantha_[9-11], but of these only _A. cyanophylla_, _A. podalyriifolia_ and _A. pycnantha_ have been studied extensively. All of these were placed by Bentham in Series 1, sub-series 6F (Uninerves Racemosae) with the exception of _A. harpophylla_ which has been placed in sub-series 7F (Plurinerves Nervosae).

A detailed analytical study has now been carried out on the gum exudates from a further six species of the Phyllodineae together with methylation data for four of them to provide details of structural features. The gums studied were from _A. difformis_, _A. falcata_, _A. mabellae_, _A. retinodes_ and _A. rubida_ (which are placed in Bentham's Series 1 sub-series 6F) and _A. calamifolia_ which is placed in Series 1 sub-series 4C (Calamiformes Uninerves).

III.2. ORIGIN OF GUM SPECIMENS

Gum from _Acacia calamifolia_ Sweet ex Lindl. (Bentham No.57) was collected by Mr. R.D. Croll from a bush 10 ft. high, 10 miles west of Rankin's Springs, New South Wales on 24 January 1970. Gum from

A. difformis R.T. Bak. was collected by Mr Croll from a bush infested with beetle-borers at Rankin's Springs, New South Wales on 23 January 1970. Reference vouchers for both these species have been verified and are lodged in the Herbarium, Royal Botanic Gardens, Kew. Gum samples from A. falcata Willd. (Bentham No 123) were obtained on 15 September 1970 and 25 June 1971, from pruning wounds on a large specimen of this species growing in the tropical house, Royal Botanic Gardens, Edinburgh. Gum from A. mabellae Maiden was collected by Mr. J. Pickard at The Vines, 8 miles SSW of Sassafras, New South Wales, on 13 June 1970 (Voucher No NSW 105156). The gum exudate from A. retinodes Schlechtd. (Bentham No 126) was collected by Dr. P. Moyna at Montevideo, Uruguay in May 1970. Gum from A. rubida A. Cunn. was collected by Mr. A. Rodd at Burbong, New South Wales, on 25 April 1968 (No NSW 99697).

III.3. EXTRACTION AND PURIFICATION OF GUM SAMPLES

Crude gum (3-4 g) were dissolved in distilled water (2% solution) over 2 days. In the case of A. calamifolia, A. difformis, A. falcata and A. retinodes, almost all of the gum dissolved but with A. mabellae and A. rubida a great deal of gum remained undissolved and required mild treatment with sodium borohydride for 2 days to facilitate almost-complete dissolution. The solutions were filtered through No 42 and No 1 filter papers, dialysed against running tap water for 2 days (4 days in the case of samples treated with borohydride) refiltered and freeze dried.

III.4.   ANALYTICAL COMPARISON

Analytical data for the six samples are shown in Table III.A.

Hydrolysis with N-sulphuric acid followed by chromatographic
examination of the hydrolysates showed the presence of large amounts
of galactose, some arabinose, and trace amounts of rhamnose together
with the aldobiuronic acid 6-$\underline{O}$-(β-$\underline{D}$-glucopyranosyl uronic acid)-$\underline{D}$-
galactose [R_{gal} 0.27 in solvent (c), 0.61 in solvent (d)] . This is
consistent with results found for A. cyanophylla[5] and A. pycnantha[9].
Also detectable were small amounts of what appeared to be, from
chromatographic evidence, 4-$\underline{O}$-methyl glucuronic acid [R_{gal} 1.76 in
solvent (c); orange/pink spot] . The presence of this acidic sugar
is unusual in a 1N-hydrolysate; 2N-hydrolysis with sulphuric acid
is normally required to break uronosyl linkages.

TABLE III,A.

ANALYTICAL DATA FOR PURIFIED GUM POLYSACCHARIDES
FROM ACACIA SPECIES OF THE PHYLLODINEAE SERIES

	Acacia calamifolia	Acacia difformis	Acacia falcata	Acacia mabellae	Acacia retinodes	Acacia rubida
Recovery from crude gum	82	93	92	70	81	88
Moisture, %	12.9	10.2	9.8	11.5	11.4	12.6
Ash, %[a]	2.0	1.5	1.8	1.7	2.1	2.0
Nitrogen, %[a]	0.26	0.28	0.21	0.23	0.48	0.50
Hence protein, % (N x 6.25)[a]	1.6	1.8	1.3	1.4	3.0	3.1
Methoxyl, %[b]	0.87	0.64	0.49	0.41	0.41	0.25
$[\alpha]_D$ in water, (degrees)[b]	+4	-5	+9	+4	+1	-25
$[\alpha]_D$ in 7M-urea, (degrees)[b]	+8	-6	+9	+6	+3	-18
Intrinsic viscosity, $[\eta]$, ml/gm[a]	5.8	6.2	5.1	5.8	9.5	9.7
Molecular weight, (MW x 10^4)[a]	24	4.7	7.9	12	73	32
Equivalent weight[b]	2430	3420	2290	2870	1770	3010
Hence uronic anhydride, (%)[b+c]	7	5	8	6	10	6
Formic acid realeased, mM/gm	n.d.	3.11	2.98	2.83	4.06	2.91
Periodate consumed, mM/gm	n.d.	9.9	9.9	9.8	12.0	8.7
Periodate consumed / Formic acid released	n.d.	3.18	3.32	3.46	2.96	2.99
Sugar composition[b] after hydrolysis						
4-O- Methylglucuronic acid[d]	5	3.5	3	2.5	2.5	1.5
Glucuronic acid	2	1.5	5	3.5	7.5	4.5
Galactose	84	75	85	76	76	63
Arabinose	8	19	7	17	12	30
Rhamnose	1	1	trace	1	2	1

a Corrected for moisture content.　　　b Corrected for moisture and protein content.

c If all acidity arises from uronic acids.　　d If all methoxyl groups located in this acid.

n.d. - not determined

Although there are a few divergences such as the relatively
high negative rotation of A. rubida (-25°), the relatively high
molecular weight (730,000) and uronic anhydride (10%) of
A. retinodes, the nitrogen content and intrinsic viscosity of
A. retinodes and A. rubida, and the galactose-arabinose ratio (2:1)
of A. rubida, the gum exudates from these six species are analytic-
ally very similar, particularly when it is appreciated that the
specimens studied were collected in such widely differing geographical
locations as Australia, Scotland and Uruguay. There is evidence
from the literature[12] that the exudates from Acacia species may vary
with geography and to obtain further evidence on this point gum
specimens from two other species of the Phyllodineae, A. cyanophylla
and A. pycnantha have been examined. The composition and properties
of an African specimen (supplied by the Botanical Research Institute,
Pretoria) and of a Western Australian sample (supplied by the
Curator, Perth Botanic Gardens) of A. cyanophylla gum did not differ
significantly from the values published for a South African sample.
A similar conclusion was reached when analytical data for two
specimens of A. pycnantha gum from New South Wales (supplied by
Mr. R.D. Croll) were compared with the values given in the
literature[10] for a South Australian sample.

There is virtually a complete correlation between taxonomy
and the analytical data for the exudates from A. calamifolia,
A. difformis, A. falcata, and A. mabellae. These species form a
distinct group in terms of their exudate composition. The galactose-
arabinose ratio in A. calamifolia and A. falcata is greater than in

any Acacia gum exudate studied so far[13]. A. podalyriifolia[6,8,14]
($[\alpha]_D$ = +5.3°, equiv. wt. = 3585, Uronic Anhydride 4.6%,
galactose-arabinose-rhamnose 83:16:1, M.W 74,000) would also fit
into this group, as would A. retinodes neglecting the slightly
higher uronic acid and the much higher molecular weight, which
may be due to the strong yellow-red colour of the gum.

The only previous reference to A. difformis was published in
1897 by Baker[15], who commented that it was one of the few Australian
Acacias to exude a soluble form of gum. The brief chemical details
quoted referred only to the solubility of the gum, the dark colour
of its solutions (exceptionally dark solutions were not found in
this study), a negative optical rotation (no value specified), and
a low ash content. Baker concluded[15] that A. difformis gum would be
of commercial value if obtainable in quantity but he classified it
with "the second-class wattle gums of which that from A. pycnantha
is a type". The results of the present study do not substantiate
the possible commercial use of the gum as its viscosity of 6 ml/g
compares most unfavourably with that of a good commercial Acacia
grade, viscosity 15 ml/g.

Baker[15] stated that Australian Acacias often give optically
inactive gum exudates. This is in error and possibly arose from
his inability to detect the low positive or negative rotations of
some of the Phyllodineae due to lack of a sensitive polarimeter.
Although the majority of Australian Acacia species studied
recently[13] have specific rotations that fall in the range -10° to
+10°, A. cyanophylla (-35°) and A. rubida (-25°) in the
Phyllodineae and several species of the Botryocephalae Series have

strongly negative rotations, eg:- A. deanei (-66°),
A. parramattensis (-49°), A. parvipinnula (-54°) and
A. trachyphloia (-57°).

From a chemotaxonomic[2] point of view, it is becoming
increasingly obvious that the most anomalous Acacia species studied
so far are A. pycnantha and A. cyanophylla. No two species differ
so widely in terms of their exudates, yet Bentham placed them next
to each other in his Series and Tindale has stated "A. pycnantha
appears to be quite closely related morphologically to
A. cyanophylla, although a noteworthy difference is that the
flowerheads of the former have 50-100 flowers in each capitulum,
whereas in the latter there are about 40". It is therefore
surprising that the gum exudates of these species should differ
so markedly. According to Hirst and Perlin[9], A. pycnantha has
$[\alpha]_D$ -8° and glucuronic acid - galactose - arabinose - rhamnose
= 1-5:65:27:1-2; for A. cyanophylla[8] the corresponding sugar ratios
are 24:46:8:21 and $[\alpha]_D$ = -20°. At the present time, the gum
from A. cyanophylla has the highest content of glucuronic acid
and rhamnose recorded for the Acacia genus; the present study has
verified that the values quoted in the literature[16] are
substantially correct, because of the importance of the issue and
the possibility of an earlier analytical error.

The six gum exudates studied resemble A. podalyriifolia and
A. pycnantha. The latter still remains the least acidic of all
Acacia species studied to date[13], and A. cyanophylla must be
regarded as atypical of this series of Acacias.

Recently it was suggested[17] that comparisons of the specific rotations of polysaccharides in water and 7M urea solution provide a new parameter for the conformational analysis of polysaccharide chains. The optical rotation in water is considered to be the resultant of the contributions from the primary and tertiary structures; the latter exists in aqueous solution but not in concentrated urea or guanidine solutions. The values obtained for the present species are shown in Table III, A. Of these, only A. rubida, which contains the highest proportion of arabinose, appears to have significant contributions from tertiary structure to its specific rotation in aqueous solution.

III.5. PARTIAL ACID HYDROLYSIS

The polysaccharides (60-100 mg) were hydrolysed with 0.5N sulphuric acid (10 ml) for 1 hour on a boiling water bath. The cooled solutions were neutralised with barium carbonate, filtered, deionised with Amberlite IR - 120(H) resin, reduced to a syrup and chromatograms run in solvent (b). Galactose, arabinose and trace amounts of rhamnose were found together with $3 - O - \beta - D -$ galactopyranosyl - D - galactose (R_{gal} 0.49) and small amounts of $6 - O - \beta - D -$ galactopyranosyl - D - galactose (R_{gal} 0.27)[10,16].

III.6. METHYLATION OF SAMPLES

Four of the samples were methylated successively by the Haworth and Purdie methods. Yields, specific rotations and methoxyl contents of the methylated products are shown in Table III,B.

TABLE III,B.

METHYLATION DATA FOR FOUR ACACIA SAMPLES OF THE SERIES PHYLLODINEAE

	A. difformis	A. mabellae	A. retinodes	A. rubida
Amount of poly-saccharide used (mg)	296	299	232	304
Amount of methy-lated product (mg)	275	272	208	243
$[\alpha]_D$ of product (in chloroform)	-50.6°	-36.0°	-46.7°	-44.6°
% OMe	39.2	39.8	39.5	40.0

Portions (ca 50 mg) of the methylated polysaccharides were methanolysed and the mixtures of O-methyl sugars examined by g.l.c. The methanolysates were then hydrolysed with N-sulphuric acid for 4 hours on a boiling water bath. The cooled solutions were neutralised with barium carbonate, filtered, deionised with Amberlite IR - 120(H) resin, concentrated and chromatograms run in solvents (e) and (f).

The methyl glycosides detected are shown in Table III,C. with their retention times (T), and R_g values. The relative amounts of each component are shown in Table III,D. together with the O-methyl sugars found in A. cyanophylla, A. podalyriifolia and A. pycnantha by Kaplan and Stephen[6] for comparison.

The methylated polysaccharides from the four samples show

O – METHYL SUGARS IDENTIFIED IN METHYLATED ACACIA GUMS OF THE SERIES PHYLLODINEAE

Relative retention time (T) of methyl glycosides*		R_g after hydrolysis		O – methyl sugar
column (1)	column (2)	solvent (e)	solvent (f)	
0.49	0.48	1.02	1.03	2,3,4 – tri – O – methyl – L – rhamnose
0.54,0.69	0.58,0.73	0.95	1.03	2,3,5 – tri – O – methyl – L – arabinose
0.85	1.03	0.79	0.78	2,3,4 – tri – O – methyl – L – arabinose
1.86	1.56	0.79	0.78	2,3 – di – O – methyl – L – arabinose
1.64	1.72	0.85	0.84	2,3,4,6 – tetra – O – methyl – D – galactose
2.71,3.33,(3.91)	(2.96),(3.91),(4.26)	0.71	0.48	2,3,6 – tri – O – methyl – D – galactose
3.65,3.91	3.91,4.26	0.71	0.43	2,4,6 – tri – O – methyl – D – galactose
6.42	6.58	0.71	0.36	2,3,4 – tri – O – methyl – D – galactose
9.24	9.28	0.47	0.20	2,6 – di – O – methyl – D – galactose
14.8,16.1	14.97,16.80	0.47	0.12	2,4 – di – O – methyl – D – galactose
-	-	0.31	0.05	2 – O – methyl – D – galactose
2.49,3.00	2.48,(2.96)	-	-	2,3,4 – tri – O – methyl – D – glucuronic acid**

*figures in parenthesis indicate T values of components which are not completely resolved

**as methyl ester methyl glycoside

31.

RELATIVE AMOUNTS OF O - METHYL SUGARS IN METHYLATED ACACIA GUMS OF THE SERIES PHYLLODINEAE

O - methyl sugar	A. difformis	A. mabellae	A. retinodes	A. rubida	A. pycnantha	A. podalyriifolia	A. cyanophylla
2,3,4 - tri - O - methyl - L - rhamnose	1	tr	2	1	tr	tr	20
2,3,5 - tri - O - methyl - L - arabinose	26	30	8	21	14	12	3
2,3,4 - tri - O - methyl - L - arabinose	tr	-	tr	3	-	-	-
2,3 - di - O - methyl - L - arabinose	-	-	-	8	1	-	1
3,5 - di - O - methyl - L - arabinose	-	-	-	-	-	-	-
2,5 - di - O - methyl - L - arabinose	-	-	-	-	3	-	7
2,3,4,6 - tetra - O - methyl - D - galactose	23	32	27	29	26	29	7
2,3,6 - tri - O - methyl - D - galactose	2	2	1	2	1	6	-
2,4,6 - tri - O - methyl - D - galactose	16	15	14	16	4	2	2
2,3,4 - tri - O - methyl - D - galactose	6	2	11	5	6	8	7
2,6 - di - O - methyl - D - galactose	1	-	1	2	2	-	-
2,4 - di - O - methyl - D - galactose	20	13	28	9	40	42	28
2,3,4 - tri - O - methyl - D - glucuronic acid	5	6	8	4	2	2	5
2,3 - di - O - methyl - D - glucuronic acid	-	-	-	-	tr	tr	20

similar specific rotation and methoxyl content, the former being

consistent with values found for A. cyanophylla (-48°),

A. podalyriifolia (-42°) and A. pycnantha (-50°).

The g.l.c. traces of the four samples show a marked similarity,

the main components being 2,3,5 - tri - O - methyl - L - arabinose,

2,3,4,6 - tetra - O - methyl - D - galactose, 2,4,6 - tri - O -

methyl - D - galactose, 2,4 - di - O - methyl - D - galactose and to

a lesser extent, 2,3,4 - tri - O - methyl - D - galactose and 2,3,4 -

tri - O - methyl - D - glucuronic acid. The only di - O - methyl

arabinose found was in A. rubida which contains more arabinose than

the other four samples. This means that in A. difformis, A. mabellae

and A. retinodes all the arabinose is end-group arabinose, which is

consistent with results found from methylated A. podalyriifolia.

A. rubida contains 2,3 - di - O - methyl - L - arabinose but the

amount would seem to suggest very short arabinose chains of no more

than two units long. This fits in to a certain extent with

A. pycnantha which contains small amounts of 2,3 - di - O - methyl -

L - arabinose and also 2,5 - di - O - methyl - L - arabinose[6,10].

The small amounts of 2,6 - di - O - methyl - D - galactose are

thought to arise from slight undermethylation but are not signifi-

cant. The large amounts of 2 - O - methyl - D - galactose found from

paper chromatography have been attributed to undermethylation[18]

and the fact that the results for the four samples show a marked

similarity to those found for A. podalyriifolia and A. pycnantha

except for the much smaller amounts of 2,4 - di - O - methyl

- $\underline{D}$ - galactose, suggests that the 2 - $\underline{O}$ - methyl - $\underline{D}$ - galactose

might arise from undermethylation of the 2,4 - di - O - methyl galactose.

III.7.   DISCUSSION

Analytically, A. calamifolia, A. difformis, A. mabellae,

A. retinodes and A. rubida are similar to A. pycnantha and

A. podalyriifolia. They all have a low viscosity (5-10), low protein

(1.5-3.1), ash, and uronic acid (5-10%) contents, and similar sugar

ratios with high proportions of galactose, some arabinose and trace

amounts of rhamnose. A. cyanophylla, with its high uronic acid and

rhamnose values appears to be quite distinct, despite the botanical

similarities between the species.

The four methylated samples gave similar g.l.c. traces after

methanolysis, and the proportions of the various methyl glycosides

were similar in each case. The g.l.c. traces were also similar to

those obtained from A. pycnantha and A. podalyriifolia with large

amounts of end-group galactose and arabinose, smaller amounts of

end-group glucuronic acid, and trace amounts of rhamnose. Here

again, A. cyanophylla does not fit into this general pattern as it

has large amounts of end-group rhamnose, not very much end-group

galactose, and much smaller amounts of terminal arabinose. Perhaps

even more significant is that, as well as chains of arabinose,

there would seem to be either chains of uronic acid or non-terminal

uronic acid residues as seen from the large amounts of 2,3 - di -

$\underline{O}$ - methyl - $\underline{D}$ - glucuronic acid in addition to the 2,3,4 - tri -

$\underline{O}$ - methyl - $\underline{D}$ - glucuronic acid.

A. cyanophylla therefore appears to be an unusual member of
the Phyllodineae Series analytically, although structurally, the
basic galactan core may be the same as for the others.

Neglecting A. cyanophylla as being atypical of the Series
Phyllodineae, Table III,E. shows how Series 1 Acacias compare with
those of the other series studied in any detail so far, namely
Series 4 and Series 5.

The Series 1 Acacias differ quite markedly from those of
Series 4 and Series 5: there is much more uronic acid than rhamnose;
only one detectable uronic acid compared to two in Series 5 and four
in Series 4; only two dissaccharides formed, one in very small
amounts; arabinose side chains, if present, are very small and
possibly not more than two or at the very most three units long.
Also, the structure of the Series 1 gum exudates would seem to be
vastly different from that of the Series 4 and Series 5 exudates.

When postulating a possible structure for the Series 1 Acacias
the following features must be accounted for:- all uronic acid
residues must be linked 1,6 - to galactose since the only biuronic
acid detected was 6 - O - (β - D - glucopyranosyl uronic acid) -
D - galactose; partial acid hydrolysis studies indicated predomin-
antly 1,3 - linked galactose chains with small amount of 1,6 -
linked galactose units. The fact that 40-50% of the O - methyl
sugars detected are end-group, ie:- 2,3,4 - tri - O - methyl
rhamnose, 2,3,4 - tri - and 2,3,5 - tri - O - methyl arabinose,
2,3,4,6 - tetra - O - methyl - D - galactose and 2,3,4 - tri - O -
methyl - D - glucuronic acid suggests a very highly branched

COMPARISON OF GUM EXUDATES FROM <u>ACACIA</u> SPECIES IN SERIES 1, 4 AND 5

	Properties	Series 1	Series 4	Series 5
1.	Sign of specific rotation	negative or slightly positive	positive	negative
2.	Ratio of rhamnose content to uronic acid content	much less than unity	greater than unity	unity
3.	Aldobiuronic acids yielded on acid hydrolysis	6-O-(β-D-G.A.)-D-gal	6-O-(β-D-G.A.)-D-gal 6-O-(4-Me-β-D-G.A.)-D-gal 4-O-(β-D-G.A.)-D-gal 4-O-(4-Me-α-D-G.A.)-D-gal	6-O-(β-D-G.A.)-D-gal 6-O-(4-Me-β-D-G.A.)-D-gal
4.	Neutral disaccharides obtained on mild acid hydrolysis	3-O-β-D-gal-D-gal 6-O-β-D-gal-D-gal	3-O-β-D-gal-D-gal 6-O-β-D-gal-D-gal 3-O-β-L-ara f-L-ara 3-O-β-L-ara p-L-ara	3-O-β-D-gal-D-gal 6-O-β-D-gal-D-gal 3-O-β-L-ara f-L-ara 3-O-β-L-ara p-L-ara 3-O-β-D-gal-L-ara
5.	O-methyl-L-arabinose obtained from the methylated gums	2,3,5-tri and in some cases 2,3,4-tri- and in a few cases 2,3-di- and 2,5-di-O-methyl-L-arabinose	2,3,5 and 2,3,4-tri, 2,5-, 3,5- and 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	1 or in some cases 2 units	some are at least 6 units long	longest observed is 5 units long

36.

COMPARISON OF GUM EXUDATES FROM <u>ACACIA</u> SPECIES IN SERIES 1, 4 AND 5

	Properties	Series 1	Series 4	Series 5
7.	Basis of molecular structure	highly branched 1,3- galactan framework with 1,6- linkages to the main chain	blocks of 1,3- linked galactose interspersed by blocks of 1,6- linked galactose in a branched galactan framework	branched 1,3- linked galactan framework
8.	Approximate weight - average molecular weights	$\bar{M}_w \leq 400,000$	$\bar{M}_w \geq 850,000$	$\bar{M}_w \leq 600,000$

37.

galactan - core structure. There is further indication of 1,3 -

and 1,6 - linked galactose chains from 2,4,6 - tri and 2,3,4 - tri -

O - methyl - D - galactosides. The 2,4 - di - O - methyl - D -

galactose indicates galactose residues linked at the 1,3 and 6

positions.

Aspinall, Hirst and Nicolson[10] concluded that A. pycnantha

gum was a highly branched structure consisting of a framework of

D - galactopyranose residues with main chains linked 1→3 and with

side chains attached by 1→6 linkages. They postulated two possible

structures (figure III,1.).

FIGURE III,1.

(I)

(II)
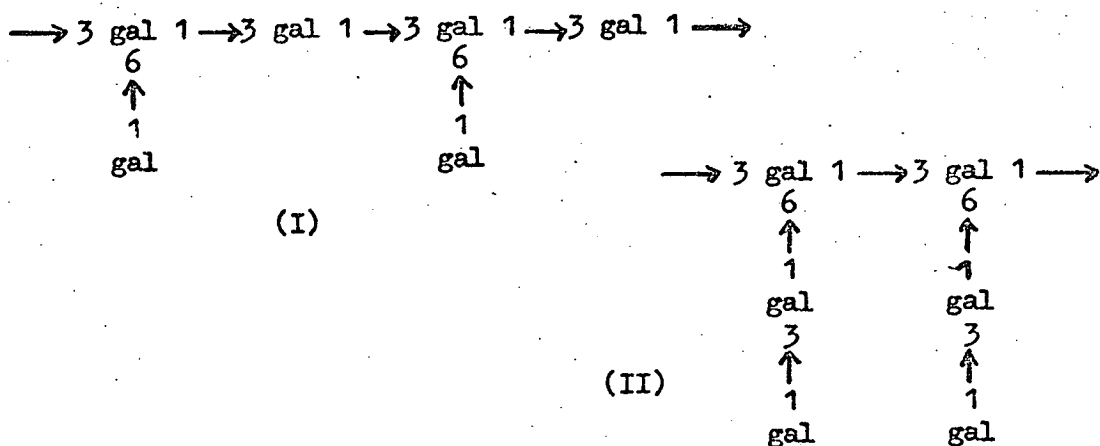
These do not sufficiently explain the large amounts of terminal

groups with respect to the rest of the molecule and in particular

to the amount of 1,3 - linked galactose (shown as 2,4,6 - tri -

O - methyl - D - galactose on methylation).

A possible structural fragment to fit the facts is shown in

```
                                           gal
                                            1
                                            ↓
                                            3
      gal                          R1——>6 gal                    gal                      gal
       1                  R                 1                      1                        1
       ↓                  1                 ↓                      ↓                        ↓
       6                  ↓                 6                      6                        3
R1——>3 gal               6                  3            gal 1——>3 gal          gal 6 ←—— R
       ↓         gal 1——>3 gal             gal            R ——>3 gal                        1
       3                  1                 1                      1                        ↓
      gal                 ↓                 ↓                      ↓                        3
       1                  6                 3                      6              R ——>6 gal
       ↓                 gal               gal                    gal                       1
       6        gal 3 ←—— 1R                1                      1                        ↓
      gal                 1                 ↓                      ↓                        6
       1                  ↓                 6                      6
       ↓                  6
       6
------->3 gal 1——>3 gal 1——>3 gal 1——>3 gal 1——>3 gal 1——>3 gal 1——>3 gal 1——>3 gal 1——>3 gal 1——> ------
                         6               6                6                6               6
                         ↑               ↑                ↑                ↑               ↑
                         1               1                1                1               1
              R ——>6 gal        R ——>6 gal               gal      R1——>6 gal
                         3               3                6                3
                         ↑               ↑                ↑                ↑
                         1               1                1                1
                        gal             gal              gal              gal
                         3                                6                3
                         ↑                                ↑                ↑
                         1                                1                1
              R ——>6 gal                                 R⁼     gal 1——>6 gal
                         3                                                3
                         ↑                                                ↑
                        gal                                               1
                                                                          R
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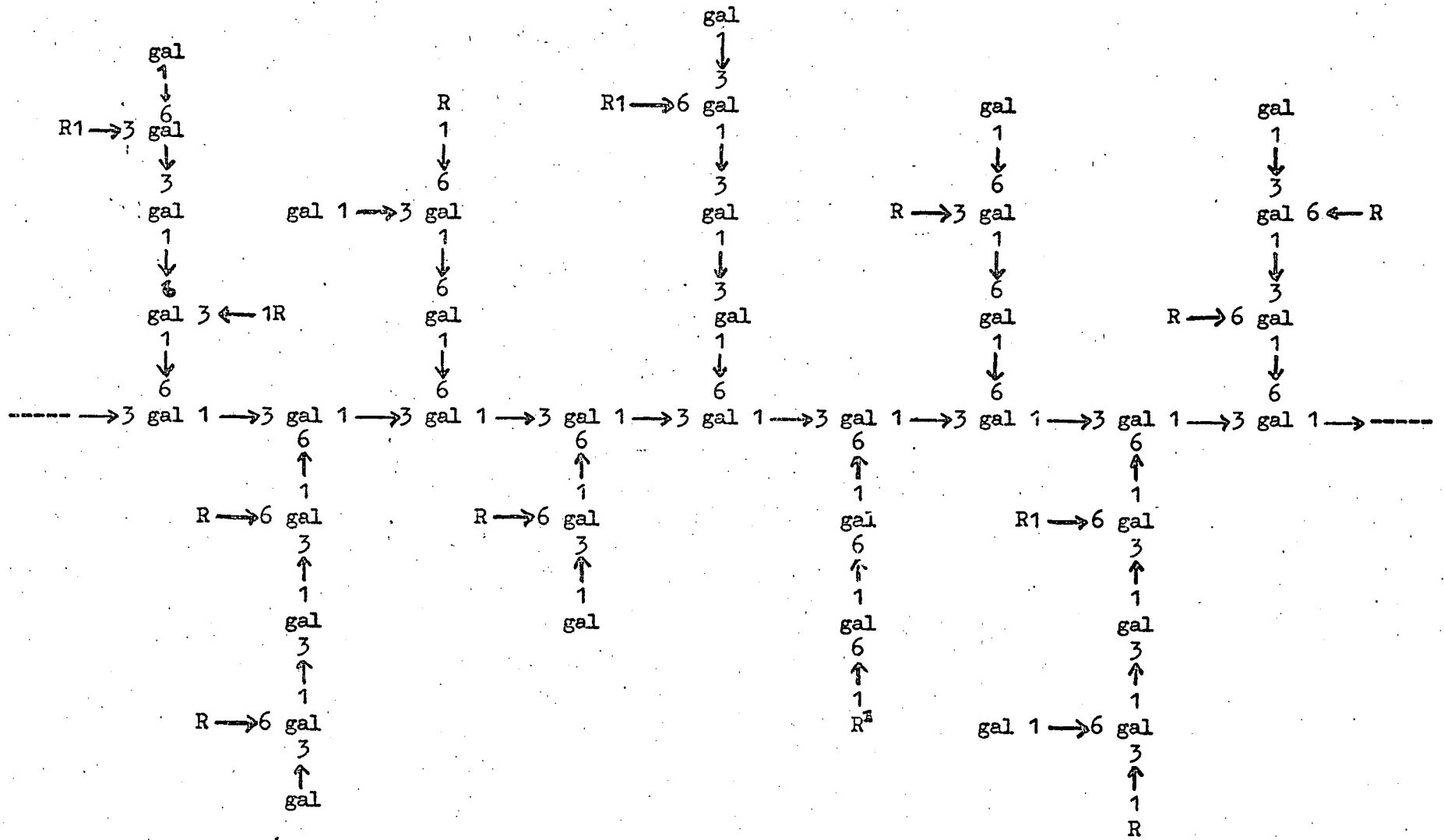
Figure III,2.

R = galactose, rhamnose, glucuronic acid, arabinose
or arabinose side chains.

R^1 = galactose or glucuronic acid.

The above structure fits the facts and agrees with the structural findings of Aspinall et al. It also explains the difficulty in methylating main chain 1,3,6 - linked galactose. A Smith degradation study would be required to help clarify this picture.

A. cyanophylla would fit into this picture if the majority of end-group galactose residues and some of the end-group arabinose residues were substituted by rhamnose and uronic acid or a uronic acid — rhamnose terminal end-grouping. However it remains the most atypical of the Series 1 Acacias.

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SECTION IV

ANALYTICAL AND STRUCTURAL FEATURES OF

THE GUM EXUDATE FROM ANACARDIUM OCCIDENTALE

SECTION IV.A.

ANALYTICAL COMPARISON OF TWO SAMPLES OF GUM FROM ANACARDIUM OCCIDENTALE

IV.A.1.   INTRODUCTION

Anacardium occidentale Linn., more commonly known as the cashewnut tree, is a member of the family Anacardiaceae. As well as its use as an adhesive, the gum from Anacardium occidentale also has insecticidal properties. Although little work has been carried out on the Anacardiaceae, Biswas and Bose[1,2] have examined the gum from Anacardium occidentale in certain detail. They have reported that the gum is composed of $\underline{D}$ - galactose, $\underline{L}$ - arabinose, $\underline{L}$ - rhamnose and $\underline{D}$ - galacturonic acid. One aldobiuronic acid, reputed to be 6 - $\underline{O}$ - (β - $\underline{D}$ - galactopyranosyluronic acid) - $\underline{D}$ - galactose was found.

This section reports the analytical data for two samples of Anacardium occidentale gum from widely differing geographical locations and will dispute certain of the findings of the above authors. This study also makes the first ever report of the presence of $\underline{D}$ - glucose as one of the constituent sugars in a plant gum exudate.

IV.A.2.   ORIGIN OF SPECIMENS

Sample 1 was collected by Mr. T.P. Baskaradoss, Forest Utilisation Officer, Forest Department, Tamilnadu, Madras 6, India on 13 October 1969.

Sample 2 was collected by Mr. S.J. Colwell of Forest Products
Research Centre, Boroko, Papua after wounding the bark and sapwood
at Laloki Agricultural Quarantine Station, Port Moresby on 4 June
1971.

IV.A.3.   PURIFICATION OF SAMPLES

Both samples dissolved in cold water. After 2 days the
solutions were filtered, dialysed for 2 days, refiltered and
freeze-dried. Reasonably high yields of freeze-dried polysaccharide
were obtained in both cases (approx 80%).

IV.A.4.   RESULTS

Each sample (50 mg) was hydrolysed with N - sulphuric acid for
7.5 hours on a boiling water bath. The solutions were neutralised,
deionised and concentrated. Paper chromatography in solvents (b)
and (c) indicated that Anacardium occidentale gum was very complex
and not as straightforward as the genus Acacia. Four main sugar
components were detectable, corresponding to galactose, arabinose,
rhamnose and one which apparently corresponded to $\underline{D}$ - glucose.
Small amounts of mannose were observed in both samples on chromato-
grams run in solvent (c) and sample 1 was also found to contain
trace amounts of xylose. Another chromatogram was run in
solvent (c); instead of development with aniline oxalate spray, it
was sprayed with glucose oxidase reagents, this test being specific
for glucose. A purple/green spot was obtained for both the
Anacardium samples as well as for a glucose standard. Secondary
development of this chromatogram with aniline oxalate indicated

that the glucose spot was in fact the component originally thought to be glucose. Typical R_{gal} values for the neutral sugars found are shown in Table IV,A.

TABLE IV,A.

R_{gal} VALUES OF NEUTRAL SUGARS IN
ANACARDIUM OCCIDENTALE GUM

Sugar	R_{gal} in solvent (b)	R_{gal} in solvent (c)	Colour
galactose	1.00	1.00	brown
glucose	1.21	1.15	brown
mannose	1.42) not	1.27	light brown
	) distinguish-		
arabinose	1.42) able	1.46) one merging	pink
		) pink spot	
xylose	1.73	1.44)	pink
rhamnose	2.25	2.07	yellow

Chromatograms run in solvent (c) and solvent (d) also indicated the presence of one main aldobiuronic acid (R_{gal} 0.28 solvent (c), 0.61 solvent (d)). This is characterised in Section IV.B. There were also slight traces of another component (R_{gal} 0.62 solvent (c), 0.83 solvent (d)).

Each sample (50 mg) was hydrolysed with 2N - sulphuric acid for 7.5 hours on a boiling water bath. After neutralisation, deionisation and concentration, paper chromatography of the hydrolysates in solvent (f) indicated the neutral sugars found

previously together with a brown spot, (R_{gal} 0.75) consistent with

$\underline{\underline{D}}$ - glucuronic acid. $\underline{\underline{D}}$ - galacturonic acid (R_{gal} 0.64) appears as a

reddish-brown spot. The presence of $\underline{\underline{D}}$ - glucuronic acid and not

galacturonic acid was carefully checked by running the 2N -

hydrolysate in solvent (f) against standard $\underline{\underline{D}}$ - glucuronic acid and

$\underline{\underline{D}}$ - galacturonic acid; the distinction was complete and unambiguous,

both in terms of the R_{gal} value and spot colour.

The analytical data for the two samples are shown in Table IV,B.

The analytical methods used were as described in Section II with the

exception that the sugar ratios were determined by running each

hydrolysate in two solvents (b) and (c) rather than just in one.

Solvent (b) allowed the calculation of the ratio galactose:glucose:

(arabinose + mannose):xylose:rhamnose. Solvent (c) gave the ratio

(galactose + glucose):mannose:(arabinose + xylose):rhamnose. So by

combination of both these ratios, the relative sugar ratio for each

gum was calculated. Neutral sugars were also calculated on the

assumption that uronic acid residues are attached to galactose. The

percentage of 4 - $\underline{O}$ - methyl - glucuronic acid in each sample was

calculated on the assumption that all the methoxyl groups are located

on the 4 - $\underline{O}$ - methyl - $\underline{\underline{D}}$ - glucuronic acid residues.

Molecular weights were calculated using the average value for

dn/dc found[3] for a series of <u>Acacia</u> species.

TABLE IV.B.

ANALYTICAL DATE FOR TWO SAMPLES OF THE GUM
FROM ANACARDIUM OCCIDENTALE

	Sample 1	Sample 2
Moisture, (%)	9.5	7.9
Ash,[a] (%)	1.3	1.1
Nitrogen,[a] (%)	0.45	0.16
Hence protein (%) (N x 6.25)[a]	2.8	1.0
Methoxyl, (%)[b]	0.31	0.21
$[\alpha]_D$ in water (degrees),[b]	+24.2	+23.6
$[\alpha]_D$ in 7M-urea (degrees)[b]	+26.5	n.d.
Intrinsic viscosity $[\eta]$ (ml g^{-1})[a]	6.3	9.4
Molecular weight, (MW x 10^4)	26	18
Equivalent weight,[b]	2814	3089
Hence uronic anhydride, (%),[bc]	6.2	5.7
% Sugar composition after hydrolysis		
4 - O-Methylglucuronic acid,[d]	1.9	1.2
glucuronic acid,	4.3	4.5
galactose,	61	63
arabinose,	14	15
rhamnose,	7	7
xylose,	2	-
mannose,	2	1
glucose,	8	9

a   Corrected for moisture content.

b   Corrected for moisture and protein content.

c   If all acidity arises from uronic acids.

d   If all methoxyl groups located in this acid.

IV.A.5.   EXTRACTION AND IDENTIFICATION OF GLUCOSE

Purified gum (4.46 g, dry weight) was hydrolysed with 1N - sulphuric acid (250 ml) for 8 hours on a boiling water bath.   After cooling, the solution was neutralised with barium carbonate, filtered, deionised with Amberlite IR - 120(H) resin, concentrated to a syrup (1-2 ml) and chromatographed on Whatman 3MM papers for 24 hours in solvent (b). Side strips were cut and developed using a glucose oxidase spray, and the glucose was eluted from the chromatograms. After concentration to a syrup (1 ml), the glucose solution was again chromatographed in solvent (b) for 36 hours on Whatman 3MM papers. Side strips were again cut and developed by the glucose oxidase spray. As before, the glucose was eluted, concentrated to a syrup (0.5-1.0 ml) and chromatography was carried out in solvent (c) for 36 hours. The glucose was eluted as before, the solution concentrated to a very thick syrup and ethanol (3ml) added. An off-white precipitate formed at this stage. The ethanol solution was refluxed at $60^{\circ}C$ for 30 minutes and cooled. The product was filtered and dried at $60^{\circ}C$ giving a pale brown amorphous solid (0.061 g, yield 1.3%).

Some of the solid was dissolved in a drop of water and chromatographed in solvent (c) for 24 hours. On development with aniline oxalate, one large spot (R_{gal} 1.09) was observed, which gave a positive reaction with glucose oxidase. However, as glucose runs only slightly in front of galactose, it is difficult to separate these two sugars completely by paper chromatography and the possibility of $\underline{D}$ - galactose impurity in the glucose was expected.

A melting point was carried out on a hot stage microscope. The sample started to melt at 146°C, and although extensive melting occurred in the range 146-148°C, melting continued up to 166°C. The melting points of pure anhydrous $\underline{D}$ - glucose and $\underline{D}$ - galactose are 146°C and 166°C respectively: this would seem to imply that both glucose and galactose are present.

Some of the sample was dissolved in water (C = 0.66), a trace of dilute ammonia added and the specific rotation determined. The value found was +73.2°. The $[\alpha]_D$ for pure anhydrous $\underline{D}$ - glucose was confirmed to be +52.8° and for $\underline{D}$ - galactose, +81.7°. Since no other sugar components are present, this gives further evidence that the extracted product is in fact a mixture of $\underline{D}$ - glucose and $\underline{D}$ - galactose. A study of specific rotation against the proportions of two components having distinctly different rotations in a mixture has shown there to be a linear relationship (see appendix). So from the rotation of the product and the rotations of $\underline{D}$ - galactose and $\underline{D}$ - glucose, it was calculated that the product contained about 30% glucose.

X-ray diffractograms of $\underline{D}$ - glucose, $\underline{D}$ - galactose, a 30:70 mixture of glucose and galactose and the extracted product from Anacardium occidentale gum were run using a Philips PW 1050 diffractometer (34 kV, 20 mA). This gave final confirmation of the presence of $\underline{D}$ - glucose in the extract in admixture with galactose.

IV.A.6.   DISCUSSION

The two samples are remarkably similar analytically. The only difference involves the small amount of xylose found in sample I but not in sample II.

Anacardium occidentale gum is an acidic polysaccharide composed mainly of galactose but also containing arabinose, rhamnose and significant amounts (approximately 8%) of glucose. Trace amounts of mannose and xylose may be present, the latter having been reported in previous work[4]. The intrinsic viscosity is low (6-9 ml/g) as are the protein, methoxyl and ash contents. The molecular weight is also not very high, being around 200,000. The uronic acid content, which is about 6%, arises mainly from $\underline{D}$ - glucuronic acid, but the fact that the gum has a methoxyl content indicates that small amounts of $4 - \underline{O}$ - methyl - $\underline{D}$ - glucuronic acid may be present which would account for the trace amounts of the biuronic acid (R_{gal} 0.62 solvent (c), 0.83 solvent (d)), which, from its R_{gal} values would be expected to be of the $4 - \underline{O}$ - methyl - $\underline{D}$ - glucuronic acid - $\underline{D}$ - galactose type.

Bose and Biswas[1,2] report only galactose, arabinose and rhamnose as the neutral sugars present with a galactose: arabinose ratio of 17:1. Bose and Soni[5,6] also state that the results found for the gum from Anacardium occidentale are identical with those obtained for the polysaccharide from cashewnut shell. It seems possible that their exceptionally high galactose/arabinose ratio may be due to their failure to identify the two components having similar R_f values, namely galactose and glucose. They would seem to have just recognised one large spot which they have assigned as

galactose. In the present study, the galactose + glucose: arabinose ratios would fit in better with the previous values quoted by Bose and Biswas although their arabinose content would seem to be rather low; further evidence for this is found in Section IVB where structural features of the gum are examined.

$\underline{D}$ - glucuronic acid was identified conclusively both by chromatography of a 2N-hydrolysate of the gum against uronic acid standards and by 2N-hydrolysis of the main aldobiuronic acid found (see Section IVB). This is in disagreement with the findings of Bose and Biswas who stated that $\underline{D}$ - galacturonic acid was the only uronic acid present although they also stated that immuno-chemical studies of the gum with Pneumococcus antisera[2] suggest that the gum contains end-group glucuronic acid or its 4 - 0 - methyl derivative. Moreover, Bose and Biswas stated that the gum did not have a methoxyl content ncr contain nitrogen, but this study confirms that both nitrogen and methoxyl are present in small amounts.

The most important feature of <u>Anacardium</u> <u>occidentale</u> gum is the presence of glucose. There have been no reports of the presence of glucose in any gum studied previously. Extraction of pure glucose from a glucose/galactose mixture by chromatography is very difficult; attempts to extract pure glucose proved unsuccessful as the product extracted from a hydrolysate of the gum was found to contain galactose also. Both melting point and specific rotation studies indicated the presence of glucose which was confirmed by an X-ray diffraction study along with glucose oxidase and chromato-

graphic evidence. Further confirmation of the presence of glucose in the gum was obtained during a structural study of the gum; methylation of the purified polysaccharide gave 2,3,4,6 - tetra - O - methyl - D - glucose (see Section IVB).

To ascertain that the glucose was present in the original gum and was not a degradation product, a mixture containing the same proportions of galactose, arabinose, rhamnose and glucuronic acid as found in the gum was prepared, hydrolysed and chromatographed under the same conditions as used for the gum. Treatment with the glucose oxidase spray proved negative, indicating that glucose is not a breakdown product of glucuronic acid under such conditions. Hence it is concluded that Anacardium occidentale gum contains glucose as one of its constituent sugars.
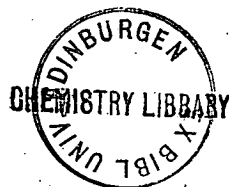
SECTION IV.B.

A STRUCTURAL STUDY OF THE GUM FROM
ANACARDIUM OCCIDENTALE

IV.B.1.   INTRODUCTION

As stated earlier, very little work has been done on the gum

from Anacardium occidentale, (Cashewnut tree). Much of the data

published by Bose and Biswas[2] has been contradicted in this present

study eg. the presence of D - galacturonic acid has not been confirmed,

and this disproves their findings that the only aldobiuronic acid

present was 6 - O - (β - D - galactopyranosyluronic acid) - D -

galactose. The structural features of the gum have not been examined,

although Bose and Soni[5,6] carried out hydrolysis studies on Cashew-

nut shell polysaccharide and found that the sugars and sugar ratios

found were almost identical. The galactose:arabinose:rhamnose ratio

for the shell polysaccharide was stated to be 17:2:1, compared with

a ratio of galactose:arabinose of 17:1 for the gum.

Bose and Soni[5] characterised the one disaccharide found on

mild hydrolysis of Cashewnut polysaccharide as 3 - O - β - D -

galactopyranosyl - D - galactose. They also found galactose 1→6

galactose linkages present[5].

This section reports the structural features of the gum from

Anacardium occidentale, sample I, and includes a complete Smith

Degradation study. The gum has been found to contain large amounts

of galactose, much smaller amounts of arabinose, rhamnose, and

glucose, and trace amounts ($<2\%$) of mannose. Xylose was also

detected in one sample. 2N - acid hydrolysis has indicated that

glucuronic acid is present, not galacturonic acid as stated by previous workers, and the fact that the gum was found to have a methoxyl content indicates that 4 - $\underline{O}$ - methyl - $\underline{\underline{D}}$ - glucuronic acid may be present.

IV.B.2. RESULTS

PURIFICATION OF GUM

Crude gum (140 g) was dissolved in distilled water (3 l) for 2 days giving a brown coloured solution which was filtered through Whatman No. 41 filter papers and then through No. 1 filter papers to remove bark and other insoluble debris. The solution was dialysed for 2 days, refiltered and freeze dried (76 g, yield 54%).

TESTS FOR HOMOGENEITY

Electrophoresis of the purified gum on cellulose acetate strips in 0.1M - ammonium carbonate buffer (pH 8.9) and 0.1M - acetate buffer (pH 4.7) and also on phoroslides in 0.1M - ammonium carbonate buffer (pH 8.9) and 0.05M - borate buffer (pH 9.2) showed one broad distinct band. Ion exchange chromatography on a DEAE - cellulose column using a sodium chloride gradient (0.0-0.5M) in 0.02M - acetate buffer gave a single symmetric peak and ultracentrifugation also showed a single peak. Molecular sieve chromatography on a Bio-gel A-5 column showed a very small peak at the void volume and a large peak at a greater elution volume. A study of the molecular sieve pattern of Anacardium occidentale with the time of standing after purification has been carried out[7] and has shown the peak at the void volume to increase in size with increased standing time. This has prompted the postulation that the peak at the void volume indicates aggregation of smaller molecules[8,9].

All these tests indicate that the gum behaves as a homo-geneous polymer system.

IDENTIFICATION OF NEUTRAL SUGARS

Purified gum (3 g) was hydrolysed with N-sulphuric acid (150 ml) for 7.5 hours on a boiling water bath. On cooling, the solution was neutralised with barium carbonate, filtered, deionised with Amberlite IR - 120(H) resin and concentrated to a syrup. The syrup was applied to a column (41 x 2.6 cm) of Duolite A-4 resin in the formate form. Elution with distilled water (750 ml) yielded the neutral sugars of _Anacardium occidentale_. After concentration to a syrup, the neutral fraction was chromatographed in solvents (b) and (c). Large amounts of galactose were found along with some arabinose, glucose and rhamnose; trace amounts of mannose and xylose were also detected. (see Table IV,A.for R_{gal} values).

IDENTIFICATION OF ACIDIC COMPONENTS

Following elution of the neutral sugars from the column with distilled water, elution with 5% formic acid (400 ml) gave the acidic fraction. The acidic solution was concentrated to a syrup (formic acid being removed by repeated addition of water followed by reconcentration to a syrup), and chromatographed in solvents (c) and (d). Galactose was observed along with a large amount of an acidic component having R_{gal} 0.28 in solvent (c) and 0.61 in solvent (d) and trace amounts of another component having R_{gal} 0.62 in solvent (c) and 0.83 in solvent (d).

The trace component was not present in sufficient quantity for its identity to be determined, although it appears to have

contained 4 - $\underline{O}$ - methyl - $\underline{D}$ - glucuronic acid and galactose.

The acidic fraction was chromatographed in solvent (c) on a Whatman 3MM paper and the main component (R_{gal} 0.28) was eluted and concentrated. The value of $[\alpha]_D$ was +20° ($\underline{c}$, 0.90), which suggested the presence of a β - $\underline{D}$ - linkage. Chromatography of the eluted biuronic acid in solvent (c) indicated some galactose impurity which would account for the higher than expected $[\alpha]_D$ value, since normally a β - $\underline{D}$ - linkage has an $[\alpha]_D$ value of about zero. The syrup (8 mg) was hydrolysed with 2N - sulphuric acid for 8 hours on a boiling water bath and paper chromatography of the hydrolysate in solvents (b), (c) and (g) indicated the presence of galactose and glucuronic acid only. The aldobiuronic acid (6 mg) was methylated by the Kuhn method. Methanolysis followed by g.l.c. examination of the methyl glycosides on column (1) indicated the presence of 2,3,4 - tri - $\underline{O}$ - methyl - $\underline{D}$ - glucuronic acid (T 2.59, 2.99), 2,3,5 - tri - $\underline{O}$ - methyl - $\underline{D}$ - galactose (T 4.12, 4.74) and 2,3,4 - tri - $\underline{O}$ - methyl - $\underline{D}$ - galactose (T 6.28). Also present was 2,3,4,6 - tetra - $\underline{O}$ - methyl - $\underline{D}$ - galactose from methylation of the galactose impurity. The methanolysate was reduced with sodium borohydride and the methyl glycosides removed by 1N - acid hydrolysis. Chromatography in solvent (c) revealed 2,3,4 - tri - $\underline{O}$ - methyl - $\underline{D}$ - galactose (R_G 0.72, red spot), 2,3,5 - tri - $\underline{O}$ - methyl - $\underline{D}$ - galactose (R_G 0.83, brown/black spot) and 2,3,4 - tri - $\underline{O}$ - methyl - $\underline{D}$ - glucose (R_G 0.88, red spot). This identifies the aldobiuronic acid as 6 - $\underline{O}$ - (β - $\underline{D}$ - glucopyranosy-

luronic acid) - $\underline{D}$ - galactose.

PARTIAL ACID HYDROLYSIS OF THE GUM

Hydrolysis of the polysaccharide (100 mg) with 0.5N - sulphuric acid (10 ml) for 1 hour on a boiling water bath, followed by neutralisation, deionisation, concentration to a syrup and chromatography in solvent (b) showed the presence of the disaccharides 3 - $\underline{O}$ - β - $\underline{D}$ - galactopyranosyl - $\underline{D}$ - galactose (R_{gal} 0.48) (major component) and 6 - $\underline{O}$ - β - $\underline{D}$ - galactopyransyl - $\underline{D}$ - galactose (R_{gal} 0.24) (minor component) together with other higher oligosaccharides.

METHYLATION OF ANACARDIUM OCCIDENTALE GUM

The gum (287 mg) was methylated to give a product (178 mg); $[\alpha]_D$ - 20.3° ($\underline{C}$, 0.97 in chloroform), OMe 42.6%. Methanolysis of a portion of the methylated product followed by g.l.c. examination of the methyl glycosides gave the results shown in Table IV,C. Hydrolysis of the methyl glycosides, followed by paper chromatography in solvents (e) and (f) indicated the presence of 2 - $\underline{O}$ - methyl - $\underline{D}$ - galactose (R_G 0.31; solvent (e), 0.05, solvent (f)) in addition to the $\underline{O}$ - methyl sugars found by g.l.c. examination. The relative amounts of the $\underline{O}$ - methyl sugars could not be estimated completely because of incomplete resolution of several of the components. However, the values quoted are only approximate and serve to give an indication of the proportions of the components present. It is not possible to quote a value for the amount of 2,3,6 - tri - $\underline{O}$ - methyl - $\underline{D}$ - galactose, but it is shown to be present in small but significant amounts, by paper chromatography of the hydrolysed methyl glycosides.

TABLE IV.C.

METHYL GLYCOSIDES FROM METHYLATED ANACARDIUM OCCIDENTALE GUM

Relative retention time (T)* of methyl glycosides		R_g after hydrolysis solvents		O-methyl sugar identified	Relative amounts
Column (1)	Column (2)	(e)	(f)		
(0.45)	(0.46)	1.02	1.03	2,3,4-tri-O-methyl-L-rhamnose	(9)
(0.45),(0.54)	(0.46),(0.56)	1.02	1.03	2,3,4-tri-O-methyl-D-xylose	
(0.99),(1.38)	(1.00),(1.42)	0.99	0.98	2,3,4,6-tetra-O-methyl-D-glucose	(10)
(1.38)	(1.42)	0.87	0.83	2,3,4,6-tetra-O-methyl-D-mannose	
(0.54),0.67	(0.56),0.71	0.94	1.03	2,3,5-tri-O-methyl-L-arabinose	(9)
(0.97)	(1.02)	0.80	0.74	2,3,4-tri-O-methyl-L-arabinose	(4)
1.14,(2.59)	1.18,(2.45)	0.80	0.74	3,5-di-O-methyl-L-arabinose	(3)
1.91	2.13	0.80	0.74	3,4-di-O-methyl-L-arabinose	(2)
1.59	1.68	0.87	0.77	2,3,4,6-tetra-O-methyl-D-galactose	(25)
(2.90),(3.60),(3.90)	(2.98),(3.85),(4.18)	0.75	0.61	2,3,6-tri-O-methyl-D-galactose	
(3.60),(3.90)	(3.85),(4.18)	0.75	0.43	2,4,6-tri-O-methyl-D-galactose	(20)
6.45	6.42	0.75	0.37	2,3,4-tri-O-methyl-D-galactose	(3)
9.43	9.48	0.55	0.20	2,6-di-O-methyl-D-galactose	trace
15.5,16.4	15.36,16.95	0.49	0.13	2,4-di-O-methyl-D-galactose	(10)
(2.59),(2.90)	(2.45),(2.98)	-	-	2,3,4-tri-O-methyl-D-glucuronic acid**	(7)

*   figures in parenthesis indicate T values of components which are not completely resolved

**   as methyl ester glycoside

The main components are 2,3,4,6 - tetra, 2,4,6 - tri and
2,4 - di - $\underline{O}$ - methyl - $\underline{\underline{D}}$ - galactose, along with end-group rhamnose,
arabinose, glucose and glucuronic acid.

PREPARATION OF DEGRADED GUM A

Purified gum (6.45 g, dry weight) was hydrolysed with 0.01N -
sulphuric acid (350 ml) on a boiling water bath for 96 hours. After
cooling, the solution was neutralised with barium carbonate, filtered,
deionised with Amberlite IR - 120(H) resin, concentrated and dialysed
against distilled water (1 l) for 24 hours. After further dialysis
against running tap-water for 48 hours, degraded gum A was recovered
as a pale brown freeze-dried product (3.22 g, yield 50%).

The distilled water dialysate of degraded gum A was concentrated
to a syrup and portions were chromatographed in solvents (b) and (c).
Galactose, arabinose, mannose, rhamnose and trace amounts of xylose
were detected. A chromatogram developed with glucose oxidase spray
gave a negative result indicating that glucose was not present in the
dialysate. Also present were small amounts of the neutral disacc-
haride 6 - $\underline{O}$ - β - $\underline{\underline{D}}$ - glucopyranosyl - $\underline{\underline{D}}$ - galactose (R_{gal} 0.31
solvent (b), 0.28 solvent (c), brown spot with aniline oxalate) and
trace amounts of the neutral disaccharide 3 - $\underline{O}$ - β - $\underline{\underline{D}}$ - galacto-
pyranosyl - $\underline{\underline{D}}$ - galactose (R_{gal} 0.50 solvent (b), 0.46 solvent (c),
brown spot). No arabinose disaccharides, indicated by pink spots
with aniline oxalate, were detected.

EXAMINATION OF DEGRADED GUM A

Degraded gum A (30 mg) was hydrolysed with 1N - sulphuric acid
for 7.5 hours. After neutralisation, deionisation and concentration,

chromatography in solvents (b) and (c) followed by development with both aniline oxalate and glucose oxidase spray reagents, indicated the presence of only two neutral sugar components, namely galactose and glucose, with galactose the major component. 2N - Sulphuric acid hydrolysis followed by chromatography in solvent (g) indicated that glucuronic acid was also present.

Degraded gum A had $[\alpha]_D$ = +29.1°; a methoxyl content of 0.37% indicated the presence of 2.2% of 4 - O - methyl - D - glucuronic acid. The equivalent weight of 2029 indicated that 8.7% uronic acid was present. The molecular weight, $\bar{M}_n$ by end-group analysis was found and confirmed to be 1640, which would seem to be very low.

Partial acid hydrolysis of degraded gum A with 0.5 N - sulphuric acid for 1 hour at 100°C revealed the presence of 3 - O - β - D - galactopyranosyl - D - galactose (R_{gal} 0.46 solvent (b)) as the only disaccharide.

Degraded gum A (198 mg) was methylated to give a product (120 mg) (Found: $[\alpha]_D$ - 22.3° (c 0.6, chloroform); OMe 40.2%). A portion of this was methanolysed and examined by glc. The methyl glycosides found are shown in Table IV,D. Hydrolysis of the mixture of methyl glycosides followed by paper chromatographic examination in solvents (e) and (f) also indicated the presence of substantial amounts of 2 - O - methyl - D - galactose in addition to the O - methyl sugars identified as their methyl glycosides by g.l.c.

TABLE IV.D.

METHYL GLYCOSIDES FROM METHYLATED DEGRADED GUM A

Relative retention time (T) of methyl glycosides		R_g in solvent (f)	Relative amounts	O-methyl sugar identified
Column (1)	Column (2)			
0.59,0.73	0.58,0.75	1.01	trace	2,3,5-tri-O-methyl-L-arabinose
1.00,1.39	1.00,1.40	0.98	5	2,3,4,6-tetra-O-methyl-D-glucose
1.62	1.72	0.80	35	2,3,4,6-tetra-O-methyl-D-galactose
(2.89),(3.57),(3.89)	3.03,3.88,4.35	0.56	1	2,3,6-tri-O-methyl-D-galactose
(3.57),(3.89)	3.88,4.35	0.47	24	2,4,6-tri-O-methyl-D-galactose
6.42	6.64	0.41	6	2,3,4-tri-O-methyl-D-galactose
9.24	9.48	0.25	1	2,6-di-O-methyl-D-galactose
14.8,16.0	15.36,16.95	0.16	20	2,4-di-O-methyl-D-galactose
2.32,(2.89)	2.42,3.03	-	8	2,3,4-tri-O-methyl-D-glucuronic acid

PREPARATION OF DEGRADED GUM B

Degraded gum A (1.7 g) was dissolved in water (50 ml) and 0.5 M - sodium metaperiodate (50 ml) added. After 72 hours in darkness at room temperature, 13.2 m moles periodate/g polysaccharide had been reduced and 6.1 m moles formic acid/g polysaccharide released. The reaction was stopped by the addition of ethylene glycol (4 ml) and the solution dialysed against running tap water for 2 days. Sodium borohydride (1.0 g) was added, and after 30 hours at room temperature, the solution was dialysed for a further 2 days. The solution was then made 1N with respect to sulphuric acid and the polyalcohol hydrolysed for 48 hours at room temperature, then dialysed for 2 days. Degraded gum B was isolated as the freeze-dried product (0.6 g, yield 31%).

EXAMINATION OF DEGRADED GUM B

Degraded gum B was hydrolysed with 1N - and 2N - sulphuric acid. Chromatography indicated that galactose was the only sugar present. Partial acid hydrolysis with 0.5 N - sulphuric acid, followed by chromatography in solvent (b) indicated that $3 - \underline{O} - \beta - \underline{D}$ - galactopyranosyl - $\underline{D}$ - galactose (R_{gal} 0.46) was also present together with higher oligosaccharides.

Degraded gum B had $[\alpha]_D = +38.3^\circ$ and the molecular weight, $\bar{M}_n$ was found (by end-group analysis) to be 2,000.

Degraded gum B (182 mg) was methylated to give a product (139 mg), (Found: $[\alpha]_D - 3.7^\circ$ ($\underline{C}$ 0.5, chloroform); OMe 40.7%). A portion of this was methanolysed and examined by g.l.c. The methyl glycosides found are shown in Table IV,E. Hydrolysis of the mixture

TABLE IV.E.

METHYL GLYCOSIDES FROM METHYLATED DEGRADED GUM B

Relative retention time (T) of methyl glycosides		R_g in solvent (f)	Relative amounts	O-methyl sugar identified
Column (1)	Column (2)			
1.62	1.69	0.80	13	2,3,4,6-tetra-O-methyl-D-galactose
2.89,(3.60),(3.96)	2.90,(3.69),(4.14)	0.55	4	2,3,6-tri-O-methyl-D-galactose
(3.60),(3.96)	(3.69),(4.14)	0.47	70	2,4,6-tri-O-methyl-D-galactose
9.36	8.79	0.20	7	2,6-di-O-methyl-D-galactose
14.3,15.2	14.6,15.8	0.12	6	2,4-di-O-methyl-D-galactose

of methyl glycosides followed by paper chromatography in solvents

(e) and (f) indicated the presence of 2 - O - methyl - D - galactose

in addition to the methyl glycosides found from g.l.c. examination.

PREPARATION OF POLYSACCHARIDE I

Preliminary small-scale experiments indicated that 0.25 M -
sodium metaperiodate solution and an oxidation time of 72 hours were
required for Anacardium occidentale gum.

Purified gum (40.7 g) was dissolved in water (1125 ml) and
0.50 M - sodium metaperiodate (1125 ml) was added. The solution was
kept in darkness at room temperature and the oxidation followed by
measuring the release of formic acid with time; aliquots (1 ml) of
the solution were titrated with 0.1081 N - sodium hydroxide, using
methyl red as indicator (Figure IV,1). After 72 hours, 9.79 m moles
periodate/g polysaccharide had been reduced and 3.89 m moles formic
acid/g polysaccharide released. The reaction was stopped by the
addition of ethylene glycol (23.9 ml) and the solution dialysed
for 2 days. Sodium borohydride (13.3 g) was added and the mixture
kept at room temperature for 30 hours, then dialysed for a further
2 days. By this time the brown colour had almost completely dis-
appeared, leaving a pale solution which was made 1 N with respect
to sulphuric acid by the addition of 4 N acid, and the polyalcohol
was hydrolysed at room temperature for 48 hours. Following dialysis
for a further 2 days, polysaccharide I was isolated as a freeze-
dried product (11.6 g, yield 28.5%).
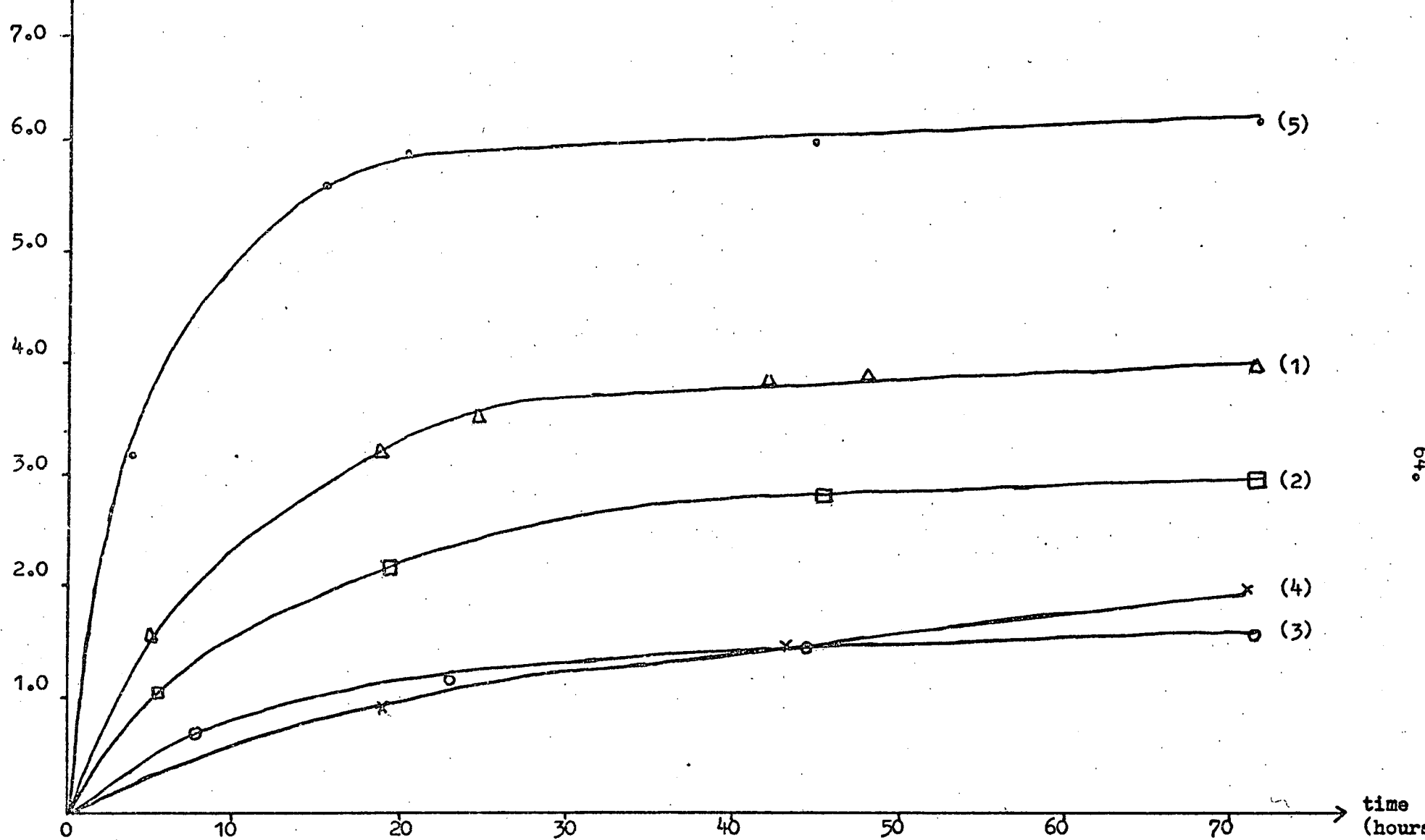
FIGURE IV,1.   Formic acid released (m moles/g) with time (hours) on periodate oxidation of:-

(1)   Anacardium occidentale gum
(2)   Polysaccharide I
(3)   Polysaccharide II
(4)   Polysaccharide III
(5)   Degraded gum A

EXAMINATION OF POLYSACCHARIDE I

Polysaccharide I (50 mg) was hydrolysed with 1 N - sulphuric

acid for 7.5 hours on a boiling water bath. After neutralisation,

filtration, deionisation and concentration, chromatography in

solvents (b) and (c) indicated the presence of galactose and arabi-

nose only, with galactose the major component. Treatment of one

chromatogram with glucose oxidase spray proved negative, indicating

that glucose was absent as well as rhamnose and the small amounts of

mannose and xylose originally present in the gum. The actual sugar

ratios are shown in Table IV.F.

Hydrolysis of Polysaccharide I with 2 N - sulphuric acid for

7.5 hours indicated that a very small amount of glucuronic acid was

present in addition to the galactose and arabinose present. Partial

acid hydrolysis (0.5 M - sulphuric acid, 1 hour at $100^{\circ}C$) also

indicated the presence of much 3 - $\underline{O}$ -β- $\underline{D}$ - galactopyranosyl - $\underline{D}$ -

galactose.

The value of $[\alpha]_D$ was +41.6; the equivalent weight of 10,000

was indicative of a uronic acid content of 1.8% which is as expected,

very low, and the molecular weight, $\overline{M}_w$ was found by light scattering

measurements to be 38,800.

Polysaccharide I (270 mg) was methylated to give a product

(153 mg), (Found: $[\alpha]_D$ - 1.8° ($\underline{C}$ 0.75, chloroform); OMe 40.8%).

A portion of this was methanolised and examined by g.l.c. The methyl

glycosides found are shown in Table IV,G. Hydrolysis of the mixture

of methyl glycosides followed by paper chromatography in solvents

(e) and (f) indicated small amounts of 2 - $\underline{O}$ - methyl - $\underline{D}$ - galactose

TABLE IV.F.

SUGAR RATIOS FOR ANACARDIUM OCCIDENTALE AND ITS DEGRADATION PRODUCTS

Polysaccharide	Constituent sugars							
	gal.	ara.	rha.	glu.	mann.	xyl.	glu. acid	4-O-Me glu. acid
Anacardium occidentale gum	61	14	7	8	2	2	4.3	1.9
Degraded gum A	84	trace	-	7	-	-	6.5	2.2
Degraded gum B	100	-	-	-	-	-	-	-
Polysaccharide I	76	22	-	-	-	-	1.8	n.d.
Polysaccharide II	90	10	-	-	-	-	-	-
Polysaccharide III	97	3	-	-	-	-	-	-
Polysaccharide IV	100	trace	-	-	-	-	-	-
Polysaccharide V	100	-	-	-	-	-	-	-

in addition to the methyl glycosides found from g.l.c. examination.

PREPARATION BY SEQUENTIAL SMITH – DEGRADATION OF POLYSACCHARIDES II-V

The following weights of polysaccharides, in a sequence of Smith - degradations, were oxidised with periodate (0.125 M), reduced with borohydride, hydrolysed with sulphuric acid, and the corresponding products recovered, all as already described for Polysaccharide I. Polysaccharide I (9.2 g) gave Polysaccharide II (7.8 g); Polysaccharide II (4.6 g) gave Polysaccharide III (2.8 g); Polysaccharide III (2.2 g) gave Polysaccharide IV (1.03 g); and Polysaccharide IV (494 mg) gave Polysaccharide V (48 mg). All weights are corrected for moisture. The analytical data for each product (including the molecular weight, $\bar{M}_n$ values found by end-group analysis experiments), is shown in Tables IV.F and IV.H and the formic acid released with time for Polysaccharides I, II, III, IV and Degraded Gum A is shown in Figure VI,1.

Partial acid hydrolysis (0.5 N - sulphuric acid for 1 hour at 100°C) of Polysaccharides II, III and IV indicated the presence in each of large amounts of 3 - O-β-D - galactopyranosyl - D - galactose.

All of the Smith-degradation products were methylated in the normal way. The yields, $[\alpha]_D$ values and methoxyl contents of the methylated polysaccharides are shown in Table IV,I. Portions of each product were methanolysed and examined by g.l.c. The methyl glycosides found together with their relative amounts are shown in Table IV.G. Hydrolysis of the methyl glycosides followed by chromatography in solvents (e) and (f) indicated the presence of

TABLE IV.G.

RELATIVE PROPORTIONS OF O - METHYL SUGARS
PRESENT IN POLYSACCHARIDES I-V

O - methyl sugar	POLYSACCHARIDE				
	I	II	III	IV	V
2,3,5-tri-O-methyl-L-arabinose	12	6	2	1	-
2,3,4-tri-O-methyl-L-arabinose	trace	-	-	-	-
3,5-di-O-methyl-L-arabinose	11	5	1	-	-
3,4-di-O-methyl-L-arabinose	2	-	-	-	-
2,3,4,6-tetra-O-methyl-D-galactose	17	16	14	14	12
2,3,6-tri-O-methyl-D-galactose	4	2	2	2	3
2,4,6-tri-O-methyl-D-galactose	37	52	66	73	77
2,3,4-tri-O-methyl-D-galactose	4	trace	-	-	-
2,6-di-O-methyl-D-galactose	3	13	13	10	8
2,4-di-O-methyl-D-galactose	8	5	3	trace	trace
2,3,4-tri-O-methyl-D-glucuronic acid	4	-	-	-	-

TABLE IV.H.

ANALYTICAL DATA FOR ANACARDIUM OCCIDENTALE AND ITS DEGRADATION PRODUCTS

Polysaccharide	Yield* (%)	$[\alpha]_D$* (degrees)	Periodate reduced* after 72 hours (m moles/g)	Formic acid released after 72 hours (m moles/g)	Molecular weight** ($\times 10^3$)
Anacardium occidentale gum	54	+24.6	9.79	3.89	260
Degraded gum A	49	+29.1	13.2	6.14	1.64
Degraded gum B	31	+38.3	n.d.	n.d.	2.00
Polysaccharide I	29	+41.6	2.50	2.32	38.8
Polysaccharide II	77	+39.9	1.46	1.25	3.24
Polysaccharide III	53	+35.2	1.30	1.58	5.92
Polysaccharide IV	48	+38.1	2.01	0.98	3.74
Polysaccharide V	8	n.d.	n.d.	n.d.	n.d.

* corrected for moisture

** $\overline{M}n$ by end-group analysis except for Polysaccharide I and the gum itself

n.d. - not done

69.

2 - O - methyl - D - galactose in each product in addition to the

other methyl glycosides identified from g.l.c.

TABLE IV,I.

METHYLATION DATA FOR SMITH - DEGRADED POLYSACCHARIDES I-V

	Polysaccharide				
	I	II	III	IV	V
Amount of polysaccharide used (mg)	270	208	253	110	30
Amount of methylated product (mg)	153	130	152	59	Not isolated
$[\alpha]_D$ of product, (degrees)	-1.8	-8.2	-8.2	-8.9	-
OMe of product, %	40.8	42.8	41.6	41.4	-

IV.B.3. DISCUSSION

Purified gum from Anacardium occidentale was examined by zone

electrophoresis, thin-layer electrophoresis, ultracentrifugation and

ion-exchange chromatography; each of these experiments indicated a

one-component system. This is consistent with the results found by

Bose and Biswas[2]. Molecular sieve chromatography on a Bio-gel A5

column showed, in addition to the large peak as expected, a small

peak near the void volume. Further experiments by a colleague

indicated that the size of this peak at the void volume was related

to the time of standing after purification[7]; peak size increased

with increase in time of standing. This is similar to the results found during molecular sieve experiments with <u>Acacia</u> <u>campylacantha</u>[9], and it is thought that the peak at the void volume is due to aggregation of smaller molecules. So, from these tests to establish homogeneity, it would seem probable that <u>Anacardium occidentale</u> exhibits the same type of heteropolymolecularity as certain <u>Acacia</u> gums[11,12,13] and the gum from <u>Lannea humilis</u>[10].

1N - Hydrolysis studies showed the gum to consist of galactose (61%), arabinose (14%), rhamnose (7%), glucose (8%), mannose and xylose (small amounts approximately 2% each), glucuronic acid (5%) and its 4 - O - methyl derivative (2%). Partial acid hydrolysis showed the presence of two detectable disaccharides namely 3 - O - β - D - galactopyranosyl - D - galactose (major component) and 6 - O - β - D - galactopyranosyl - D - galactose (minor component). No arabinobioses were detected.

Separation of a large-scale 1N - hydrolysate on a Duolite A4 ion-exchange resin column into neutral and acidic fractions showed the presence of one major aldobiuronic acid and trace amounts of another. The major aldobiuronic acid was characterised as 6 - O - (β - D - glucopyranosyluronic acid) - D - galactose by hydrolysis and methylation experiments. The minor component was not present in sufficient quantity for it to be characterised; but from its R_{gal} values in various solvents and the fact that only one other aldobiuronic acid is present, it would seem reasonable to assume that it was the aldobiuronic acid 6 - O - (4 - O - methyl -β- D - gluco- pyranosyluronic acid) - D - galactose.

The gum was methylated; and on subsequent methanolysis, g.l.c. and hydrolysis examination, 2,3,4 - tri - O - methyl - L - rhamnose, 2,3,4 - tri - O - methyl - D - xylose, 2,3,4,6 - tetra - O - methyl - D - glucose, 2,3,5 - and 2,3,4 - tri -, 3,4 - and 3,5 - di - O - methyl - L - arabinose, 2,3,4,6 - tetra -, 2,3,6 - 2,4,6 - and 2,3,4 - tri -, 2,4 - di and 2 - O - methyl - D - galactose and 2,3,4 - tri - D - glucuronic acid were identified. Rhamnose, xylose, mannose, glucose and glucuronic acid are therefore all present as end-group residues. Also present as end-group residues are considerable amounts of galactose and arabinose. To accommodate such a large amount of end-group residues, the gum would have to be a very highly branched structure, which is consistent with the low viscosity found (6.3 ml/g).

Mild acid hydrolysis of the gum by 0.01 N - sulphuric acid gave a 50% yield of degraded gum A, together with considerable amounts of galactose, arabinose, and rhamnose and trace amounts of mannose and xylose. Also liberated were 6 - O -β- D - galactopyranosyl - D - galactose and trace amounts of 3 - O -β- D - galactopyranosyl - D - galactose. No glucose was released from the gum during mild acid hydrolysis, as shown by a negative glucose oxidase reaction.

Degraded gum A was found to contain galactose (84%), glucose (7%) and uronic acid (9%) only. Partial acid hydrolysis showed the presence of only one disaccharide namely 3 - O -β- D - galacto-pyranosyl - D - galactose. The molecular weight, M_n was found by end-group analysis to be 1,640, a value which would seem to be very low, since the molecular weight ($\bar{M}_w$) of the whole gum was found by light scattering measurements to be 260,000. A molecular weight of

1,640 would imply the presence of only 9 residues which is obviously
incorrect. However, end-group analysis is based on the assumption of
one reducing end-group forming one molecule of formaldehyde, so it
would seem possible that more than one reducing end-group was present
thus giving more formaldehyde formation and hence a much lower value
for molecular weight. Repeating the experiment verified the above
value. So this would imply fragmentation of the gum during mild
hydrolysis, many fragments being too large to be removed by dialysis.
This seems possible since much degradation had occurred as shown by
the large amount of periodate consumed. Hydrolysis of some
galactopyranosidic bonds had also occurred as shown by the large
amounts of galactose and some galactobiose units in the diffusate
from the dialysis of Degraded gum A. Higher oligosaccharide units
were also observed in the dialysis diffusate adding further evidence
of fragmentation on quite a large scale. This is furthermore
consistent with results found during mild acid hydrolysis of many
of the <u>Acacia</u> gums[9].

Methylated Degraded gum A, on methanolysis and hydrolysis gave
2,3,4,6 - tetra - $\underline{O}$ - methyl - $\underline{D}$ - glucose, 2,3,4,6 - tetra -,2,3,6 -,
2,4,6 - and 2,3,4 - tri, 2,4 - and a small amount of 2,6 - di - and
2 - $\underline{O}$ - methyl - $\underline{D}$ - galactose as well as 2,3,4 - tri - $\underline{O}$ - methyl
- $\underline{D}$ - glucuronic acid. A trace but insignificant amount of 2,3,5 -
tri - $\underline{O}$ - methyl - $\underline{L}$ - arabinose was also detected. The presence of
2,6 - di - and 2 - $\underline{O}$ - methyl - $\underline{D}$ - galactose is ascribed to under-
methylation. 2,3,6 - Tri - $\underline{O}$ - methyl - $\underline{D}$ - galactose may arise
from undermethylation of some end-group galactose or it may indicate

that a small number of galactose residues are linked at the 4 - position. The glucose and glucuronic acid residues are present as end-groups. The fact that glucose is present is indicative of a strong galactose-glucose bond, which is unaffected by such mild acid treatment. Since galactopyranosidic bonds should not be broken by mild acid hydrolysis then galactose-glucose bonds would not be expected to be broken either. The fact that the relative amounts of 2,3,4 - tri - $\underline{O}$ - methyl - $\underline{D}$ - galactose and 2,3,4 - tri - $\underline{O}$ - methyl - $\underline{D}$ - glucuronic acid are almost the same would seem to confirm the aldobiuronic acid as being 6 - $\underline{O}$ - (β - $\underline{D}$ - glucopyranosyluronic acid) - $\underline{D}$ - galactose especially since no 1,6 - linked galactobioses were observed after partial acid hydrolysis.

Periodate oxidation of Degraded gum A gave Degraded gum B which was found to contain only galactose. Partial acid hydrolysis again only showed the presence of a 1,3 - linked galactobiose in addition to higher oligosaccharides. Methanolysis and hydrolysis of Degraded gum B indicated the presence of 2,4,6 - tri - $\underline{O}$ - methyl - $\underline{D}$ - galactose and 2,3,4,6 - tetra - $\underline{O}$ - methyl - $\underline{D}$ - galactose as the major components with the former being by far the major component. Also present were small amounts of 2,3,6 - tri -, 2,6 - and 2,4 - di - and 2 - $\underline{O}$ - methyl - $\underline{D}$ - galactose. The absence of 2,3,4 - tri - $\underline{O}$ - methyl - $\underline{D}$ - galactose confirms that galactose is linked by the 6 - position to end-group uronic acid residues.

The molecular weight, ($\overline{M}_n$) of Degraded gum B was found by end-group analysis to be 2,000. The fact that this is higher than the value obtained for Degraded gum A is possibly due to the degraded

fragments, which gave rise to the low value for Degraded gum A, being small enough to be dialysed away.

From the results found for Degraded gum A and Degraded gum B a possible structure for Degraded gum A would be:-

```
U.A.                                 ¦
 |                                   ¦
 6                                   ¦
gal                                  ¦
 1          glu                      ¦              gal
 |           |            gal        |               |
 3           6             |         |               6
gal1-6gal1-3gal1-3gal1-3gal1-3gal1-6gal3-1gal3-1gal3-1gal3-1gal
              6
              |
              1
            gal1-6gal
              3
              |
              1
            gal3-1gal3-1gal6-U.A.
              6
              |
              1
            gal
```
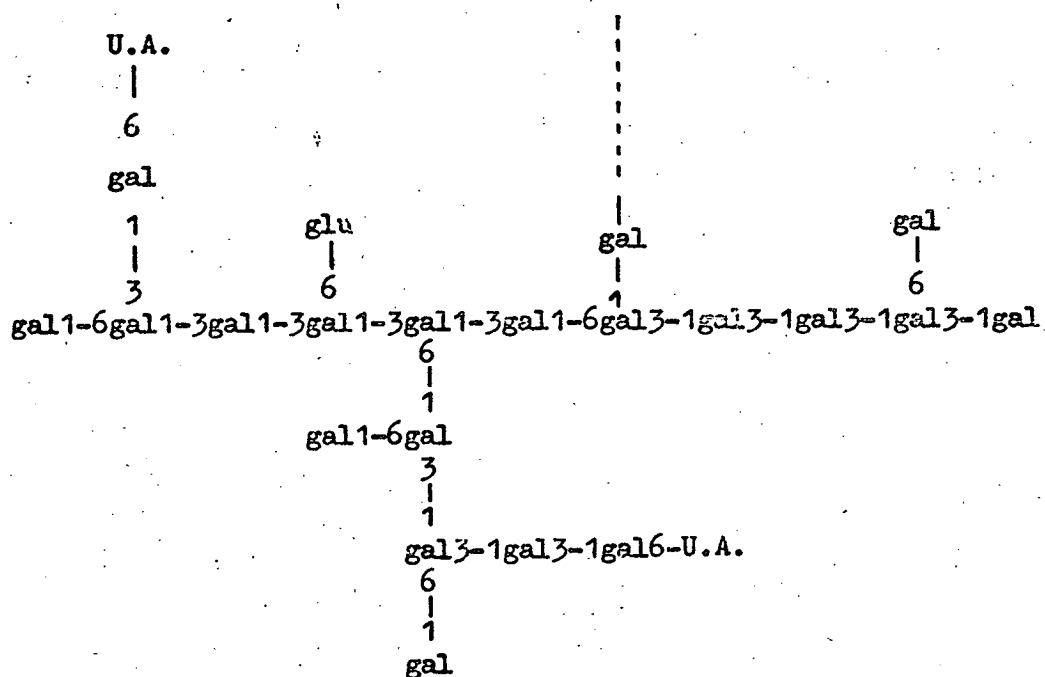
FIGURE IV,2.

where gal = galactopyanose, glu = glucopyranose, U.A. = glucuronic acid or its 4 - O - methyl derivative.

Such a structure on Smith degradation would give rise to a structure for Degraded gum B of:-

```
                                    ¦
                                    ¦
                                    ¦
                                    ¦
                                    ¦
     gal                           gal
      1                             3
      ¦                             ¦
      3                             1
gal1-3gal1-3gal1-3gal1-3gal1-6gal1-3gal1-3gal
               6
               ¦
               1
              gal
               3
               ¦
               1
         gal3-1gal3-1gal
```

FIGURE IV,3.

These structures fit in with the results found.

Anacardium occidentale gum was subjected to five successive

Smith degradations giving Polysaccharides I-V. The first degrada-

tion, which was quite drastic as shown by the large amount of

periodate reduced and the low yield obtained, removed all the xylose,

mannose and glucose which was end-group along with a lot of gala-

ctose, arabinose and glucuronic acid. Since 2,3,4 - tri - $\underline{O}$ - methyl

- $\underline{D}$ - glucuronic - acid only was observed in the methylated

polysaccharide from Anacardium occidentale implying that all the

glucuronic acid was end-group, all uronic acid was expected to be

removed in the first degradation. However, the fact that a small

amount of glucuronic acid is left in Polysaccharide I, is attributed

to incomplete oxidation due to steric hindrance [14].

$\bar{M}_n$ values were found for Polysaccharides II-IV by end-group

analysis. A very low value of 3,240 was recorded for Polysaccharide

II, indicating fragmentation similar to that suspected in Degraded

gum A. Evidence for this possibility is found in the fact that a much higher yield (77%) of Polysaccharide II was obtained than would be expected implying that fragments may have been freeze-dried along with the expected product. This is similar to results found during the degradation of Lannea humilis[10]. The $\bar{M}_n$ values for Polysaccharides III and IV would seem to be reasonable indicating that the fragments present in Polysaccharide II have been degraded sufficiently to allow them to dialyse away.

All the 1,6 - linked galactose was also removed during the first Smith degradation indicating that such galactobioses may be present at the end of galactose chains as shown in Figure IV,4.

```
- - - - gal ——— gal——— 1gal6 ———1gal
                                3
                                |
                                1
                                R
```
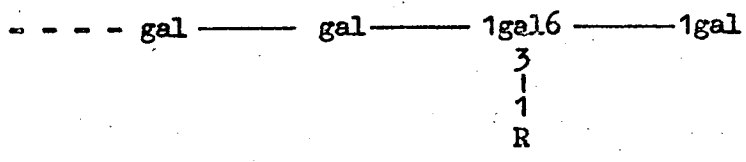
FIGURE IV,4.

Where R = arabinose, rhamnose, glucose or arabinose side chains.

Examination of methylated polysaccharides I-V gave the O - methyl sugars shown in Table IV,G. Five Smith degradations were required to remove all the arabinose, indicating that some of the arabinose side chains were 5 units long. However the small amounts of 2,3,5 - tri and 3,5 - di - O - methyl L - arabinose in Polysaccharide III and the trace amount of 2,3,5 - tri - O - methyl - L - arabinose in Polysaccharide IV indicate the presence of very few

(possibly only one) five-unit arabinose chains. Identification of 2,3,4 - tri - $\underline{O}$ - methyl - $\underline{\underline{L}}$ - arabinose and 3,4, - di - $\underline{O}$ - methyl $\underline{\underline{L}}$ - arabinose are indications of the presence of arabinopyranose residues. Arabinopyranose residues should all be removed during the first Smith degradation. Only trace amounts of 2,3,4 - tri - $\underline{O}$ - methyl - $\underline{\underline{L}}$ - arabinose were observed in Polysaccharide I confirming almost complete removal of end-group arabinopyranose residues. The presence of small amounts of 3,4 - di - $\underline{O}$ - methyl - $\underline{\underline{L}}$ - arabinose in Polysaccharide I would seem to indicate incomplete oxidation of all arabinopyranose residues present as units within an arabinose chain. This may be due to steric reasons[14] as with the case of the remaining glucuronic acid.

The fact that only 3,4 - and 3,5 - di - $\underline{O}$ - methyl arabinose were found in the gum itself and its degradation products indicates arabinose units in a chain linked 1,2 -. This differs from the majority of arabinose side chains in the _Acacia_ genus which are 1,3 - linked. 1,2 - linked arabinose side chains would be a more strained system than corresponding 1,3 - linked chains which would help to explain why all the arabinose is removed during mild acid hydrolysis and also, why no arabinobioses were observed (normally pink spots) either in the dialysate from degraded gum A or in any of the partial acid hydrolysates ($0.5N-H_2SO_4$ for 1 hour).

The $[\alpha]_D$ values for Polysaccharides I to IV were almost identical and the same as the value for Degraded gum B. This would indicate that after the first Smith degradation the basic structure of the gum remained virtually the same. So a great deal of

degradation would be expected to occur during the first treatment

with periodate. This was confirmed by the low yield (29%) of

Polysaccharide I. This identifies <u>Anacardium occidentale</u> as having

70% of its structural units susceptible to periodate oxidation

and is indicative of a highly branched structure (see Figure IV.4)

similar to that of the Group I <u>Acacias</u> (see Section III).
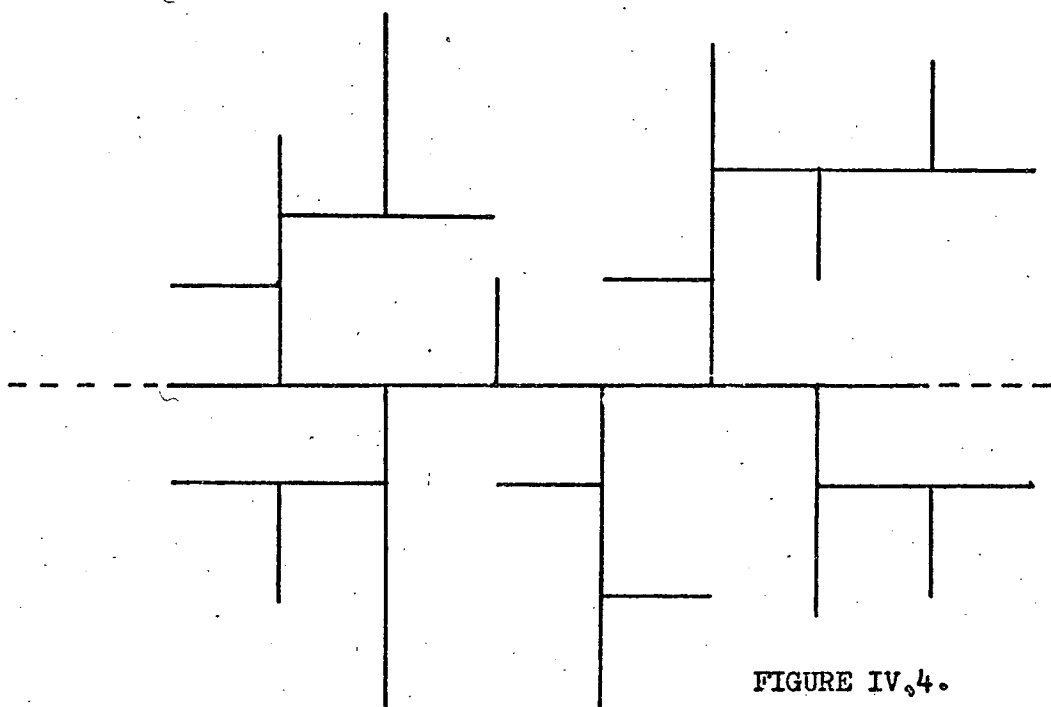
FIGURE IV.4.

Assuming that 2,3,6 - tri -, 2,6 - di - and 2 - $\underline{O}$ - methyl -

$\underline{D}$ - galactose arise from undermethylation then the main components of

methylated Polysaccharide I-V are 2,3,4,6 - tetra - and 2,4,6 - tri -

$\underline{O}$ - methyl - $\underline{D}$ - galactose with smaller amounts of 2,4 - di - $\underline{O}$ -

methyl - $\underline{D}$ - galactose. Assuming that 2 - $\underline{O}$ - methyl - $\underline{D}$ - galactose

arises from undermethylation of 2,4 - di - $\underline{O}$ - methyl - $\underline{D}$ - galactose

possibly indicating 1,3,6 branches close together (see Figure IV.5)

then this fits in with the picture of a highly branched β - 1,3 -

linked galactan framework with side chains linked β - 1,6 to the
main chain. The low viscosity found for <u>Anacardium</u> <u>occidentale</u>
would suggest a highly branched structure.

$$\begin{array}{ccccccc}
& & \downarrow & & \downarrow & & \\
& & 6 & & 6 & & \\
----\to 3\text{gal}1 & \to & 3\text{gal}1 & \to & 3\text{gal}1 & \to & 3\text{gal}1 \to ---- \\
& & 6 & & & & 6 \\
& & \uparrow & & & & \uparrow
\end{array}$$
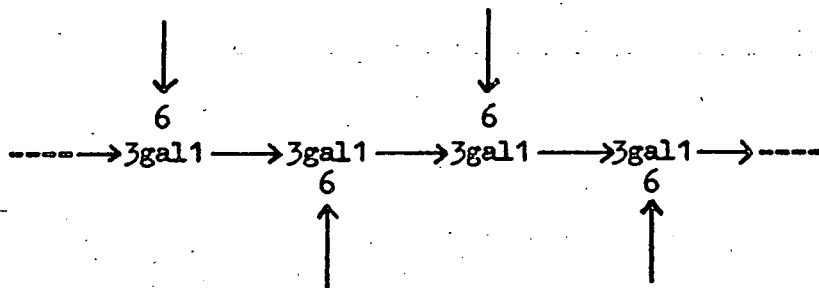
FIGURE IV,5.

A possible structural fragment of the gum is shown in Figure
IV,6. This consists of 1,3 - linked galactose chains linked through
β - 1,6 linkages. On the ends of these 1,3 - galactose branches
are rhamnose, glucose, glucuronic acid, arabinose or arabinose
side chains of one to five units long. The glucuronic acid is
always joined onto the C_6 of a galactose residue. The attachment
of glucose and rhamnose is unknown. Due to the absence of 2,3 -
di - O - methyl - D - glucuronic acid in the methylated gum,
rhamnose cannot be joined to the 4 - position of glucuronic acid
as has been established for <u>Acacia</u> gums[9,15,16]. Attachment is
possibly through the 3 - or 6 - positions of galactose. Arabinose
and its side chains are also thought to be either 1,3 - or 1,6 -
linked to galactose.

The presence of small amounts of 2,4 - di - O - methyl - D -
galactose in methylated Polysaccharide V shows that it is not a linear
molecule, but has a small amount of branching: possibly only one or
two branches.

```
                                              R"
                                              1
                                              ↓
                                              6
              gal                  gal1 ──→ 3gal
              1                               1
              ↓                               ↑
              6                               3
R1 ──→3gal1 ──→3gal                         gal6 ←── 1R
              1                               1
              ↓                               ↓
              3                               3                        R
              gal                            gal                       1
              1                               1                        ↓
              ↓                               ↓                        6
              3                               3                      gal3 ←── 1gal
              gal                            gal                       1
              1                               1                        ↓
              ↓                               ↓                        6
              6                               6
--- → gal1 ──→3gal1 ──→ 3gal1 ──→3gal1 ──→3gal1 ──→3gal1 ──→3gal1 ──→3gal1 ── ---
        6                         6                   6                    6
        ↑                         ↑                   ↑                    ↑
        1                         1                   1                    1
      gal                        glu               gal6←── 1gal          gal
        3                                             3                    3
        ↑                                             ↑                    ↑
        1                                             1                    1
     gal3 ←── 1gal3 ←── 1gal                        gal                  gal3 ←──1R
        6        6                                                         6
        ↑        ↑                                                         ↑
        1        1                                                         1
        R       gal                                                     glu.a.
```
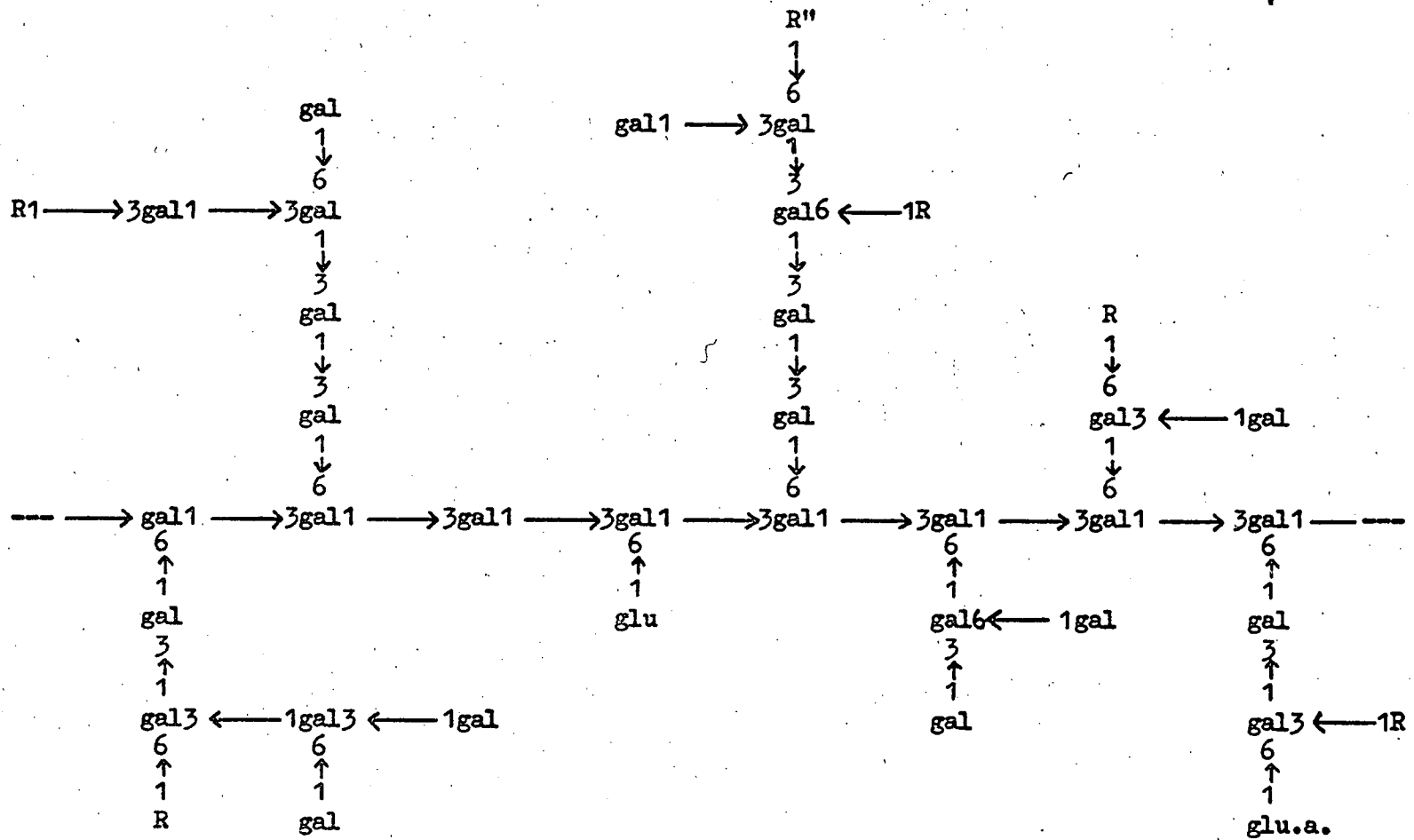
R" represents D-glucose or D-glucuronic acid.   R represents D – mannose, D – xylose, L – rhamnose,
                                                  L – arabinose or 1,2 – linked arabinose chains.

POSSIBLE STRUCTURAL FRAGMENT OF ANACARDIUM OCCIDENTALE GUM

The structural fragment shown in Figure IV,6, which, it must be emphasised, is not intended to represent any repeating structural unit within the molecule, is only one of many similar structures which can be postulated on the derived evidence. It resembles the galactose framework found for <u>Acacia</u> gums[9,11,12] of Bentham's Series 5, Vulgares. It also resembles the Series 5 <u>Acacias</u> in that it contains only two aldobiuronic acids namely $6 - \underline{O} - (\beta - \underline{D} - $ gluco-pyranosyluronic acid) $- \underline{D} -$ galactose and $6 - \underline{O} - (4 - \underline{O} -$ methyl $- \beta - \underline{D} -$ glucopyranosyluronic acid) $- \underline{D} -$ galactose, the longest observed arabinose side chains are 5 units long although none were found to be terminated by galactose, and the rhamnose to uronic acid ratio was found to be unity.

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SECTION V

ANALYTICAL AND STRUCTURAL FEATURES OF GUM EXUDATES

FROM THE COMBRETUM GENUS

SECTION V.A.

COMPARATIVE ANALYTICAL FEATURES OF GUM EXUDATES
FROM THE GENERA COMBRETUM AND TERMINALIA

V.A.1.   INTRODUCTION

PLANT GUMS OF THE FAMILY COMBRETACEAE R.Br.

The Combretaceae form an important family of tropical and sub-

tropical genera: the botanical relationship between its 20 different

genera is shown in Table V.A,1. in terms of the recent revision by

Exell and Stace[1], which involves an organisation into 2 sub-families,

2 tribes and 3 sub-tribes.

To date, only 5 species belonging to the Combretaceae have been

involved in studies of plant gum exudates, namely Anogeissus

latifolia[2-6], Anogeissus schimperi Hochst. [syn. A. leiocarpus

(DC.) Guill. et Perr.][7-11], Combretum verticillatum[12] [syn.

C. collinum Fresen. subsp. hypopilinum (Diels) Okafor] ,

Combretum leonense Engl. and Diels[13-15] [syn. C. glutinosum Perr. ex

DC.] and Terminalia tomentosa[16].

In view of the fact that Anogeissus latifolia is the origin of

gum ghatti, a gum exudate of considerable industrial importance, and

because the gum from C. leonense was found to be a very viscous

polysaccharide of potential commercial interest, it was considered

to be desirable to study as many other gums from Combretaceae species

as possible to establish their composition, properties and structural

features. Section V.A. reports the analytical data of 12 Combretum

species and 2 Terminalia species.

TABLE V.A,1.

Family ⟶ Sub-families

Combretaceae

(a) STREPHONEMATOIDEAE Engl. and Diels
[1 genus: Strephonema Hook. f.
(7 spp.)]

(b) COMBRETOIDEAE (10 genera)

Tribe

COMBRETEAE (16 genera)

Tribe

LAGUNCULARIEAE Engl. and Diels
[3 genera: Laguncularia Gaertn. f.
(1-2 spp.)
Lumnitzera Willd. (2 spp.)
Macropteranthes F. Muell
(4 spp.)]

Sub-tribe

COMBRETINAE (7 genera)

Sub-tribe

PTELEOPSIDINAE
(1 genus)

Sub-tribe

TERMINALIINAE (8 genera)

[Thiloa Eichl. (3 spp.)
Quisqualis L. (16 spp.)
Calopyxis Tul. (22 spp.)
Meiostemon Exell and
Stace (2 spp.)
Guiera Juss (1 spp.)
Calycopteris Lam
(1 spp.)
Combretum Loefl.
(approx 200 spp.)]

[Pteleopsis.
Engl.
(10 spp.)]

[Terminalia L. (approx
150 spp.)
Ramatuella Kunth (6 spp.)
Terminaliopsis Danguy
(1 spp.)
Bucida L., (9 spp.)
Buchenavia Eichl.
(22 spp.)
Anogeissus Wall.
(14 spp.)
Finetia Gagnep. (1 spp.)
Conocarpus L., (2 spp.)]

Sub-genus

Combretum

Sub-genus

Cacoucia

Sub-genus

Apetalanthum

(N.B.:- spp. = species)

BOTANICAL NOMENCLATURE OF THE GENUS COMBRETUM

The genus Combretum Loefl. is the largest in the Family COMBRETACEAE (order, MYRTALES); it is cosmopolitan in the tropics and sub-tropics, although absent[17] from Australia and the Pacific islands. The naming of species in Combretum has long been recognised[18] as a difficult problem, especially in the absence of flowers. Over 600 specific names are now known[17] to have been used to represent some 250 actual species, and consequently the synonymy is frequently unusually extensive (see origin of samples). The greatest range of structure and most of the difficult taxonomic problems are found in Africa, where approximately 180 of the known species occur[17]; there are approximately 30 Asian species[19].

There were several attempts at taxonomic classification in the nineteenth century. Engler and Diels[20] divided the species on a world-wide basis into 55 sections in 1899, and their classification still forms the basis for modern revisions, such as Exell's account[18] of the American species. A revision by Exell and Stace[1] in 1966 grouped Engler and Diel's sections, and 10 sections added by other authors, into subgenera, the major of which are subgenus Combretum, subgenus Cacoucia and subgenus Apetalanthum. In 1969, Exell and Stace[21] described 3 new sections (all African species) of the sub-genus Combretum; this brought the total number of sections in the genus to 68, of which 22 in subgenus Combretum and 5 in subgenus Cacoucia are recognised in Africa.

These taxonomic difficulties have arisen because the genus Combretum is a complex, heterogeneous population in which there

appears to be a continuous re-shuffling of genes[18]; a number of characters are found in nearly every combination. All that can be done is to give the "complexes" or "aggregates" of species the earliest legitimate name available; with new material continually being collected and ideas constantly changing, there is no way[22] of avoiding the inconvenience of changing the preferred name for species and for the extensive use of synonyms. Exell has pointed out[18] that there are three types of synonym: (a) nomenclatural synonyms indissolubly linked with the accepted name, (b) names given to plants that appear to be identical to the type, (c) names given to plants that differ from the type in certain characteristics, each of which represents one of the ways in which a number of characters can combine in a heterogeneous population.

V.A.2. NOMENCLATURE AND ORIGIN OF THE COMBRETUM GUM
        SAMPLES USED IN THIS STUDY

1. C. collinum Fresen., syns. C. binderanum Kotschy, C. mechowianum
        O. Hoffm., C. laeteviride Engl. and Gilg., C. cognatum
        Diels, C. bajonense Sim, C. gazense Swynnerton and Bak. f.,
        C. junodii Dummer, C. album De Wild., C. angustilanceolatum
        Engl., C. griseiflorum S. Moore, C. milleranum Burtt,
        C. tophamii Exell ex Burtt Davy and Hoyle, C. abercomense
        Exell, C. burtii Exell, C. eylesii Exell. (There are other
        synonyms, and also 11 subspecies.)

- Medium size, dark nodules, collected by Mr. J.H. Dick, Regional
Forest Officer, in September 1965 at Isenegaza Public Lands, Tabora,
Tanzania.   [Sect. METALLICUM]

2. C. collinum Fresen. subsp. hypopilinum (Diels) Okafor, syns.

C. verticillatum Engl., C. hypopilinum Diels,

C. kattoense Exell, C. flaviflorum Exell.

- Collected in 1955 for (the late) Professor R.J. McIlroy by

Mr. Oseni, Botanist at Western Region Department of Forestry,

Ibadan. A preliminary examination of this gum was published[12] by

Professor McIlroy, who subsequently very kindly placed his

Fraction A at our disposal for this work.   [Sect. METALLICUM]

3. C. erythrophyllum (Burch.) Sond., syns. C. glomeruliflorum Sond.,

C. riparium Sond., C. sonderi Gerr. ex Sond., C. ligustrifolium

Engl. and Diels ex Bak. f., C. lydenburgianum Engl. and Diels,

C. salicifolium sensu Monro.

- Collected in October 1970 by Mr. T. Gordon at Audley End Farm,

Darwendele, nr. Salisbury, Rhodesia.   [Sect. ANGUSTIMARGINATA]

4. C. zeyheri Sond., syns. C. tinctorum Welw. ex Laws., C. teuszii

O. Hoffm., C. glandulosum F. Hoffm., C. oblongum F. Hoffm.,

C. bragae Engl., C. lopolense Engl. and Diels.

- Small, dark nodules collected by Mr. J.H. Dick in September 1965

at Isenegaza Public Lands, Tabora, Tanzania.[Sect. SPATHULIPETALA]

5. C. molle R. Br., syns. C. velutinum DC., C. tricanthum Fresen.,

C. lepidotum A. Rich., C. gueinzii Sond., C. holosericeum

Sond., C. nyikae Engl., C. splendens Engl., C. ulugurense

Engl. and Diels, C. galpinii Engl. and Diels,

C. splendens Engl., C. arbuscula Engl. and Gilg,

C. atelanthum Diels, C. arengense Sim, C. ellipticum

Sim, <u>C. pretoriense</u> Dummer. (There are other synonyms
and some subspecies.)

- Medium-size, pale nodules, collected in September 1965 by Mr. J.H.

Dick at Isenegaza Public Lands, Tanzania.   [Sect. CILIATIPETALA]

6. <u>C. molle R. Br.</u> (as for 5.).
- Large, pale nodules collected in August 1967 by Mr. G.E. Rweyemamu,

Regional Forest Officer, at Tabora, Tanzania.

[Sect. CILIATIPETALA]

7. <u>C. apiculatum Sond.</u>, syn. <u>C. glutinosum</u> Wood non Perr. ex DC.

- Obtained from BAUCHI, Northern Nigeria, in January 1967, per

Mr. Kenyon, Tropical Products Institute, London.

[Sect. CILIATIPETALA]

8. <u>C. psidioides Welw.</u>, syns. <u>C. holosericeum</u> sensu Laws.,

<u>C. grandifolium</u> F. Hoffm. (There are 3 subspecies.)

- Medium size, dark nodules collected in September 1965 by Mr. J.H.

Dick at Isenegaza Public Lands, Tabora, Tanzania.

[Sect. CILIATIPETALA]

9. <u>C. nigricans Lepr. ex Guill. et Perr. var. elliotii</u> (Engl. and

<u>Diels) Aubrev.</u>

- Large, very dark nodules collected in 1955 by Mr. Oseqi, Botanist

at Western Region Department of Forestry, Ibadan, and placed at our

disposal by (the late) Professor R.J. McIlroy.

[Sect. CILIATIPETALA]

10.   <u>C. fragrans</u> F. Hoffm., syns. <u>C. kilossanum</u> Engl. and Diels,
            <u>C. ghasalense</u> Engl. and Diels, <u>C. ternifolium</u> Engl. and
            Diels, <u>C. tetraphyllum</u> Diels.

- Small, dark nodules collected in September 1965 by Mr. J.H. Dick

at Isenegaza Public Lands, Tabora, Tanzania.   [Sect. GLABRIPETALA]

11.   <u>C. fragrans</u> F. Hoffm. (as for 10)

- Dark, large nodules collected in May 1970 by Mr. A.G. Sief-el-Din,

Sudanese Gum Research Officer, at Rashad, Republic of the Sudan.

[Sect. GLABRIPETALA]

12.   <u>C. hartmannianum</u> Schweinf.

- Large, pale nodules collected in December 1970 by Mr. A.G. Seif-el-

Din at Umin Abdalla, Republic of the Sudan.   [Sect. GLABRIPETALA]

13.   <u>C. glutinosum</u> Perr. ex DC., syn. <u>C. leonense</u> Engl. and Diels.

- Collection details as for sample 9, <u>C. nigricans</u>.

[Sect. GLABRIPETALA]

14.   <u>C. obovatum</u> F. Hoffm., syn. <u>C. lasiocarpum</u> sensu Exell and
            Garcia.

- Large, pale nodules collected in September 1965 by Mr. J.H. Dick

at Isenegaza Public Lands, Tabora, Tanzania.   [Sect. LASIOPETALA]

15.   <u>C. obovatum</u> F. Hoffm., (as for 14).

- Large pale nodules collected in August 1967 by Mr. G.E. Rweyemamu,

Regional Forest Officer, at Tabora, Tanzania.   [Sect. LASIOPETALA]

Table V.A.2 shows a tabular representation of the botanical relationship between the sections and species examined in this study.

TABLE V.A.2.

ORDER - MYRTALES
FAMILY - Combretaceae; Sub-family - Combretoideae
Tribe - Combreteae
Sub-tribe - Combretinae
Genus: Combretum Loefl.

Subgenus

COMBRETUM

Subgenus

CACOUCIA

(Aubl.) Exell and Stace

Section: ANGUSTIMARGINATA
    Engl. and Diels :
    C. erythrophyllum

Section: LASIOPETALA Engl. and
Diels:C. obovatum

Section: CILIATIPETALA Engl. and
    Diels:C. molle, C. apiculatum,
    C. psidioides, C. nigricans

Section: GLABRIPETALA Engl. and
    Diels emend Exell and Stace :
    C. fragrans, C. hartmannianum,
    C. glutinosum

Section: METALLICUM Exell and Stace
    (syn. GLABRIPETALA Engl. and
    Diels, pro parte):C. collinum,
    C. collinum subsp. hypopilinum

Section: SPATHULIPETALA Engl. and
    Diels:C. zeyheri

V.A.3.   ORIGIN OF THE TERMINALIA SAMPLES USED IN THIS STUDY

The two Terminalia samples studied were from (a) T. sericea
Burch., collected in October 1970 by Mr. T. Gordon, Audley End Farm,

Darwendele, Salisbury, Rhodesia, and (b) T. superba Engl. and Diels,

collected in April 1970 by W. Kriek per Mr. J.J. Lawrie, Marak

Forest Reserve, Cameroun.

V.A.4. PURIFICATION OF SAMPLES

With the exception of T. superba, all the samples were supplied

in nodular form. After being crushed to pass a small mesh sieve,

small amounts (3g) were dissolved in water (300 ml) for 2 days,

after which time all samples had almost completely dissolved, except

for C. collinum, C. fragrans (sample B) and C. nigricans, which

required the addition of a small amount of sodium borohydride to

facilitate dissolution[23]. The solutions (pH 4.5), were filtered

through muslin to remove bark and other insoluble debris, then

through Whatman No. 41 filter paper and finally through No. 1

filter paper, after which the solutions were dialysed against

running tap water for 2 days, (4 days in the case of borohydride -

treated samples). The polysaccharides were obtained as the freeze-

dried products.

T. superba gum, which had been collected in the form of a soft,

plastic mass, and sent in a polythene package, was dissolved in

distilled water, filtered, and freeze-dried.

V.A.5. RESULTS

Hydrolysis with sulphuric acid (2N) for 7.5 hours showed that

all the Combretum and Terminalia samples contained galacturonic acid,

glucuronic acid and glucurono - 6,3 - lactone. Some samples showed

the presence of large amounts of 4 - $\underline{O}$ - methylglucuronic acid.
Typical R_{gal} values for uronic acids are shown in Table V.A,3.
The determination of the ratio of galacturonic acid: glucuronic acid
was similar to the method used for neutral sugars. Chromatography
of the 2N - hydrolysate was carried out in solvent (g), side-strips
cut, each component eluted and their ratio determined. Combination
of the amounts of glucuronic acid and glucurono - 6,3 - lactone gave
the total glucuronic acid content.

TABLE V.A,3.

URONIC ACIDS OBTAINED ON 2N - HYDROLYSIS OF
COMBRETUM AND TERMINALIA SAMPLES

Uronic acid	R_{gal} value	colour of spot
galacturonic acid	0.50	reddish/brown
glucuronic acid	0.67	brown
4-O-methylglucuronic acid	1.28	pink
glucurono-6,3-lactone	1.50	yellow brown

Hydrolysis with 1N - H_2SO_4 for 7.5 hours followed by chromato-
graphy in solvents (a), (b) and (c) showed that all the samples
contained galactose, arabinose and rhamnose; in addition, some were
also found to contain significant amounts of mannose and xylose.
Sugar ratios were determined for all the samples in the same way
as for Anacardium occidentale (see Section IV.A), by running two

Whatman 3MM chromatography papers for each sample, one in solvent (b) and one in solvent (c). Chromatography in solvent (b) determined the ratio of galactose: (mannose + arabinose): xylose: rhamnose, and chromatography in solvent (c) determined the ratio of mannose: (arabinose + xylose): rhamnose. By a combination of both results, the complete sugar ratios were determined as shown in Table V.A,4.

As well as there being differences in sugar content of the samples, chromatography in solvent (c) of the 1N - hydrolysate showed basic differences in biuronic acid content of certain of the samples. The majority of the samples showed biuronic acid spots at R_{gal} 0.28 and 0.88 which correspond to 6 - $\underline{O}$ - (β - $\underline{\underline{D}}$ - gluco-pyranosyluronic acid) - $\underline{\underline{D}}$ - galactose and 2 - $\underline{O}$ - (α- $\underline{\underline{D}}$ - galacto-pyranosyluronic acid) - $\underline{\underline{L}}$ - rhamnose as found previously in C. leonense by Aspinall and Bhavandan[14,15] and verified in this study (see Section V.B). Other samples which contained both the above biuronic acids also showed a spot at R_{gal} 0.48. These samples were also found to have a high methoxyl content and this spot is attributed to the presence of the biuronic acid, 6 - $\underline{O}$ - (4 - $\underline{O}$ - methyl - β - $\underline{\underline{D}}$ - glucopyranosyluronic acid) - $\underline{\underline{D}}$ - galactose (see Section V.B).

However, certain Combretum samples and both the Terminalia samples showed, as well as the spot at R_{gal} 0.28, a large spot at R_{gal} 0.51 but nothing at R_{gal} 0.88, indicating the absence of galacturonic acid - rhamnose biuronic acid. It was also noted that these samples differed from the other Combretum samples in that they contained considerable amounts of mannose and xylose. On the

TABLE V.A.4.

SUGAR RATIOS FOR COMBRETUM AND TERMINALIA SAMPLES

Section	Sample	gal.	ara.	rha.	mann.	xyl.
Angustimarginata	C. erythrophyllum	10	26.1	7.4	-	trace
Ciliatipetala	C. apiculatum	10	8.6	5.9	2.2	2.4
	C. molle (sample A)	10	9.6	3.8	-	trace
	C. molle (sample B)	10	9.4	3.6	-	trace
	C. nigricans	10	18.3	4.6	-	trace
	C. psidioides	10	5.9	3.7	1.4	trace
Glabripetala	C. fragrans (sample A)	10	12.4	5.0	1.7	trace
	C. fragrans (sample B)	10	15.4	5.5	-	trace
	C. glutinosum	10	10.6	3.2	-	sl. trace
	C. hartmannianum	10	32.4	3.2	3.2	4.4
Metallicum	C. collinum	10	35.4	5.7	3.5	2.2
	C. collinum (subsp. hypopilinum)	10	6.7	3.7	1.3	1.1
Spathulipetala	C. zeyheri	10	18.6	6.5	-	trace
Lasiopetala	C. obovatum (sample A)	10	16.9	7.5	3.0	4.0
	C. obovatum (sample B)	10	9.6	4.1	1.4	1.3
(Terminalia)	T. sericea	10	33.7	4.5	3.6	4.1
	T. superba	10	38.5	4.0	4.5	3.5

whole, these samples did not have a high methoxyl content and hence did not contain very much 4 - $\underline{O}$ - methylglucuronic acid, so the significant spot at R_{gal} 0.51 would have to be due to something other than 6 - $\underline{O}$ - (4 - $\underline{O}$ - methyl - $\underline{D}$ - glucuronosyluronic acid) - $\underline{D}$ - galactose. Also, in these samples, galacturonic acid had to be accounted for as it was not present in a biuronic acid linked to rhamnose. On the basis of experimental work (see Section V.B) the biuronic acid R_{gal} 0.51 is thought to consist of galacturonic acid and mannose.

Table V.A,5.shows the biuronic acid spots present in all the samples. The percentage of sugars present in each sample was calculated, taking the biuronic acids present into account. Tables V.A,6 - 9.show the complete analytical data for all the Combretum samples studied; Table V.A,10.shows the analytical data for the two Terminalia samples.

Several of the crude gums had a "vinegar-type" odour; an acetyl content was carried out on each sample, some of the samples showing the presence of quite substantial amounts of acetyl (4 - 7%) present.

The relationship between viscosity, $[\eta]$ and molecular weight, $\bar{M}w$, for the series of Combretum species studied was examined:-

$$[\eta] = KM^{\alpha} \text{ , where K and } \alpha \text{ are constants}$$

$$\therefore \log [\eta] = \log K + \alpha \log M$$

$$\text{ie:-} \quad y = \text{const} + mx$$

Table V.A,11.shows the value of y and x for each of the species studied.

TABLE V.A,5.

BIURONIC ACID SPOTS OBTAINED BY CHROMATOGRAPHY IN SOLVENT 18.3.1.4 AFTER HYDROLYSIS OF COMBRETUM AND TERMINALIA SAMPLES WITH 1N-H_2SO_4

Section	Sample	R_{gal} 0.28	R_{gal} 0.47	R_{gal} 0.51	R_{gal} 0.88
Angustimarginata	C. erythrophyllum	+	-	-	+
Ciliatipetala	C. apiculatum	+	+	-	+
	C. molle (sample A)	+	-	-	+
	C. molle (sample B)	+	-	-	+
	C. nigricans	+	-	-	+
	C. psidioides	+	-	-	+
Glabripetala	C. fragrans (sample A)	+	+	-	+
	C. fragrans (sample B)	+	+	-	+
	C. glutinosum	+	-	-	+
	C. hartmannianum	+	-	+	-
Metallicum	C. collinum	+	-	+	-
	C. collinum (subsp. hypopilinum)	+	+	-	+
Spathulipetala	C. zeyheri	+	-	-	+
Lasiopetala	C. obovatum (sample A)	+	-	+	-
	C. obovatum (sample B)	+	-	+	-
(Terminalia)	T. sericea	+	-	+	-
	T. superba	+	-	+	-

TABLE V.A.6.

ANALYTICAL DATA FOR GUM POLYSACCHARIDES FROM
COMBRETUM SPECIES, SUBGENUS COMBRETUM, SECTIONS
METALLICUM*, ANGUSTIMARGINATA** AND SPATHULIPETALA***

	* □ C. collinum (1)	C. collinum subsp. hypopilinum (2)	** C. erythrophyllum (3)	*** C. zeyheri (4)
DATA ON CRUDE GUM				
Moisture, %	9.8	15.7	12.0	12.5
Ash, %	4.1	13.5	3.6	6.2
Nitrogen, %	.11	.09	.12	.51
Hence, protein, % (N x 6.25)[a]	0.7	0.6	0.8	3.2
Acetyl, %[b]	0.3	0.5	1.7	3.0
DATA ON PURIFIED GUM				
Recovery from crude gum	73	87	78	81
Moisture, %	8.9	13.8	10.6	12.3
Ash, %	3.3	11.7	5.7	8.2
Nitrogen, %[a]	0.13	0.10	0.11	0.16
Hence, protein, % (N x 6.25)[a]	0.8	0.6	0.7	1.0
Methoxyl, %[b]	0.45	1.46	0.33	0.38
$[\alpha]_D$ in distilled water, (degrees)[b]	−81	+53	−54	+7
Intrinsic viscosity, $[\eta]_{25}$ ml/g[a]	312	60	110	163
Molecular Weight, (MW x 10^5)[a]	116	7.3	13	56
Equivalent weight,[b]	1405	398	745	472
Hence, uronic anhydride[b,c]	12.5	44.3	23.6	37.3
Sugar composition[b] after hydrolysis				
4-O-methylglucuronic acid[d]	2.7	8.8	2.0	2.3
glucuronic acid	6.7	23.9	13.9	25.0
galacturonic acid	3.1	11.6	7.7	10.0
galactose	22	36	27	34
arabinose	47	5	33	15
rhamnose	8	13	16	14
mannose	9	1	−	−
xylose	3	1	trace	trace

TABLE V.A.7.

ANALYTICAL DATA FOR GUM POLYSACCHARIDES FROM
COMBRETUM SPECIES SUBGENUS COMBRETUM
SECTION CILIATIPETALA

	C. molle (sample A) (5)	C. molle (sample B) (6)	C. apiculetum (7)	C. psidioides (8)	C. nigricans (9)
DATA ON CRUDE GUM					
Moisture, %	11.8	11.6	12.4	13.4	10.6
Ash, %	5.0	4.3	6.3	4.4	3.0
Nitrogen, %[a]	.19	.20	.24	.34	.35
Hence, protein, % (N x 6.25)[a]	1.2	1.2	1.5	2.1	2.2
Acetyl, %[b]	5.6	5.9	3.0	7.0	2.5
DATA ON PURIFIED GUM					
Recovery from crude gum, %	87	79	58	78	80
Moisture, %	11.3	8.4	11.9	11.1	9.1
Ash, %	4.0	3.9	7.0	5.8	3.1
Nitrogen, %[a]	0.08	0.08	0.25	0.11	0.10
Hence, protein, % (N x 6.25)[a]	0.5	0.5	1.6	0.7	0.6
Methoxyl, %[b]	0.28	0.40	0.68	0.22	0.24
$[\alpha]_D$ in distilled water, (degrees)[b]	+2	+3	+24	+47	-43
Intrinsic viscosity, $[\eta]_5$ ml/g[a]	74	70	188	146	35
Molecular weight, (MW x 10^5)[a]	5.3	6.3	30	14	4.8
Equivalent weight[b]	983	965	674	766	1244
Hence, uronic anhydride[b,c]	17.9	18.3	26.1	22.3	14.0
Sugar composition[b] after hydrolysis					
4-O-methylglucuronic acid[d]	1.7	2.4	4.1	1.3	1.4
glucuronic acid	10.5	10.3	13.9	14.3	7.7
galacturonic acid	5.7	5.6	8.1	6.7	4.9
galactose	40	40	34	42	30
arabinose	27	27	15	16	41
rhamnose	16	15	17	16	15
mannose	-	-	4	4	-
xylose	trace	trace	4	trace	trace

TABLE V.A.8.

ANALYTICAL DATA FOR GUM POLYSACCHARIDES FROM COMBRETUM SPECIES SUBGENUS COMBRETUM SECTION GLABRIPETALA

	C. fragrans sample (A) (10)	C. fragrans sample (B) (11)	C. hartmannianum (12)	C. glutinosum (13)
DATA ON CRUDE GUM				
Moisture, %	13.5	13.1	11.9	13.0
Ash, %[a]	7.0	7.7	3.8	3.5
Nitrogen, %[a]	.27	.27	.64	.25
Hence, protein, % (N x 6.25)[a]	1.7	1.7	4.0	1.6
Acetyl, %[b]	4.4	2.5	0.4	4.2
DATA ON PURIFIED GUM				
Recovery from crude gum, %	79	65	83	n.d.
Moisture, %	12.5	12.0	8.3	11.9
Ash, %[a]	7.7	8.3	3.7	3.3
Nitrogen, %[a]	0.17	0.18	0.61	0.07
Hence, protein, % (N x 6.25)[a]	1.1	1.1	3.8	0.4
Methoxyl, %[b]	0.85	1.07	0.25	0.58
$[\alpha]_D$ in distilled water, (degrees)[b]	+35	+41	-35	-9
Intrinsic viscosity, $[\eta]$, ml/g[a]	162	170	63	75
Molecular weight, (MW x 10^5)[a]	17	49	6.4	5.3
Equivalent weight[b]	505	487	1173	1073
Hence, uronic anhydride[b,c]	35.0	36.2	15.0	16.4
Sugar composition[b] after hydrolysis				
4-O-methylglucuronic acid[d]	5.1	6.4	1.5	3.5
glucuronic acid	19.7	19.8	7.4	8.9
galacturonic acid	10.2	10.0	6.1	4.0
galactose	34	34	22	40
arabinose	14	16	43	31
rhamnose	15	14	4	13
mannose	2	–	10	–
xylose	trace	trace	6	sl. trace

TABLE V.A.9.

ANALYTICAL DATA FOR GUM POLYSACCHARIDES FROM COMBRETUM SPECIES, SUBGENUS CACOUCIA SECTION LASIOPETALA

	C. obovatum (sample A)	C. obovatum (sample B)
	(14)	(15)
DATA ON CRUDE GUM		
Moisture, %	11.5	12.2
Ash, %	5.4	5.3
Nitrogen, %[a]	0.26	0.18
Hence, protein, % (N x 6.25)[a]	1.7	1.6
Acetyl, %[b]	4.4	1.7
DATA ON PURIFIED GUM		
Recovery from crude gum	84	79
Moisture, %	10.5	9.9
Ash, %[a]	5.9	5.1
Nitrogen, %[a]	0.23	0.10
Hence, protein, % (N x 6.25)[a]	1.4	0.6
Methoxyl, %[b]	0.37	0.21
$[\alpha]_D$ in distilled water, (degrees)[b]	-45	-18
Intrinsic viscosity, $[\eta]_{25}$ ml/g[a]	200	139
Molecular Weight, (MW x 10^5)[a]	141	35
Equivalent weight[b]	761	816
Hence, uronic anhydride[b,c]	23.1	21.6
Sugar composition[b] after hydrolysis		
4-O-methylglucuronic acid[d]	2.2	1.3
glucuronic acid	13.8	12.8
galacturonic acid	7.1	7.5
galactose	28	35
arabinose	23	21
rhamnose	10	9
mannose	11	10
xylose	5	3

TABLE V.A.10.

ANALYTICAL DATA FOR PURIFIED GUM POLYSACCHARIDES FROM TWO TERMINALIA SPECIES

	T. sericea	T. superba
Moisture, %	9.2	12.2
Ash, %[a]	2.4	0.6
Nitrogen, %[a]	0.46	0.18
Hence, protein, % (N x 6.25)[a]	2.9	1.1
Acetyl, %[b]	0.6	0.3
Methoxyl, %[b]	1.20	0.39
$[\alpha]_D$ in distilled water, (degrees)[b]	-13	+44
Intrinsic viscosity, $[\eta]$, ml/g[a]	145	157
Molecular weight, (MW x 10^5)[a]	21	40
Equivalent weight[b]	1615	1659
Hence, uronic anhydride[b,c]	10.9	10.6
Sugar composition[b] after hydrolysis		
4-O-methylglucuronic acid[d]	7.2	2.3
glucuronic acid	1.6	5.2
galacturonic acid	2.1	3.1
galactose	22	20
arabinose	48	51
rhamnose	6	5
mannose	7	9
xylose	6	4

KEY TO TABLES

☐   Borohydride added to facilitate dissolution

a   Corrected for moisture content

b   Corrected for moisture and protein contents

c   If all acidity arises from uronic acids

d   If all methoxyl groups located in this acid

The molecular weights shown were calculated using a dn/dc value of 0.146 as found for a series of Acacia gums. A value of dn/dc of 0.142 has been previously reported[24] for C. psidioides, so the above assumption would seem to hold fairly well considering the report that within one genus, the values of dn/dc of 23 species were almost identical[25].

TABLE V.A.11.

Sample	Ref. no.	log $[\eta]$	log $\overline{M}_w$
C. collinum	1	2.494	7.064
C. collinum subsp. hypopilinum	2	1.778	5.863
C. erythrophyllum	3	2.042	6.114
C. zeyheri	4	2.212	6.747
C. molle (sample A)	5	1.869	5.724
C. molle (sample B)	6	1.845	5.799
C. apiculatum	7	2.274	6.477
C. psidioides	8	2.164	6.146
C. nigricans	9	1.544	5.681
C. fragrans (sample A)	10	2.209	6.230
C. fragrans (sample B)	11	2.230	6.690
C. hartmannianum	12	1.799	5.806
C. glutinosum	13	1.875	5.724
C. obovatum (sample A)	14	2.301	7.149
C. obovatum (sample B)	15	2.143	6.544

Figure V.A,1. shows a plot of log $[\eta]$ against log $\overline{M}_w$. The graph shows a reasonably linear relationship considering the experimental errors in all the parameters. Taking the best straight line as shown, the values of K and α were found to be 0.10 and 0.49 respectively.

Figure V.A,2. shows a plot of ash content against uronic acid content. Within the experimental error boundaries of the two

GRAPH OF LOG [η] AGAINST LOG M̄w FOR COMBRETUM SAMPLES
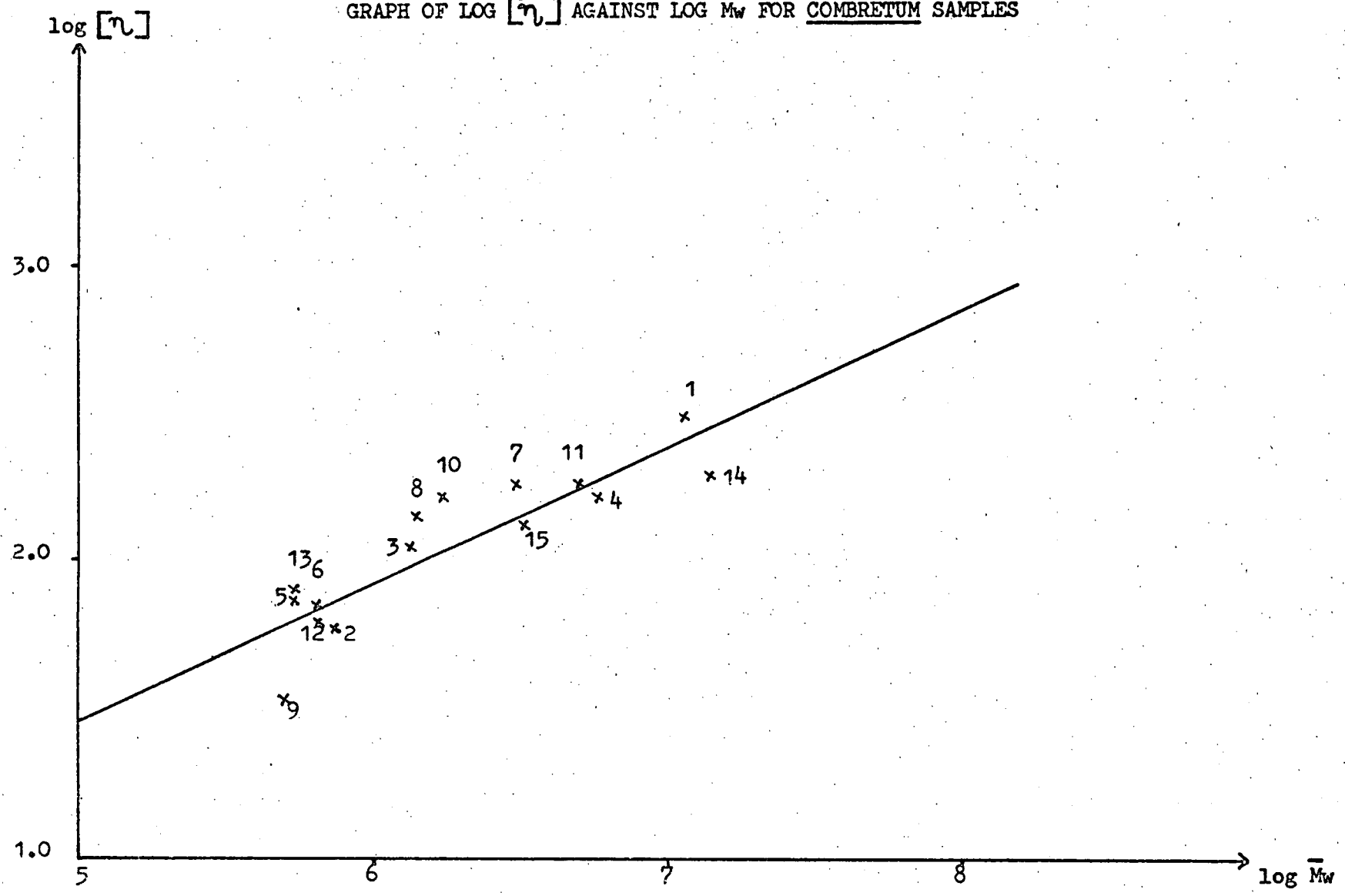
GRAPH OF URONIC ACID AGAINST ASH CONTENT FOR COMBRETUM SAMPLES

105.
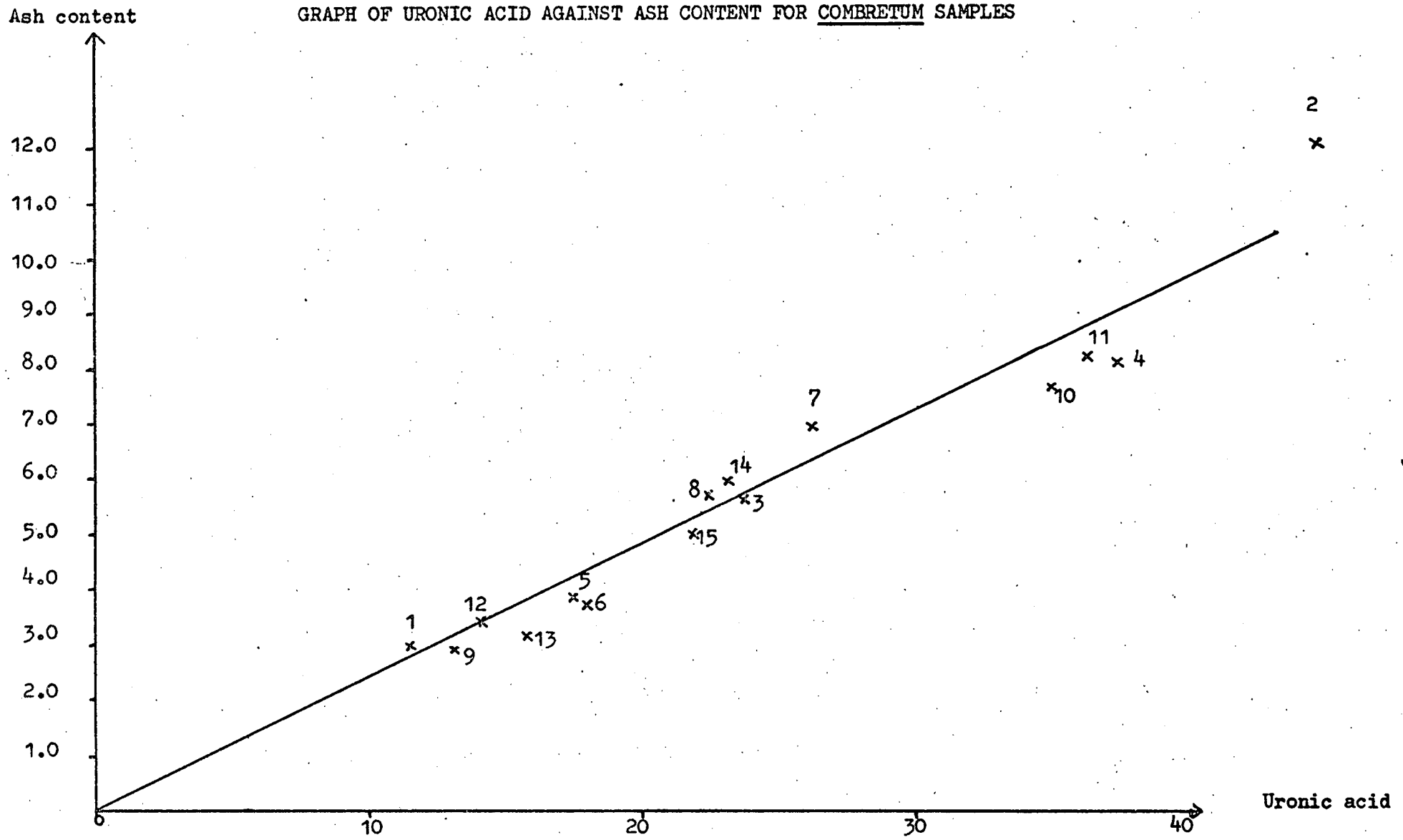
parameters, there would appear to be a very good linear relationship between uronic acid and ash content. This is as expected, since most of the -COOH groups in the gum molecule would exist in the ionised form, the negative ions would form ionic bonds with positive ions such as Na, K, Mg and Ca, a fact confirmed by the pH of the gum solutions of 4.6, which is not particularly low. The ash content, after all the carbohydrate material has been burned off mainly as CO_2 and H_2O, consists of the oxides and carbonates of the above and some other metallic elements, such as Mn, Co etc.

V.A.6. DISCUSSION

On the whole, the analytical data found for C. glutinosum, C. collinum subsp. hypopilinum, C. molle, C. psidioides and C. nigricans agree with the values found previously by Speed[24], (C. glutinosum called C. leonense and C. collinum subsp. hypopilinum called C. verticillatum) and confirm the values published for C. leonense by Anderson, Hirst and King[13]. However, McIlroy[12] reported that C. verticillatum contained galactose, arabinose and glucuronic acid as the only uronic acid present. This is disputed by the present findings although the $[\alpha]_D$ value of $+53°$ found for this gum by McIlroy agrees exactly with the value obtained for C. collinum subsp. hypopilinum.

The analytical data show some very interesting features. The majority of the gums in the purified form are virtually free of protein ($\not< 1\%$). The methoxyl contents, although showing a wide variation, are within the normal range. The specific rotations

also show a wide range (-81° to +53°), with C. collinum having the highest-recorded, negative rotation (-81°).

The outstanding features of the Combretum genus shown by this study are the high ash and hence uronic acid contents and the unusually high viscosities and molecular weights. The ash content (11.7%) and uronic acid content (44.3%) of C. collinum subsp. hypopilinum are exceptionally high and nothing comparable has been reported. Also, the viscosity of C. collinum (312 ml/g) and $\overline{Mw}$ of C. obovatum (sample A) (1.4×10^7) by far surpass the previous highest recorded values. As a comparison, the highest recorded $\overline{Mw}$ for gums of the Acacia genus are 2.3×10^6 for A. arabica, 2.3×10^6 A. nilotica and 2.4×10^6 for A. adamsonii[24]. The high viscosity and molecular weight would suggest a complicated, possibly rod-shaped, molecular structure for the gums.

The sugar ratios (see Table V.A.4) show quite a wide variation in galactose: arabinose: rhamnose content as well as differences in the sugars present, where some samples contain mannose and xylose and others do not. An interesting point to emerge from examining the sugar ratios and optical rotations is that samples with a high arabinose content have a high negative $[\alpha]_D$ value and those with a low arabinose content have a high +ve value, exemplified by C. collinum, galactose: arabinose 10: 35.4, $[\alpha]_D$ -81 and C. psidioides 10: 5.9, $[\alpha]_D$ +47. (This would seem to disprove the high arabinose sugar ratio found in C. verticillatum by McIlroy, since $[\alpha]_D$ was found to be +53°). However, although a fall in arabinose content seemed to be coupled with $[\alpha]_D$ becoming less negative and then

positive, there was no visible linear relationship. The Terminalia

samples did not fit into this pattern since, although they both had

a high arabinose content, they did not have high negative optical

rotations, in fact T. superba had a high +ve value (+44°). This would

seem to indicate structural differences between the Combretum and

Terminalia gums, which would be as expected botanically.

Hydrolysis with $1N-H_2SO_4$ also indicated structural differences

within the Combretum genus as regards the aldobiuronic acids.

Although all the samples, including the two Terminalia samples,

contained 6 - O - (β - D - glucopyranosyluronic acid) - D - galactose,

there was a difference in the galacturonic acid - containing

biuronic acid. Most of the Combretum gums contained 2 - O - (α - D -

galactopyranosyluronic acid) - L - rhamnose which had been found and

characterised previously in C. leonense[14,26] and is a common aldobiu-

ronic acid in other genera such as Sterculia[27-29], Khaya[30,31] and is

found in the gum from Cochlospermum gossypium[32]. However, some of

the samples, including the two C. obovatum samples from the

Lasiopetala subsection of Combretum, as well as the two Terminalia

gums, contained a biuronic acid of unknown structure but expected,

from experimental evidence (see Section V.B), to contain mannose and

galacturonic acid. Such a biuronic acid has not been reported

previously in any gum. However, the biuronic acid, 2 - O - (β - D -

glucopyranosyluronic acid) - D - mannose has been found in Anogeissus

samples[2,5,8,33], which were reported to contain glucuronic acid as

their only uronic acid, a fact confirmed during this present

investigation. So there is a possibility that the biuronic acid

found in the <u>Combretum</u> and <u>Terminalia</u> gums may be 2 - $\underline{O}$ - (β- $\underline{\underline{D}}$ -

galactopyranosyluronic acid) - $\underline{\underline{D}}$ - mannose. The isolation and

structural examination of this biuronic acid is the subject of a

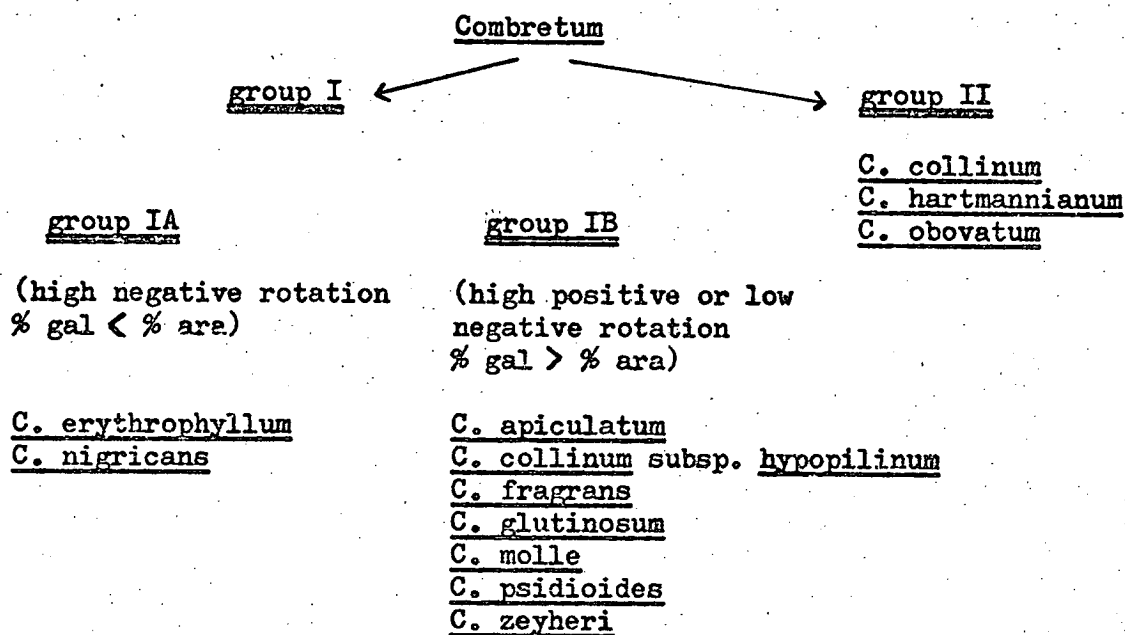present investigation[34].

However, the finding of such a biuronic acid in <u>Terminalia</u>

samples but not in <u>Anogeissus</u> gums would throw doubt on the botanical

classification, where <u>Anogeissus</u> and <u>Terminalia</u> appear together, on

chemical grounds. The two <u>Terminalia</u> samples were found conclusively

to contain galacturonic acid, whereas <u>Anogeissus</u> samples were not.

So it would seem that the gums from the <u>Terminalia</u> genus resemble

certain gums of Combretum rather than <u>Anogeissus</u>.

An interesting feature found in the study of these <u>Combretum</u>

gum exudates is the presence of two separate structural types,

identified by their different biuronic acids. Also, the samples

with the biuronic acid containing mannose and galacturonic acid

contained significant amounts of mannose and xylose as well as

galactose, arabinose and rhamnose. This disputes, on chemical

grounds, certain features of the classification shown in

Table V.A,4. <u>C. collinum</u> and its subspecies <u>hypopilinum</u> are

different both analytically and as regards biuronic acid content

and should belong to different sections. Also, <u>C. hartmannianum</u>

does not fit in with the other members of the Glabripetala but,

together <u>C. collinum</u>, resembles <u>C. obovatum</u>. <u>C. nigricans</u> seems

to resemble <u>C. erythrophyllum</u> from the Angustimarginata more than

the other members of the Ciliatipetala, whereas <u>C. collinum</u> subsp.

<u>hypopilinum</u> would fit in very well to the Ciliatipetala. <u>C. zeyheri</u>

from the Spathulipetala resembles the gums of the Glabripetala with

the exception of <u>C. hartmannianum</u>.

On the basis of this experimental work, the <u>Combretum</u> samples could be divided into two groups, with one group subdivided into two:- Table V.A,12.

TABLE V.A,12.

CLASSIFICATION OF COMBRETUM SAMPLES STUDIED ON THE BASIS OF CHEMICAL ANALYSIS

Combretum

group I ← → group II

		<u>group II</u>
		<u>C. collinum</u>
		<u>C. hartmannianum</u>
		<u>C. obovatum</u>

<u>group IA</u>

(high negative rotation % gal < % ara)

<u>C. erythrophyllum</u>
<u>C. nigricans</u>

<u>group IB</u>

(high positive or low negative rotation % gal > % ara)

<u>C. apiculatum</u>
<u>C. collinum</u> subsp. <u>hypopilinum</u>
<u>C. fragrans</u>
<u>C. glutinosum</u>
<u>C. molle</u>
<u>C. psidioides</u>
<u>C. zeyheri</u>

And so, on the basis of chemical evidence, three main points emerge from this study:- (a) <u>Anogeissus</u> and <u>Terminalia</u> have different structural features but are classed together botanically; (b) the <u>Combretum</u> genus would seem to be divisible into two sub-divisions on the basis of structural features (see Section V.B); (c) one of the <u>Combretum</u> divisions can be further subdivided into two on the basis of sugar content and rotation.

In the light of this evidence, the Family Combretaceae is a very complex family and not as straightforward as other families studied to date.

SECTION V.B

A PRELIMINARY STUDY OF THE STRUCTURAL FEATURES OF
THE PLANT GUMS FROM COMBRETUM ERYTHROPHYLLUM
AND COMBRETUM HARTMANNIANUM

V.B.1. INTRODUCTION

Unlike the Acacia genus, where extensive work has been carried out
in the field of chemical taxonomy, relatively little is known about
the chemistry of gums from the Combretum genus. The only structural
investigations so far have been carried out by Aspinall and
Bhavanandan[14,15] on the gum from C. leonense (referred to here by its
botanically preferred synonym C. glutinosum); they carried out
hydrolysis studies on the gum and characterised the fragments
obtained. The neutral fragments were found to be 3 - $\underline{O}$ -β- $\underline{L}$ -
arabinopyranosyl - $\underline{L}$ - arabinose, 3 - $\underline{O}$ -β- $\underline{D}$ - galactopyranosyl -
$\underline{L}$ - arabinose and 6 - $\underline{O}$ -β- $\underline{D}$ - galactopyranosyl - $\underline{D}$ - galactose.
The aldobiuronic acids isolated were found to be 6 - $\underline{O}$ - (β- $\underline{D}$ -
glucopyranosyluronic acid) - $\underline{D}$ - galactose and 2 - $\underline{O}$ - (α- $\underline{D}$ -
galactopyranosyluronic acid) - $\underline{L}$ - rhamnose.

On the basis of the findings of Section V.A, which proposes the
existence of two distinct structural types within the genus
Combretum, it was decided to examine the structural features of both
types to see if the differences proposed were slight or significant.
Section V.B therefore examines structural features of (a) the gum
from C. erythrophyllum, on the basis of a Smith degradation and
hydrolysis studies, to determine its similarity or otherwise to
C. leonense, and (b) the gum from C. hartmannianum, which on the

basis of sugar content and aldobiuronic acids differs markedly from both C. erythrophyllum and C. leonense. Both C. erythrophyllum gum and C. hartmannianum gum have very complex structures and it was decided that the limited time and material available should be used to establish some of the broad features of the gums and their degree of complexity, rather than minor details. With the present preliminary study as a guide, it is hoped that some future investigator will be able to start work on a scale necessary to allow full chemical characterisations of all isolated components to be undertaken.

SECTION V.B PART A

STRUCTURAL FEATURES OF THE GUM FROM C. ERYTHROPHYLLUM (BURCH.) SOND

V.B.2. PURIFICATION OF C. ERYTHROPHYLLUM GUM

Crude gum (102.7 g) was stirred in distilled water (1.5 l). After 2 days, solubilisation was incomplete. The gum solution was decanted off and more water (2 l) was added to the undissolved gum; after a further 2 days, all the gum had dissolved. The decanted solutions were filtered through Whatman No. 41 and then through No. 1 filter papers and dialysed against running tap water. The total gum solution (volume ~9 l) was concentrated to 4 l, filtered, and the pure gum obtained as the freeze-dried product (70.3 g, yield 69%). Values for the moisture content (8%), intrinsic viscosity (102 ml/g) and specific rotation (-57°) were similar to the values obtained previously (see Section V.A).

V.B.3.   RESULTS

The polysaccharide migrated as a single band on electrophoresis on cellulose acetate film in 0.1M - ammonium carbonate buffer (pH 8.9) and 0.1M - acetate buffer, and on thin-layer electrophoresis of dyed and undyed polysaccharide on Phoroslides in 0.05M - ammonium carbonate buffer and 0.05M borate buffer (pH 9.2). Ion exchange chromatography on a DEAE - cellulose column using a sodium chloride gradient (0.0 - 0.5M in 0.02M - acetate buffer) produced one single symmetrical peak, as did ultracentrifugation in 0.5M sodium chloride. Molecular-sieve chromatography on Bio-gel A-5 and A-15 columns in each case showed a sharp peak at the void volume, indicating that the high molecular weight polysaccharide was excluded from the gel. M-SC on a Bio-gel A150 column, however, gave a single symmetric peak at greater than the void volume. All these experiments indicate that the gum from C. erythrophyllum shows no distinct heterogeneity.

IDENTIFICATION OF NEUTRAL SUGARS

C. erythrophyllum gum (1 g) was hydrolysed with 1N - sulphuric acid for 7.5 hours on a boiling water bath. The cooled solution was neutralised, filtered, deionised and concentrated to a syrup, which was applied to a column (41 x 2.6 cm) of Duolite A-4 resin in the formate form. Elution with distilled water (400 ml) yielded the neutral sugars present in the hydrolysate. After concentration of the neutral fraction to a syrup, chromatography in solvents (a), (b) and (c) showed the presence of galactose, arabinose and rhamnose; arabinose was the major constituent. A slight trace of xylose was also present.

IDENTIFICATION OF THE ALDOBIURONIC ACIDS

Elution of the Duolite A-4 column with 5% formic acid (500 ml) yielded the acidic fraction of the hydrolysate. This acidic solution was concentrated to a syrup and chromatography was carried out in solvents (c), (d) and (g). Trace amounts of galactose and arabinose, two major spots (R_{gal} 0.28 and 0.88) and a trace spot at R_{gal} 0.47 (all in solvent (c)) were detected. Solvent (g) also showed the presence of small amounts of galacturonic acid and glucuronic acid; these may have arisen from cleavage of a few uronosyl linkages.

Chromatography was carried out on Whatman 3MM papers in solvent (c); side strips were cut and the two major acidic fractions eluted as in sugar ratio determinations. After elution, each fraction was concentrated to a syrup, and the purity of each was checked by chromatography in solvent (c). Then in each case the syrup was transferred to a dry tared 50 ml round-bottom flask; the syrup was taken to dryness. After drying in a vacuum desiccator, the flask was reweighed to find the weight of aldobiuronic acid present. Distilled water (2 ml) was added to dissolve the acid and its specific rotation was determined. Each sample was again taken to dryness, 2N - sulphuric acid (5 ml) added, and hydrolysis carried out for 8 hours to cleave the uronosyl linkages.

Fraction (a) had R_{gal} 0.28, solvent (c), and R_{gal} 0.56, solvent (d); it was indistinguishable chromatographically from 6 - O - (β - D - glucopyranosyluronic acid) - D - galactose. After elution, the $[\alpha]_D$ value was found to be +20°. However, chromatography showed a trace of galactose impurity. This is consistent with the results

found for <u>Anacardium occidentale</u> gum (see Section IV.B). Hydrolysis,

followed by chromatography in solvents (c) and (g) showed the pres-

ence of galactose and glucuronic acid. These results indicate that

fraction (a) is 6 - $\underline{O}$ - (β - $\underline{\underline{D}}$ - glucopyranosyluronic acid) - $\underline{\underline{D}}$ -

galactose; this is consistent with the findings of Bhavanandan[26] for

<u>C. leonense</u> gum.

<u>Fraction (b)</u>, which gave a yellow/brown spot with aniline oxalate,

had R_{gal} 0.88, solvent (c), R_{gal} 0.89, solvent (d), R_{gal} 0.46,

solvent (g) and R_{gal} A 0.80, solvent (g). After elution from

chromatograms run in solvent (c), re-chromatography in solvents (c)

and (g) showed that fraction (b) contained some galactose impurity

as expected since galactose runs close to this aldobiuronic acid in

solvent (c). Also present were slight traces of galacturonic and

glucuronic acids. Using the same procedure as for fraction (a),

the specific rotation was +63°. Hydrolysis followed by chromato-

graphy in solvents (c) and (g) showed the presence of rhamnose and

galacturonic acid, with traces of glucuronic acid and galactose

impurity. These results also agree with those found for <u>C. leonense</u>

by Bhavanandan[25], who characterised this aldobiuronic acid as 2 - $\underline{O}$ -

(α - $\underline{\underline{D}}$ - galacturonosyluronic acid) - $\underline{\underline{L}}$ - rhamnose.

<u>Fraction (c)</u>, which appeared as a faint pink spot having R_{gal}

0.47 in solvent (c) was not present in sufficient amount to allow

its examination. However, investigation of the acidic fraction of

<u>C. hartmannianum</u> gum (see later), together with the fact that

<u>C. erythrophyllum</u> gum was shown to have a methoxyl content, point

to this spot being due to a galactose/4 - $\underline{O}$ - methylglucuronic acid,

possibly $6 - \underline{O} - (4 - \underline{O} - \text{methyl} - \beta - \underline{\underline{D}} - \text{glucopyranosyluronic acid}) - \underline{\underline{D}} - \text{galactose}$.

PREPARATION OF DEGRADED GUM A

C. erythrophyllum gum (9.2 g) was dissolved in 0.01N - sulphuric acid (500 ml) and hydrolysed for 96 hours on a boiling water bath. At intervals, portions (6 ml) were withdrawn and intrinsic viscosities determined (see Figure V.B,1). After 35 hours a brown precipitate formed, possibly due to denatured protein. After 96 hours the intrinsic viscosity had dropped from 102 ml/g to less than 1 ml/g and the brown solution was concentrated, dialysed against distilled water (2 l) for 24 hours then against running tap water for a further 48 hours, and freeze-dried to give Degraded Gum A (1.8 g; yield 19.2%).

The 24-hour dialysate was yellow coloured; after concentration to a syrup, the solution was chromatographed in solvents (a), (b) and (c). A large amount of arabinose was observed, with smaller amounts of galactose and rhamnose, and a trace of xylose. Major components were also observed at R_{gal} 0.87, solvent (a); 0.78, solvent (b); and 0.86, solvent (c), (pink spot, suspected from its R_{gal} values and colour to be an arabinobiose); and at R_{gal} 0.37, solvent (a); 0.27, solvent (b); and 0.34, solvent (c), large diffuse brown spot). Also present was a component at R_{gal} 0.26, solvent (a); 0.16, solvent (b); and 0.12, solvent (c); which from the low mobility was probably a triose or higher oligosaccharide. Chromatograms run in solvent (c) also showed small amounts of a component with R_{gal} 1.15 (pink spot), corresponding to $3 - \underline{O} - \beta - \underline{\underline{L}} - \text{arabinofuranosyl} - \underline{\underline{L}} - \text{arabinose}$. These components were not present in sufficient concentration to allow their characterisation. The two major

INTRINSIC VISCOSITY MEASURED WITH TIME DURING 0.01N ACID
AND AUTOHYDROLYSIS OF C. ERYTHROPHYLLUM GUM
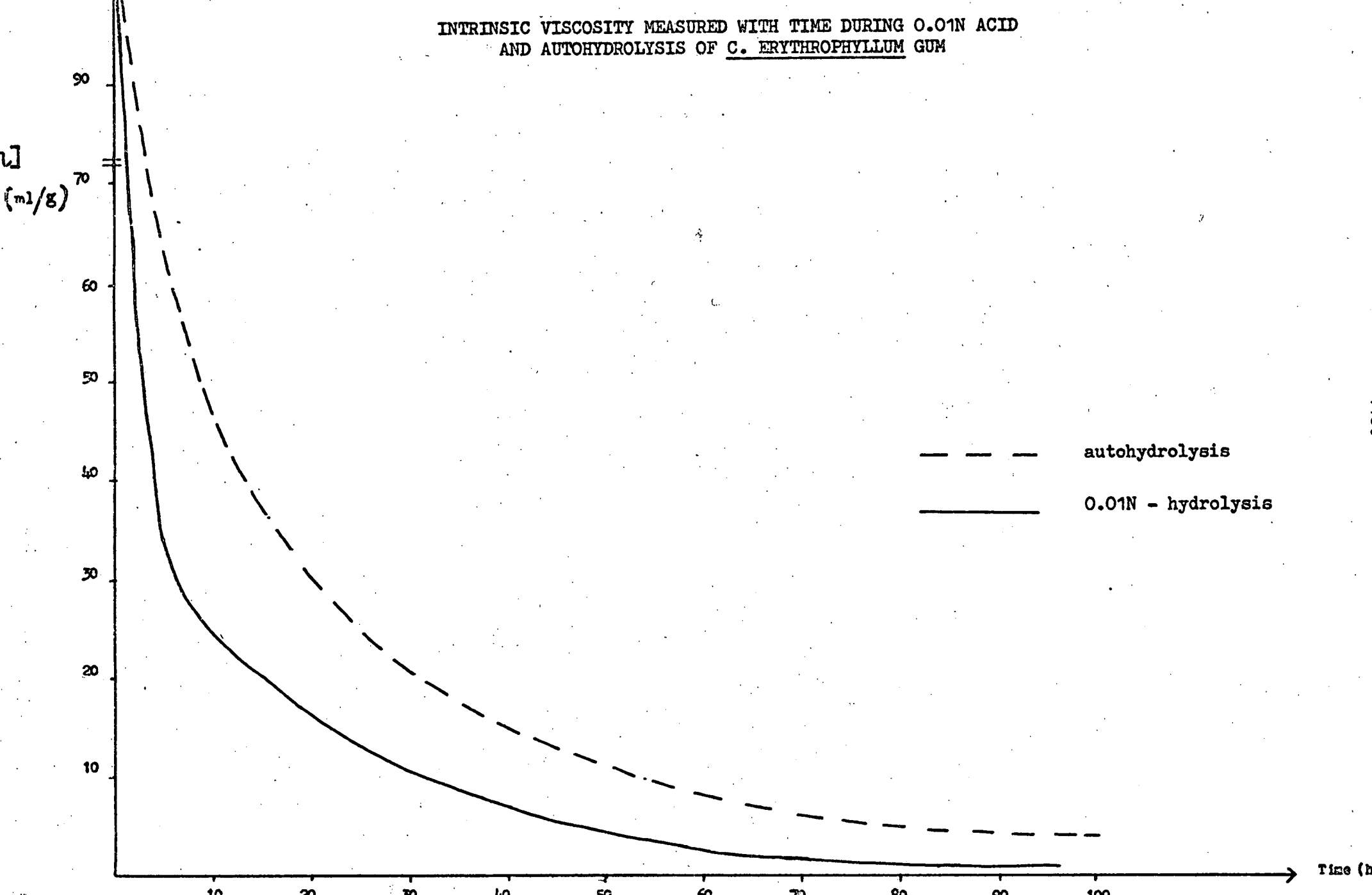
components, however, were isolated from Whatman 3MM papers run in solvent (c).

Component A, the suspected arabinobiose, had $[\alpha]_D$ +112°. Methylation with methyl iodide, N,N - dimethylformamide and silver oxide, followed by methanolysis and g.l.c. showed the presence of 2,3,5 - tri - O - methyl - L - arabinose (T 0.56, 0.71, column (1); 0.54, 0.86, column (2)); 2,3,4 - tri - O - methyl - L - arabinose (T 0.97, column (1); 1.01, column (2)); 2,3 - di - O - methyl - L - arabinose (T 1.68, column (1); 1.54 column (2)); 2,5 - di - O - methyl - L - arabinose (T 1.21, 2.54, column (1); 1.92, 3.00, column (2)) and 2,4 - di - O - methyl - L - arabinose (T 1.92, column (1); 2.21 column (2)). These results indicate that Component A is not a simple arabinobiose.

Component B had $[\alpha]_D$ +36°. Hydrolysis with 2N - sulphuric acid and chromatography in solvent (c) showed a small amount of arabinose in addition to galactose. Methylation of component B by the Kuhn method followed by g.l.c. using column (2) showed 2,3,4,6 - tetra - O - methyl - D - galactose, T 1.76 to be the main O - methyl sugar present. Significant amounts of 2,3,6 - tri - O - methyl - D - galactose, T 2.98, 4.14, 2,3,4 - tri - O - methyl - D - galactose, T 6.52 and 3,5 - di - O - methyl - L - arabinose, T 1.04, 2.32 were also present. This implies that component B is also complex, possibly being composed of two galactobioses and an arabinofuranosyl - D - galactose.

EXAMINATION OF DEGRADED GUM A

The brown colour of its solution made the optical rotation of

Degraded Gum A difficult to determine. However, by using dilute solutions, the specific rotation was found to be $+53^{\circ}$. The molecular weight, determined by light scattering, was 3.2×10^{4}; a low value such as this was indicated by the extensive structural fragmentation shown in the examination of the dialysate from the preparation of Degraded Gum A. The equivalent weight, found by titration of the free gum acid prepared by electrodialysis was 502, indicating a uronic acid content of 35.1%.

Hydrolysis with 1N - sulphuric acid followed by chromatography in solvents (b) and (c) indicated that galactose was the major component, with large amounts of rhamnose and much smaller amounts of arabinose. The ratio gal:ara:rha was 10:3.7:5.4. Complete sugar ratios are shown in Table V.B,6. Chromatography in solvent (c) also showed the presence of the three acidic fragments at R_{gal} 0.28, 0.46 and 0.85 corresponding to 6 - $\underline{O}$ - (β - $\underline{D}$ - glucopyranosyluronic acid) - $\underline{D}$ - galactose (major component), 6 - $\underline{O}$ - (4 - $\underline{O}$ - methyl - - $\underline{D}$ - glucopyranosyluronic acid) - $\underline{D}$ - galactose (minor component) and 2 - $\underline{O}$ - (α - $\underline{D}$ - galactopyranosyluronic acid) - $\underline{L}$ - rhamnose respectively. The assignment of the spot at R_{gal} 0.46 is based on the fact that Degraded Gum A had a small methoxyl content.

Partial acid hydrolysis with 0.5N - sulphuric acid followed by chromatography in solvents (b) and (c) showed one major component (R_{gal} 0.30, solvent (b); 0.26, solvent (c), brown spot), which from its R_{gal} value and the detection of large amounts of 1,6 - linked galactose residues on methylation (see below), is almost certainly 6 - $\underline{O}$ - β - $\underline{D}$ - galactopyranosyl - $\underline{D}$ - galactose. A faint pink spot

was observed at $R_{gal} \sim 0.50$ in both solvents indicating a galacto-arabinobiose. A faint brown spot R_{gal} 0.33 solvent (c) was also found.

Degraded gum A (177 mg) was methylated to give a product (74 mg), (Found: $[\alpha]_D$ +39.6°; OMe 37.2%, not increased by further methylation). A portion of the product was methanolysed and the mixture of methyl glycosides examined by g.l.c. The methanolysate was then hydrolysed and the resulting syrup examined by paper chromatography in solvents (e) and (f). The O - methyl sugars identified are shown in Table V.B,1. The major components identified were 2,3,4,6 - tetra - and 2,3,4 - tri - O - methyl - D - galactose, with substantial amounts of 2,3,5 - tri - O - methyl - L - arabinose, 2,3,6 - and 2,4,6 - tri - O - methyl - D - galactose, 3 - O - methyl - L - rhamnose and end-group glucuronic and galacturonic acid. Chromatography of the hydrolysed methanolysate also showed the presence of a fairly substantial amount of 2 - O - methyl - D - galactose.

EXAMINATION AND METHYLATION OF C. ERYTHROPHYLLUM GUM

Hydrolysis of the purified gum with 1N - sulphuric acid confirmed the ratio of galactose:arabinose:rhamnose (10:26.1:7.4) found in the analytical study. Partial acid hydrolysis with 0.5N - sulphuric acid followed by chromatography in solvent (b) indicated large amounts of galactose and arabinose, smaller amounts of rhamnose, two major components at R_{gal} 0.90 (pink spot), and R_{gal} 0.26 (brown spot) and a minor component at R_{gal} 0.43 (brown spot). The major component at R_{gal} 0.90 is an arabinobiose of some sort and the component at R_{gal} 0.26 would be 6 - O - β - D - galactopyranosyl - D - galactose.

TABLE V.B.1.

O – METHYL SUGARS IDENTIFIED IN METHYLATED DEGRADED GUM A

Relative retention time (T) of methyl glycosides*		R_g after hydrolysis		O – methyl sugar	Relative** amounts
Column (1)	Column (2)	solvent (e)	solvent (f)		
0.51	0.46	0.98	1.01	2,3,4-tri-O-methyl-L-rhamnose	1
0.69, 1.04	0.73, (1.01)	0.85	0.68	3,4-di-O-methyl-L-rhamnose	1.5
3.36	3.34	0.65	0.29	3-O-methyl-L-rhamnose	3
0.58, (0.69)	0.56, (0.73)	0.98	1.01	2,3,5-tri-O-methyl-L-arabinose	2
0.93	(1.01)	0.85	0.78	2,3,4-tri-O-methyl-L-arabinose	0.5
1.86	1.43	0.85	0.78	2,3-di-O-methyl-L-arabinose	1
1.58	1.67	0.91	0.82	2,3,4,6-tetra-O-methyl-D-galactose	10
(2.88), (3.87)	(2.83), (4.24)	0.75	0.48	2,3,6-tri-O-methyl-D-galactose	4
(3.36), (3.87)	3.78, (4.24)	0.75	0.44	2,4,6-tri-O-methyl-D-galactose	4
6.35	6.42	0.75	0.33	2,3,4-tri-O-methyl-D-galactose	10
7.15	9.30	0.54	0.20	2,6-di-O-methyl-D-galactose	trace
11.0, 13.3, 14.5	12.5, 14.4, 15.4	0.54	0.14	2,3-di-O-methyl-D-galactose	1
2.24, (2.88)	2.32, (2.83)	-	-	2,3,4-tri-O-methyl-D-glucuronic acid**	3
4.74	4.73	-	-	2,3,4-tri-O-methyl-D-galacturonic acid**	1.5
2.53	2.57			unknown component	1

* figures in parenthesis indicate T values of components which are not completely resolved

** as methyl ester methyl glycoside

*** this is only a very rough estimate due to incomplete resolution of the majority of the components

122.

C. erythrophyllum gum (315 mg) was methylated to give a product (207 mg), (found: $[\alpha]_D$ - 58.9°, OMe 36.9%). The O - methyl sugars identified are shown in Table V.B,2. Hydrolysis of the methanolysate followed by chromatography in solvents (e) and (f) showed the presence of fairly substantial amounts of 2 - O - methyl - D - galactose in addition to the methyl glycosides identified. The major component identified was 2,3,5 - tri - O - methyl - L - arabinose, with 2,3 - di - O - methyl - L - rhamnose and 2,3,4,6 - tetra - O - methyl - D - galactose also present in large amounts.

PREPARATION AND EXAMINATION OF POLYSACCHARIDE I

Preliminary, small-scale experiments established that 0.125M - sodium metaperiodate solution and oxidation time of 48 hours were required for a Smith degradation of Combretum erythrophyllum gum.

C. erythrophyllum gum (41.5 g) was dissolved in distilled water (1125 ml) and 0.25M - sodium metaperiodate solution (1125 ml) added. Oxidation was carried out in darkness at room temperature and the reaction was followed by measuring the release of formic acid with time (figure V.B,2). After 48 hours, 5.84 m moles periodate/g polysaccharide had been reduced and 1.8 m moles formic acid/g polysaccharide released. The reaction was stopped by the addition of ethylene glycol (22.1 ml), and the solution was dialysed against running tap water for 2 days. Sodium borohydride (13.3 g) was added and the mixture kept at room temperature for 30 hours, then dialysed for 2 days. The solution was made 1N with respect to sulphuric acid, and the polyalcohol was hydrolysed for 48 hours at

O – METHYL SUGARS IDENTIFIED IN METHYLATED C. ERYTHROPHYLLUM GUM

Relative retention time (T) of methyl glycosides*		R_g after hydrolysis		O – methyl sugar	Relative** amount
Column (1)	Column (2)	solvent (e)	solvent (f)		
0.47	0.46	0.97	0.99	2,3,4-tri-O-methyl-L-rhamnose	1
(0.68),1.02	(0.73),(1.00)	0.86	0.59	3,4-di-O-methyl-L-rhamnose	2
3.24	3.26	0.62	0.27	3-O-methyl-L-rhamnose	1.5
0.55,(0.68)	(0.56),(0.73)	0.97	0.99	2,3,5-tri-O-methyl-L-arabinose	10
0.94	(1.00)	0.86	0.80	2,3,4-tri-O-methyl-L-arabinose	1.5
1.15,(2.34)	1.16,(2.38)	0.83	0.80	3,5-di-O-methyl-L-arabinose	1
1.45	(1.89)	0.83	0.80	2,5-di-O-methyl-L-arabinose	1.5
1.85	1.53	0.83	0.80	2,3-di-O-methyl-L-arabinose	3.5
1.60	1.79	0.89	0.84	2,3,4,6-tetra-O-methyl-D-galactose	4
2.60,3.79	(3.26),4.32	0.74	0.48	2,3,6-tri-O-methyl-D-galactose	1
6.04	6.85	0.74	0.35	2,3,4-tri-O-methyl-D-galactose	0.5
(10.7),13.8,15.0	(12.9),15.0,15.9	0.56	0.14	2,3-di-O-methyl-D-galactose	1.5
(2.34),2.96	(2.38),(3.26)	-	-	2,3,4-tri-O-methyl-D-glucuronic acid**	0.5
7.10,8.32	7.40,8.60	-	-	2,3-di-O-methyl-D-glucuronic acid**	1
4.63	4.94	-	-	2,3,4-tri-O-methyl-D-galacturonic acid**	0.5
(10.7)	(12.9)	-	-	2,3-di-O-methyl-D-galacturonic acid**	1.5

*   figures in parenthesis indicate T values of components which are not completely resolved

**   as methyl ester methyl glycoside

*** this is only a very rough estimate due to incomplete resolution of the majority of the components

room temperature. A portion of this solution (1/10 of total volume) was neutralised with barium carbonate, filtered, dialysed against distilled water (4 1) for 24 hours and against running tap water for 2 days, then mixed with the main portion of the solution, which had been dialysed against running tap water for 3 days. Polysaccharide I was isolated as the freeze-dried product (13.4 g; yield 32%).

The dialysate obtained after the hydrolysis stage in the preparation was concentrated, deionised with Amberlite IR - 120 (H) resin and concentrated to a syrup. Paper chromatography in solvents (b) and (c) indicated the presence of large amounts of arabinose [R_F 0.32 solvent (b), 0.19 solvent (c) (pink spot)], with glycerol [major component:- R_F 0.50 solvent (b), 0.37 solvent (e) (brown spot)], threitol in smaller amounts [R_F 0.41 solvent (b), 0.30 solvent (e) (yellow spot)] and a small amount of ethylene glycol [R_F 0.56 solvent (b), 0.46 solvent (e) (light brown spot)]. Chromatograms sprayed with silver nitrate and permanganate also showed traces of glycolaldehyde [R_F 0.63 solvent (b), 0.58 solvent (e)].

Polysaccharide I was found to have $[\alpha]_D$ - 25.1° and molecular weight 4.2 x 10^5 (light scattering) which corresponds well with the 32% yield from the gum itself. Hydrolysis with sulphuric acid (2N -), followed by chromatography in solvents (b), (c) and (g) showed the presence of galactose, arabinose and a significant amount of rhamnose as well as galacturonic acid, glucuronic acid and glucurono - 6,3 lactone. The ratio gal:ara:rha was 10:11.4:5.5. Hydrolysis (1N -) followed by chromatography in solvent (c) revealed the two major aldobiuronic acids, 6 - O - (β - D - glucopyranosyluronic acid)

- $\underline{\underline{D}}$ - galactose and 2 - $\underline{\underline{O}}$ - (α - $\underline{\underline{D}}$ - galacturonosyluronic acid) -

$\underline{\underline{L}}$ - rhamnose in addition to a minor spot at R_{gal} 0.49, but again this

component was not present in sufficient quantity to allow its isolation

and identification.

A sample of Polysaccharide I was electrodialysed. The equivalent

weight was 725 indicating a uronic acid content of 23.9% (almost the

same as that of the gum itself). Using this figure and determining

the uronic acid ratios, the complete sugar content of Polysaccharide I

was calculated as shown in Table B.V,6.

Partial acid hydrolysis of Polysaccharide I with 0.5N - sulphuric

acid followed by chromatography in solvents (b) and (c) showed two

major components having R_{gal} 0.27 in both solvents (brown spot) and

R_{gal} 0.90, solvent (b); 0.85, solvent (c) (reddish/brown spot)

indicating 6 - $\underline{\underline{O}}$ - β - $\underline{\underline{D}}$ - galactopyranosyl - $\underline{\underline{D}}$ - galactose and an

arabinobiose component respectively. A minor component at R_{gal}

0.44, solvent (b); 0.41, solvent (c) (brown spot) was also present.

Polysaccharide I (262 mg) was methylated to give a product

(155 mg), (Found: $[\alpha]_D$ - 7.5°, OMe 37.8%). A portion of the

product was methanolysed and the mixture of methyl glycosides

examined by g.l.c. The methanolysate was then hydrolysed and the

resulting syrup examined by paper chromatography in solvents (e)

and (f). The $\underline{\underline{O}}$ - methyl sugars identified are shown in Table V.B,3.

The major component found was 2,3,5 - tri - $\underline{\underline{O}}$ - methyl - $\underline{\underline{L}}$ - arabinose.

No end-group rhamnose was found although 2,3 - di - and 3 - $\underline{\underline{O}}$ - methyl

rhamnose were present. Large amounts of end-group galactose,

galacturonic acid and glucuronic acid were also found together with

TABLE V.B.3.

$\underline{O}$ - METHYL SUGARS IDENTIFIED IN METHYLATED POLYSACCHARIDE I

Relative retention time (T) of methyl glycosides*		R_g after hydrolysis		$\underline{O}$ - methyl sugar	Relative*** amount
Column (1)	Column (2)	solvent (e)	solvent (f)		
(0.66),(1.02)	(0.75),(1.08)	0.86	0.61	3,4-di-$\underline{O}$-methyl-L-rhamnose	2
3.42	3.46	0.63	0.24	3-$\underline{O}$-methyl-L-rhamnose	4
0.54,(0.66)	0.59,(0.75)	0.95	0.99	2,3,5-tri-$\underline{O}$-methyl-L-arabinose	10
0.92	(1.08)	0.83	0.81	2,3,4-tri-$\underline{O}$-methyl-L-arabinose	1
1.16,2.33	(1.08),(2.44)	0.83	0.81	3,5-di-$\underline{O}$-methyl-L-arabinose	2
1.84	1.59	0.83	0.81	2,3-di-$\underline{O}$-methyl-L-arabinose	3
1.64	1.76	0.89	0.85	2,3,4,6-tetra-$\underline{O}$-methyl-D-galactose	5
(2.90),(3.97)	(3.04),(4.32)	0.74	0.48	2,3,6-tri-$\underline{O}$-methyl-D-galactose	4
3.63,(3.97)	3.97,(4.32)	0.74	0.44	2,4,6-tri-$\underline{O}$-methyl-D-galactose	4
6.42	6.61	0.74	0.35	2,3,4-tri-$\underline{O}$-methyl-D-galactose	1
13.2,15.1,16.3	12.9,15.0,16.1	0.54	0.15	2,3-di-$\underline{O}$-methyl-D-galactose	1
2.58,(2.90)	(2.44),(3.04)	-	-	2,3,4-tri-$\underline{O}$-methyl-D-glucuronic acid**	3
4,34	5.02	-	-	2,3,4-tri-$\underline{O}$-methyl-D-galacturonic acid**	2

* figures in parenthesis indicate T values of components which are not complete resolved

** as methyl ester methyl glycoside

*** this is only a very rough estimate due to incomplete resolution of the majority of the components

2,3,6-, 2,4,6 - and 2,3,4 - tri - $\underline{O}$ - methyl - $\underline{D}$ - galactose and

2,3 - di - $\underline{O}$ - methyl - $\underline{L}$ - arabinose. 3,5 - Di - $\underline{O}$ - methyl - $\underline{L}$ -

arabinose was also present in significant amounts. Chromatography

in solvents (e) and (f) also showed the presence of a small amount

of 2 - $\underline{O}$ - methyl - $\underline{D}$ - galactose in addition to the $\underline{O}$ - methyl

sugars already identified.

PREPARATION AND EXAMINATION OF POLYSACCHARIDE II

Preliminary, small-scale experiments established that a 0.125M -
sodium metaperiodate solution and oxidation time of 48 hours were
required for polysaccharide I.

Polysaccharide I (11.3 g) was oxidised in darkness for 48 hours
after which time 6.73 m moles periodate/g polysaccharide had been
reduced and 1.1 m moles formic acid/g polysaccharide released.
Addition of ethylene glycol (6.8 ml) stopped the reaction and after
dialysis against running tap water for 2 days the solution was
reduced with sodium borohydride (3.9 g) for 30 hours, dialysed, made
1N with respect to sulphuric acid, then hydrolysed for 2 days. During
this hydrolysis, a white precipitate formed and settled on the bottom
of the container. The supernatant was decanted off and freeze-dried
to give Polysaccharide II (3.3 g, yield 29.2%).

Polysaccharide II ($[\alpha]_D$, 0°) was hydrolysed with 2N - sulphuric
acid for 7.5 hours. Chromatography in solvent (g) showed that no
uronic acid was present. Hydrolysis with 1N - sulphuric acid,
followed by chromatography in solvents (b) and (c) showed that
Polysaccharide II contained large amounts of galactose and rhamnose
as well as smaller amounts of arabinose and a component running between

galactose and arabinose in solvent (c) (yellow/brown spot) and
with arabinose in solvent (b) (giving the pink arabinose spot a
yellow colouration). Another chromatogram was run in solvent (c);
instead of developing with aniline oxalate, the chromatogram was
sprayed with glucose oxidase reagent (see Section IV). A negative
result was obtained; the component was therefore not glucose. Tests
with mannose showed it to behave chromatographically in the same way
as the unknown component. Also certain subsequent methanolysates
(see later) showed a component, T 1.42, columns (1) and (2) which is
attributed to 2,3,4,6 - tetra - $\underline{O}$ - methyl - $\underline{D}$ - mannose. However
there was not enough starting material to carry out a large-scale
hydrolysis to isolate the component and characterise it positively.
The ratio gal:mann (?):ara:rha was 10:5.3:7.5:15.0.

Polysaccharide II was methylated by the Haworth method but on
pouring the chloroform syrup into petrol ether (60-80) only a cloud-
iness was obtained. Another three attempts achieved the same result.
The four solutions were therefore allowed to evaporate after which
the fine white residue was dissolved in methanol and a Purdie
methylation carried out. Instead of pouring the chloroform syrup
into petrol ether again, the solution was evaporated to dryness,
methanolic HCl added, and methanolysis carried out in the normal way.
The methanolysate was examined by g.l.c. and the methyl glycosides
identified are shown in Table V.B,4. Hydrolysis of the methanolysate,
followed by chromatography in solvents (e) and (f) showed the presence
of small amounts of 2 - $\underline{O}$ - methyl - $\underline{D}$ - galactose.

Unfortunately, these methylation attempts used up so much material
that insufficient polysaccharide remained for a partial acid hydrolysis

O – METHYL SUGARS IDENTIFIED IN METHYLATED POLYSACCHARIDES II AND III

Relative retention time (T) of methyl glycosides		R_g after hydrolysis		O – methyl sugar	Relative Amount	
					Polysaccharide II	Polysaccharide III
Column (1)	Column (2)	solvent (e)	solvent (f)			
0.48	0.49	0.93	0.99	2,3,4-tri-O-methyl-L-rhamnose	5	2
(0.68),1.06	(0.72),1.02	0.80	0.66	3,4-di-O-methyl-L-rhamnose	3	-
(3.52)	(3.33)	0.62	0.28	3-O-methyl-L-rhamnose	1.5	?
0.57,(0.68)	0.58,(0.72)	0.93	0.99	2,3,5-tri-O-methyl-L-arabinose	2	3
0.97	1.02	0.80	0.81	2,3,4-tri-O-methyl-L-arabinose	1.5	1.5
1.16,2.00	1.17,2.08	0.80	0.81	3,5-di-O-methyl-L-arabinose	1.5	4
1.89	(1.43)	0.80	0.81	2,3-di-O-methyl-L-arabinose	2	4
1.59	1.66	0.86	0.85	2,3,4,6-tetra-O-methyl-D-galactose	10	10
2.96,(3.97)	2.86,4.08	0.71	0.46	2,3,6-tri-O-methyl-D-galactose	1	2
(3.52),(3.97)	3.74,4.0	0.71	0.42	2,4,6-tri-O-methyl-D-galactose	0.5	-
6.57	6.12	0.71	0.33	2,3,4-tri-O-methyl-D-galactose	1	0.5
13.0,15.1,16.3	12.9,15.0,16.4	0.48	0.13	2,3-di-O-methyl-D-galactose	1.5	trace
(1.42)	(1.43)	0.86	0.95	2,3,4,6-tetra-O-methyl-D-mannose	1	?
(3.52)	(3.33)	0.80	0.66	2,4,6-tri-O-methyl-D-mannose	2.5	5

or molecular weight determination.

PREPARATION AND EXAMINATION OF POLYSACCHARIDE III

Polysaccharide II was oxidised with sodium metaperiodate in the normal way. After 72 hours 5.4 m moles periodate/g polysaccharide had been reduced and 2.66 m moles formic acid/g polysaccharide released. Polysaccharide III was obtained as the freeze-dried product (188 mg, yield 7.2%). The very low yield is indicative of extensive structural degradation of Polysaccharide II.

Polysaccharide III was hydrolysed with 1N - sulphuric acid. Chromatography of the hydrolysate in solvents (b) and (c) showed the presence of galactose, smaller amounts of arabinose and rhamnose, and the suspected mannose as the major component.

Polysaccharide III was methylated in a similar manner to Polysaccharide II. Methanolysis followed by g.l.c. gave the mixture of methyl glycosides shown in Table V.B,4. The main components found were 2,3,4,6 - tetra - O - methyl - D - galactose, 3,5 - and 2,3 - di - O - methyl - L - arabinose and 2,4,6 - tri - O - methyl - D - mannose. Chromatography of the hydrolysed methanolysate in solvents (e) and (f) showed a trace amount of 2 - O - methyl - D - galactose.

These results show Polysaccharide III to be very complex, which is most unusual since it is such a small part of the original molecule. An unusual feature of the methylation pattern is the large proportion of end-groups compared with the small amount of 2,3 - di - O - methyl - D - galactose which is the only branching residue-type present.

PREPARATION OF DEGRADED GUM B - AUTOHYDROLYSIS PRODUCT

Purified gum (9.2 g) was dissolved in distilled water (500 ml) and hydrolysed on a boiling water-bath for 100 hours, during which time the intrinsic viscosity fell from 102 ml/g to 4 ml/g (see figure V.B.1). At various time intervals, samples were withdrawn from the solution and chromatographed in solvents (b) and (c). After 53 hours, some arabinose was observed together with a suspected arabinobiose (R_{gal} 0.81, solvent (c), pink spot). The amounts of these two components increased with time up to 100 hours, by which time some $3 - O - \beta - L - arabinofuranosyl - L - arabinose$ (R_{gal} 1.13, solvent (c), pink spot) and a trace amount of rhamnose were also found. After 100 hours, the solution was neutralised, filtered, deionised with Amberlite IR - 120 (H) resin, then concentrated and dialysed against running tap water for 72 hours. After re-filtration, Degraded Gum B was obtained as the freeze-dried product (4.73 g, yield 51%).

Degraded Gum B had $[\alpha]_D$ - 22° and molecular weight 2.7 x 10^5 (light scattering). An equivalent weight of 732 indicated a uronic acid content of 23.4%. The ratio gal:ara:rha was 10:22.7:5.2, which does not vary much from that of the gum itself. Chromatography of a 1N - hydrolysate, followed by chromatography in solvent (c) showed the presence of the three aldobiuronic acid components described previously. Partial acid hydrolysis with 0.5N - sulphuric acid showed three major biose components (R_{gal} 0.83, solvent (b); 0.86, solvent (c), pink spot - arabinobiose), (R_{gal} 0.43, solvents (b) and (c), brown spot) and (R_{gal} 0.25, solvent (b); 0.27, solvent (c), brown spot), the latter being $6 - O - \beta - D - galactopyranosyl - D - galactose$.

Degraded Gum B (257 mg) was methylated to give a product (162 mg),
(found: $[\alpha]_D$ +9.0°; OMe 36.4%). Methanolysis followed by g.l.c.
showed a similar O - methyl glycoside pattern to that obtained from
the gum itself with 2,3,5 - tri - O - methyl - L - arabinose being
by far the major component. Large amounts of 2,3,4,6 - tetra - O -
methyl - D - galactose, 2,3 - di - O - methyl - L - arabinose and
3 - O - methyl - L - rhamnose were also found. Slightly larger amounts
of end-group galacturonic acid and glucuronic acid than in the
methylated gum itself were also found.

PREPARATION AND EXAMINATION OF DEGRADED GUM C

A large-scale autohydrolysis was carried out on C. erythrophyllum
gum to give an amount of Degraded Gum B suitable for further degrada-
tion studies. Preliminary small-scale experiments established that a
0.125M - sodium metaperiodate solution and an oxidation time of 48
hours were required for Degraded Gum B.

Degraded Gum B (32.8 g) was dissolved in distilled water (900 ml)
and 0.25M - sodium metaperiodate solution (900 ml) was added.
Oxidation was carried out for 48 hours after which time 8.75 m moles
periodate/g polysaccharide had been reduced and 1.65 m moles formic
acid/g polysaccharide released. Ethylene glycol (17.2 ml) was added
to stop the reaction and the solution dialysed for 2 days. Sodium
borohydride (10.6 g) was added, the solution left for 30 hours,
dialysed for 2 days and made 1N with respect to sulphuric acid.
After leaving the solution for a further 2 days a whitish precipitate
had formed, which is similar to what happened in the preparation of
Polysaccharide II. The precipitate was filtered off, the solution

dialysed for 2 days and Degraded Gum C was obtained as the freeze-dried product (12.5 g, yield 38%).

Degraded Gum C had $[\alpha]_D$ -9.5° and molecular weight 3.13 x 10⁴ (light scattering). An equivalent weight of 1073 indicated a uronic acid content of 16.4%. Hydrolysis with 2N – sulphuric acid followed by chromatography in solvent (g) showed the presence of glucuronic acid and galacturonic acid. Hydrolysis with 1N – sulphuric acid showed the three aldobiuronic acid components and gave the ratio gal:ara:rha as 10:11.1:4.9 (this compares with the ratio for Polysaccharide I). Partial acid hydrolysis with 0.5N – sulphuric acid followed by chromatography in solvents (b) and (c) showed 6 – O – β – D – galactopyranosyl – D – galactose (R_{gal} 0.25, solvent (b); 0.27, solvent (c), brown spot) as the major biose component.

Degraded Gum C was methylated in the normal way, but, as in the case of Polysaccharide II, the methylated polysaccharide would not precipitate in petrol ether (60-80). Because of this, methylation and subsequent methanolysis were carried out in the same way as for Polysaccharide II. The methyl glycosides were examined by g.l.c. and are shown in Table V.B,5. Hydrolysis of the methanolysate, followed by chromatography in solvents (e) and (f) showed the presence of a small amount of 2 – O – methyl – D – galactose in addition to the methyl glycosides identified from g.l.c.

PREPARATION AND EXAMINATION OF DEGRADED GUM D

Degraded Gum C (9.9 g) was oxidised with sodium metaperiodate solution (0.125M –) for 72 hours after which time 9.4 m moles periodate/g polysaccharide had been reduced and 3.7 m moles formic

$\underline{O}$ - METHYL SUGARS IDENTIFIED IN METHYLATED DEGRADED GUM C

Relative retention time (T) of methyl glycosides*		R_g after hydrolysis		$\underline{O}$ - methyl sugar	Relative*** amount
Column (1)	Column (2)	solvent (e)	solvent (f)		
0.47	0.45	0.97	1.00	2,3,4-tri-$\underline{O}$-methyl-$\underline{L}$-rhamnose	1
0.68,1.00	0.72,(0.98)	0.85	0.66	3,4-di-$\underline{O}$-methyl-$\underline{L}$-rhamnose	2
(3.46)	3.36	0.67	0.29	3-$\underline{O}$-methyl-$\underline{L}$-rhamnose	4
0.56,0.68	0.56,0.72	0.97	1.00	2,3,5-tri-$\underline{O}$-methyl-$\underline{L}$-arabinose	10
0.91	(0.98)	0.85	0.77	2,3,4-tri-$\underline{O}$-methyl-$\underline{L}$-arabinose	1
1.46	(1.69)	0.85	0.77	2,5-di-$\underline{O}$-methyl-$\underline{L}$-arabinose	1
1.89	1.42	0.85	0.77	2,3-di-$\underline{O}$-methyl-$\underline{L}$-arabinose	1.5
1.59	(1.69)	0.90	0.82	2,3,4,6-tetra-$\underline{O}$-methyl-$\underline{D}$-galactose	5
(2.81),(3.94)	(2.86),(4.24)	0.77	0.47	2,3,6-tri-$\underline{O}$-methyl-$\underline{D}$-galactose	5
(3.46),(3.94)	3.84,(4.24)	0.77	0.42	2,4,6-tri-$\underline{O}$-methyl-$\underline{D}$-galactose	4
6.53	6.7	0.77	0.37	2,3,4-tri-$\underline{O}$-methyl-$\underline{D}$-galactose	3
9.46	9.22	0.56	0.20	2,6-di-$\underline{O}$-methyl-$\underline{D}$-galactose	trace
13.2,15.1,16.3	13.4,15.3,16.8	0.56	0.12	2,3-di-$\underline{O}$-methyl-$\underline{D}$-galactose	1.5
2.58,(2.81)	2,38,(2.86)	-	-	2,3,4-tri-$\underline{O}$-methyl-$\underline{D}$-glucuronic acid**	1.5
4.63	4.78	-	-	2,3,4-tri-$\underline{O}$-methyl-$\underline{D}$-galacturonic acid**	1

*   figures in parenthesis indicate T values of components which are not completely resolved

**   as methyl ester methyl glycoside

***   this is only a rough estimate due to incomplete resolution of the majority of the components

acid/g formic acid/g polysaccharide released. The solution was treated in the normal way for a Smith degradation and Degraded Gum D was obtained as the freeze-dried product, (0.55 g, yield 6%).

Degraded Gum D had $[\alpha]_D$ +13° and a molecular weight ($\overline{M}n$) of 2.13 x 10^3 found by end-group analysis. Hydrolysis with 2N - sulphuric acid followed by chromatography in solvents (b) and (c) showed that galactose was the main component along with arabinose and large amounts of rhamnose. Chromatography in solvent (c) showed the presence of a substantial amount of mannose also. The ratio gal: ara:mann:rha was 10:5.1:2.8:6.9. Table V.B,6 shows the percentage of sugars present in Degraded Gum D and all the other degradation products of C. erythrophyllum gum.

Degraded Gum D was methylated in the same way as Polysaccharide II. Methanalysis followed by g.l.c. on column (1) showed that the major component was 2,3,4,6 - tetra - O - methyl - D - galactose (T 1.58). Large amounts of 2,3,4 - tri -, 3,4 - di - O - methyl - L - rhamnose, 2,3,6 - tri - and 2,3 - di - O - methyl - D - galactose, 2,3,4 - tri - and 2,5 - and 2,3 - di - O - methyl - L - arabinose were also present along with a small amount of 2,3,4 - tri - O - methyl - D - galactose. A small amount of end-group mannose (T 1.42) was confirmed chromatographically in solvent (f) (R$_{gal}$ 0.97), and a component (T 2.66) is thought to be 3,4,6 - tri - O - methyl - D - mannose. The large component (T 3.48) is thought to be a mixture of 2 - O - methyl - L - rhamnose and 2,4,6 - tri - O - methyl - D - mannose; the latter may be present in small amounts in all of the methanolysates.

TABLE V.B.6.

SUGAR CONTENT OF C. ERYTHROPHYLLUM AND ITS DEGRADATION PRODUCTS

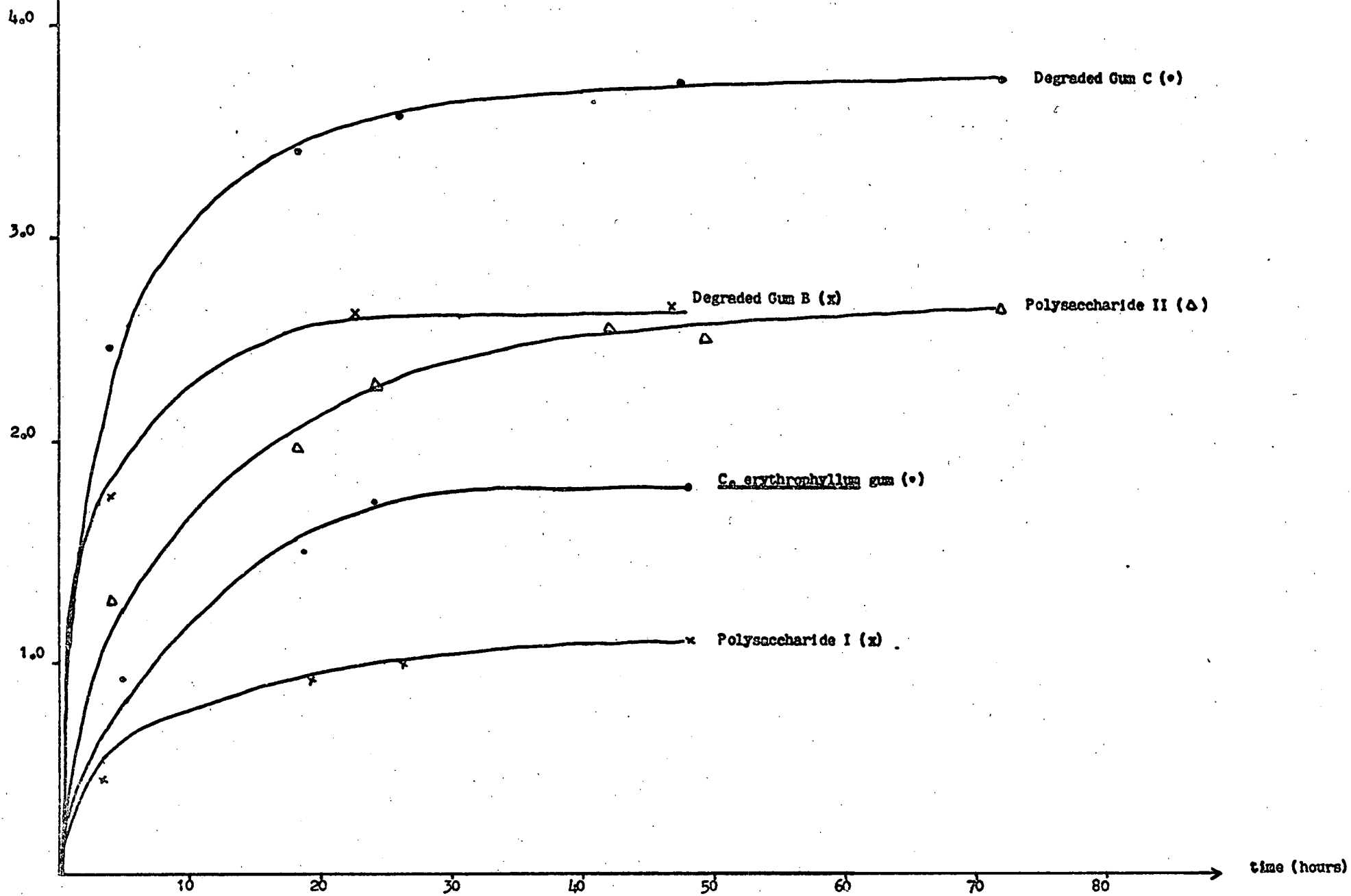| Polysaccharide	gal.	ara.	rha.	mann.	gal.A.	glu.A.	$[\alpha]_D$ degrees
C. erythrophyllum gum	27	33	16	-	8	14	-57
Polysaccharide I	32	24	21	-	11	12	-25
Polysaccharide II	27	18	41	14	-	-	0
Polysaccharide III	26	20	23	31	-	-	n.d.
Degraded gum A	37	6	21	-	14	22	+53
Degraded gum B	30	33	14	-	7	16	-22
Degraded gum C	37	29	18	-	6	10	-10
Degraded gum D	40	21	28	11	-	-	+13

n.d. - not done

Figure V.B,2.shows the formic acid released with time on periodate oxidation of C. erythrophyllum gum and its degradation products.

V.B.4. DISCUSSION

C. erythrophyllum gum contains galactose (27%), arabinose (33%), rhamnose (16%) and uronic acid (24%). The uronic acid content consisted of glucuronic acid, galacturonic acid, 4 - 0 - methylglucuronic acid and glucurono - 6,3 - lactone, with glucuronic acid being the major acidic sugar present.

Hydrolysis with 1N - sulphuric acid followed by chromatography in solvent (c) indicated the presence of three aldobiuronic acids at R_{gal} 0.88, 0.48, and 0.28. The component at R_{gal} 0.28, which was the major component, was chromatographically indistinguishable from 6 - $\underline{0}$ - (β - $\underline{D}$ - glucopyranosyluronic acid) - $\underline{D}$ - galactose. Its $[\alpha]_D$ value and hydrolysis experiments showed that the component is in fact 6 - $\underline{0}$ - (β - $\underline{D}$ - glucopyranosyluronic acid) - $\underline{D}$ - galactose. The component at R_{gal} 0.88 gave a yellow spot colouration with aniline oxalate spray reagent. Hydrolysis experiments indicated that this aldobiuronic acid contained rhamnose and galacturonic acid which together with the $[\alpha]_D$ value found indicates that this component is 2 - $\underline{0}$ - (α - $\underline{D}$ - galactopyranosyluronic acid) - $\underline{L}$ - rhamnose, which has been found and characterised by Aspinall and Bhavanandan in C. leonense gum. The third component, R_{gal} 0.48, was not present in sufficient amount to allow its characterisation, but from its R_{gal} value, and the fact that C. erythrophyllum gum has a small methoxyl content, this trace aldobiuronic acid may be 6 - $\underline{0}$ - (4 - $\underline{0}$ - methyl -β - $\underline{D}$ - glucuronosyluronic acid) - $\underline{D}$ - galactose.

FORMIC ACID RELEASED (m moles/gm) WITH TIME (HOURS) ON PERIODATE OXIDATION
OF C. ERYTHROPHYLLUM GUM AND ITS DEGRADATION PRODUCTS

Degraded Gum C (•)

Degraded Gum B (x)

Polysaccharide II (Δ)

C. erythrophyllum gum (•)

Polysaccharide I (x)

time (hours)

C. erythrophyllum gum was methylated. Methanolysis followed by
g.l.c. examination showed the presence of large amounts of end-group
arabinofuranose and galactose residues. Small amounts of end-group
arabinopyranose, rhamnose and uronic acid were also present. 2,3 -
Di - O - methyl - L - arabinose was present in substantial amounts in
addition to smaller amounts of 2,5 - and 3,5 - di - O - methyl - L -
arabinose. In addition to 3,4 - di - and 3 - O - methyl - L - rhamnose
substantial amounts of 2,3 - di - O - methyl glucuronic and galacturonic
acid were present indicating intra-chain uronic acid residues linked
in the 4-position to end-group rhamnose, arabinose or galactose.
2,3 - Di - O - methyl - D - galactose and 3 - O - methyl - L - rhamnose
were the only branch-point residues observed. The 2 - O - methyl - D
- galactose may be due to undermethylation or may indicate galactose
linked in the 1-, 3-, 4- and 6- positions as has been postulated for
C. leonense by Aspinall and Bhavanandan[15].

Hydrolysis of C. erythrophyllum gum with 0.01N - sulphuric acid
for 96 hours on a boiling water bath gave Degraded Gum A. The
distilled-water dialysate from the preparation of Degraded Gum A was
concentrated and examined by chromatography. Small amounts of gala-
ctose and rhamnose were observed together with large amounts of
arabinose and two major components (R_{gal} 0.86, solvent (c), pink spot)
and (R_{gal} 0.34, solvent (c), large diffuse brown spot). The two major
biose components were eluted from Whatman 3MM papers and examined by
hydrolysis and methanolysis. Fraction (a), (R_{gal} 0.86) which contained
only arabinose, was a mixture of arabinobiose components. Fraction (b)
(R_{gal} 0.34), which contained a small amount of arabinose in addition
to galactose was also very complex, containing two galactobioses and
a suspected arabinosyl-galactobiose.

Degraded Gum A contained galactose, rhamnose and a small amount of arabinose in addition to a large amount of uronic acid, which is as expected, since C.01N - hydrolysis does not break uronosyl linkages. Partial acid hydrolysis showed 6 - $\underline{O}$ -β- $\underline{D}$ - galacto-pyranosyl - $\underline{D}$ - galactose to be the major biose present, a fact confirmed by the presence of large amounts of 2,3,4 - tri - $\underline{O}$ - methyl - $\underline{D}$ - galactose in the methanolysate of Degraded Gum A. The methanolysate also showed large amounts of end-group galactose, arabinofuranose, rhamnose, glucuronic and galacturonic acid. 2,3 - Di - $\underline{O}$ - methyl - $\underline{L}$ - arabinose indicated the presence of intra-chain arabinose, and intra-chain rhamnose was also indicated by 3,4 - di - and 3, - $\underline{O}$ - methyl - $\underline{L}$ - rhamnose.

C. erythrophyllum gum was Smith degraded to give Polysaccharide I which contained galactose, arabinose and rhamnose in large amounts and uronic acid (24%). A similar uronic acid content to the gum itself indicates that some uronic acid is end-group in the gum itself but most of it is intra-chain. Polysaccharide I contained the same three aldobiuronic acid components as the gum itself.

Methylation of Polysaccharide I showed large amounts of end-group galactose and arabinofuranose in addition to end-group glucuronic and galacturonic acid. 2,3 - Di - $\underline{O}$ - methyl - $\underline{L}$ - arabinose was the major di - $\underline{O}$ - methyl arabinose found, with smaller amounts of 3,5 - di - $\underline{O}$ - methyl - $\underline{L}$ - arabinose, but no 2,4 - or 2,5 - di - $\underline{O}$ - methyl - $\underline{L}$ - arabinose was found showing that Polysaccharide I does not contain any 3 - $\underline{O}$ -β- arabinosyl - $\underline{L}$ - arabinose. The 3 - $\underline{O}$ - methyl - $\underline{L}$ - rhamnose and 2,3 - di - $\underline{O}$ - methyl - $\underline{D}$ - galactose are indicative of chain branch points.

A Smith degradation of Polysaccharide I gave Polysaccharide II which had rhamnose as its major sugar. Galactose and arabinose were also present together with a substantial amount of a component behaving chromatographically like mannose. No uronic acid was present. The presence of another neutral sugar in the latter stages of a Smith degradation study is very unusual and opens speculation as to the location of the mannose in the gum structure. Two possible theories are:- (a) it is present in a very small amount in the gum but attached to uronic acid, thus not appearing in a 1N - hydrolysate until all the uronic acid has been removed; (b) mannose is in the central core of the gum structure and is protected from hydrolysis until a great deal of the peripheral material has been removed. Mannose, however, is only postulated on chromatographic evidence; insufficient material was available to allow its isolation and identification.

Polysaccharide II was methylated and g.l.c. examination of the methanolysate showed the main components to be end-group rhamnose and galactose as well as end-group arabinose (furanose and pyranose). 3,4 - Di - O - methyl - L - rhamnose, 3,5 - and 2,3 - di - O - methyl - L - arabinose and 2,3,6 - and 2,3,4 - tri - O - methyl - D - galactose were also present in substantial amounts. 2,3 - Di - O - methyl - D - galactose and 3 - O - methyl - L - rhamnose are the only branch-point residues found. 2,4,6 - Tri - O - methyl - D - mannose is thought to be present but unfortunately this has approximately the same T value as 3 - O - methyl - L - rhamnose[6,35] (see Section V.B, Part B), which prompts the speculation that the tri - O - methylmannose may have been obscured by the 3 - O - methyl - L - rhamnose in other g.l.c. traces.

Polysaccharide II was Smith-degraded to give Polysaccharide III in a very small yield (7.2%), indicating large-scale structural degradation of Polysaccharide II [confirmed by the amount of formic acid released (see figure V.B,2)]. The suspected mannose was the major sugar found together with galactose, arabinose, and rhamnose. Partial acid hydrolysis showed the presence of $6 - \underline{O} - \beta - \underline{D} -$ galactopyranosyl $- \underline{D} -$ galactose.

Methylation of Polysaccharide III showed large amounts of end-group galactose. A significant amount of $2,3 - \text{di} - \underline{O} - \text{methyl} - \underline{L} -$ arabinose indicated some intra-chain arabinose residues. The facts that (a) Polysaccharide III was obtained in very small yield and (b) only a small amount of $2,3 - \text{di} - \underline{O} - \text{methyl} - \underline{D} -$ galactose relative to end-group residues was found, indicate that whole side-chains present in Polysaccharide II may have been cleaved in the preparation of Polysaccharide III.

An autohydrolysis of C. erythrophyllum gum followed by a subsequent Smith-degradation study confirmed the above findings with mannose appearing in 1N - hydrolysates only after all uronic acid had been removed.

As is obvious from the complexity of the results obtained, no definite structural type can be postulated for C. erythrophyllum gum. It is very unusual for such a complex neutral sugar mixture to be present after such extensive degradation of a gum structure has been carried out. Also extremely unusual is the appearance during a Smith-degradation sequence of what, from chromatographic evidence, seems to be mannose. A significant fact is that it is only detectable by

chromatography _after_ all uronic acid has been removed from the gum
structure. This prompts the postulation of a side chain of the
type shown in figure V.B,3.

<div align="center">

FIGURE V.B,3.

</div>

------ mannose ——— uronic acid —⫽— X   C. erythrophyllum gum

------ mannose —⫽— uronic acid   Polysaccharide I

------ mannose   Polysaccharide II
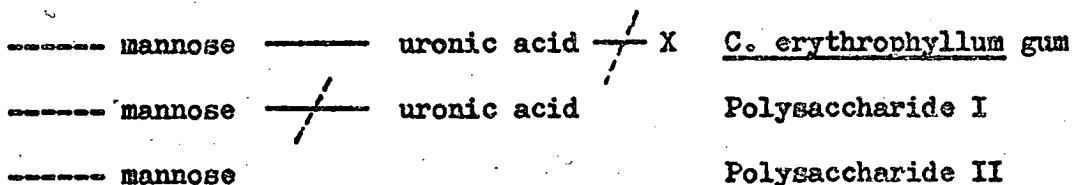
Apart from arabinobiose components which may, from the presence of
2,3 - di - O - methyl - L - arabinose in all methanolysates, contain
1,5 - linked arabinose residues, the main biose components are 6 -
O - β - D - galactopyranosyl - D - galactose and a component (R_{gal}
0.40, brown spot), which from its low R_{gal} value is expected to
be a galactobiose but is not 1,3 - or 1,6 - linked galactose residues.
From the presence of substantial amounts of 2,3,6 - tri - O - methyl -
D - galactose in all methanolysates, this component could well be
4 - O - (α or β) - galactopyranosyl - D - galactose, although further
experiments would be required to isolate and characterise it.

The main structural features to emerge from this present work are:
(1) C. erythrophyllum has a 1,6 - linked galactose backbone with
1,4 - linked galactose chains present also; (2) the majority of uronic
acid residues are intra-chain, the second residue from the end of a
chain. The end-groups attached to the uronic acid may be either
arabinose, rhamnose and galactose and they are attached to the 4 -
position of the uronic acid. Galacturonic acid is linked 1,2 - to
rhamnose, whereas glucuronic acid and its 4 - O - methyl analogue are
attached 1,6 - to galactose (figure V.B,4).

FIGURE V.B.4.

- - - - - - -   galactose 6 ⟵————— 1 glucuronic acid 4 ⟵————— X

- - - - - - -   rhamnose 2 ⟵————— 1 galacturonic acid 4 ⟵——— X
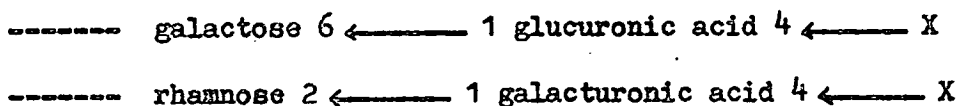
In the case of the galacturonic acid/rhamnose species the rhamnose
may be linked to something other than the uronic acid, which would
explain the presence of 3 - O - methyl - L - rhamnose. The presence
of such a system has been postulated for C. leonense by Aspinall and
Bhavanandan[14] (see figure V.B.5);

FIGURE V.B.5.

⟵- - - - - - 1 rhamnose 2 ⟵————— 1 galacturonic acid 4 ⟵——— X
                4
                ↑
                |
                |
                |
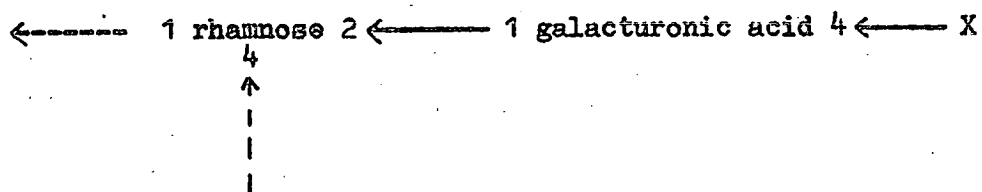
(3) The structure contains a lot of peripheral arabinofuranosyl units.
Also on the periphery are 1,3 - linked arabinopyranose and arabino-
furanose biose units. There are no long chains of 1,3 - linked
arabinose units shown by the absence of any 2,4 - or 2,5 - di - O -
methyl - L - arabinose in methylated Degraded Gum A or Polysaccharide I.
There may be, however, long chains of 1,5 - linked arabinose units as
shown by the presence of 2,3 - di - O - methyl - L - arabinose; The
presence of 3,4 - di - and 3 - O - methyl - L - rhamnose indicate
intra-chain rhamnose residues and the possibility of rhamnose side
chains cannot be ruled out.

All these results indicate that C. erythrophyllum gum has a very

complex structure, confirming the genetic complexity of the

Combretum genus. Extensive work will have to be carried out in

much greater detail and with larger amounts of starting material to

clarify the significance of these results and allow the postulation

of a complete structure for the gum.

SECTION V.B   PART B

SOME STRUCTURAL FEATURES OF THE GUM FROM
C. HARTMANNIANUM SCHWEINF

V.B.5. **PURIFICATION AND EXAMINATION OF C. HARTMANNIANUM**
GUM AND ITS DEGRADATION PRODUCTS

Crude gum (32.3 g) was dissolved in distilled water (2.5 l)

giving a clear, yellow solution which was easily filtered. After

filtration, the solution was dialysed for 2 days, concentrated, and

obtained as the freeze-dried product (23.4 g, yield 72%).

The polysaccharide was found to contain the neutral sugars

galactose, arabinose, mannose xylose and rhamnose in approximately

the same proportions as found previously in the analytical study.

Galacturonic acid, glucuronic acid and its 4 - $\underline{O}$ - methyl analogue,

and glucuronolactone were also found in a 2N - hydrolysate.

IDENTIFICATION OF THE BIURONIC ACIDS

Chromatography of the 1N - hydrolysate in solvent (c) showed

two spots at R_{gal} 0.28 and 0.50 respectively indicating the presence

of two aldobiuronic acid components. No spot was observed just behind

galactose ($R_{gal} \sim 0.90$) indicating the absence of 2 - $\underline{O}$ - (α - galacto-

pyranosyluronic acid) - $\underline{L}$ - rhamnose. Chromatography was carried out

on Whatman 3MM papers in solvent (c) and the two acidic components were

eluted as described for C. erythrophyllum. Specific rotations were recorded for each component and hydrolysis with 2N acid was carried out to determine the constituents.

Component (a) (R_{gal} 0.28) was found to have $[\alpha]_D$ +23° and was chromatographically indistinguishable from 6 - O - (β - D - glucopyranosyluronic acid) - D - galactose. Hydrolysis with 2N - sulphuric acid, followed by chromatography in solvents (c) and (g), indicated the presence of galactose and glucuronic acid in approximately equal amounts. These results are consistent with those found for component (a) from C. erythrophyllum, and so component (a) from C. hartmannianum is 6 - O - (β - D - glucopyranosyluronic acid) - D - galactose.

Component (b) (R_{gal} 0.50) had $[\alpha]_D$ -27°. Hydrolysis (2N - acid) followed by chromatography in solvents (c) and (g) indicated the presence of mannose and galacturonic acid as the major components, with smaller amounts of galactose, glucuronic acid and 4 - O - methylglucuronic acid. From these results it appears that component (b) is a mixture of at least two aldobiuronic acids, containing mannose and galacturonic acid and galactose and 4 - O - methylglucuronic acid respectively. A mannose-glucuronic acid with $[\alpha]_D$ -32° has been found in Anogeissus gum exudates and has been characterised as 2 - O - (β - D - glucopyranosyluronic acid) - D - mannose. This was confirmed for a sample of Anogeissus latifolia during this present study where hydrolysis of the component in question with 2N - acid gave mannose and glucuronic acid. So it is possible that the galacturonic acid and mannose may arise from 2 - O - (β - D - galactopyranosyluronic acid) - D - mannose. The

presence of glucuronic acid in the 2N - hydrolysate of component (b)
may arise from the loss of a methyl group from certain 4 - $\underline{O}$ - methyl-
glucuronic acid residues, although the possibility of it being present
in a glucuronic acid - mannose biuronic acid species cannot be ruled
out. Attempts to separate the two fractions of component (b) by
chromatography in solvent (c) for 48 hours and in solvent (b) for
96 hours were unsuccessful, indicating that the two components
behave similarly chromatographically.

Partial acid hydrolysis of C. hartmannianum with 0.5N - sulphuric
acid for 1 hour followed by chromatography in solvents (b) and (c)
showed the presence of three components present in approximately equal
proportions at R_{gal} 0.84 solvent (b) 0.87 solvent (c), 0.51 solvents
(b) and (c) and 0.28 solvent (b) 0.24 solvent (c). The first of
these components, on the basis of its R_{gal} value and pink colouration,
appears to be an arabinobiose of some kind; a pinkish spot at R_{gal}
0.51 could be of the galactose-arabinose type; and a brown component
at R_{gal} 0.28 solvent (b) 0.24 solvent (c) could be 6 - $\underline{O}$ -β- $\underline{D}$ -
galactopyranosyl - $\underline{D}$ - galactose.

C. hartmannianum polysaccharide (239 mg) was methylated to give
a product (123 mg), (Found: $[\alpha]_D$ -32°; OMe 39.1%). A portion of
the product was methanolysed and the mixture of methyl glycosides
examined by g.l.c. The methanolysate was then hydrolysed and the
resulting syrup examined by paper chromatography in solvents (e)
and (f). The $\underline{O}$ - methyl sugars identified are shown in Table V.B, 7.
The major component identified was 2,3,5 - tri - $\underline{O}$ - methyl - $\underline{L}$ -
arabinose. Also present were large amounts of 2,3,4 - tri - $\underline{O}$ -
methyl - $\underline{L}$ - rhamnose, 2,3,4,6 - tetra - $\underline{O}$ - methyl - $\underline{D}$ - galactose

TABLE V.B.7.

O - METHYL SUGARS IDENTIFIED IN METHYLATED C. HARTMANNIANUM GUM

Relative retention time (T) of methyl glycosides*		R_g after hydrolysis		O - methyl sugar	Relative*** amount
Column (1)	Column (2)	solvent (e)	solvent (f)		
(0.49)	(0.47)	0.96	1.04	2,3,4-tri-O-methyl-L-rhamnose	2
(0.49),(0.52)	(0.47),(0.56)	0.96	1.04	2,3,4-tri-O-methyl-D-xylose	2
(0.52),0.67	(0.56),0.71	0.96	1.04	2,3,5-tri-O-methyl-L-arabinose	10
0.90	(1.01)	0.85	0.82	2,3,4-tri-O-methyl-L-arabinose	3
1.72	(2.26)	0.85	0.82	2,4-di-O-methyl-L-arabinose	4
1.72	1.57	0.85	0.82	2,3-di-O-methyl-L-arabinose	2
1.52	1.77	0.90	0.96	2,3,4,6-tetra-O-methyl-D-galactose	4
(3.02),3.87	(3.12),4.29	0.73	0.48	2,3,6-tri-O-methyl-D-galactose	2
6.33	6.22	0.73	0.38	2,3,4-tri-O-methyl-D-galactose	2
8.45	9.28	0.51	0.22	2,6-di-O-methyl-D-galactose	2
11.0,13.3,14.5	12.5,14.8,15.8	0.51	0.15	2,3-di-O-methyl-D-galactose	2
1.36	1.40	0.90	0.96	2,3,4,6-tetra-O-methyl-D-mannose	trace
2.72	2.93	0.73	0.69	3,4,6-tri-O-methyl-D-mannose	2
3.42	3.37	0.64	0.69	2,4,6-tri-O-methyl-D-mannose	2
7.42	7.52	0.51	0.22	2,6-tri-O-methyl-D-mannose	1
2,38,(3.02)	(2.26),(3.12)	-	-	2,3,4-tri-O-methyl-D-glucuronic acid**	2
5.27	4.96	-	-	2,3,4-tri-O-methyl-D-galacturonic acid**	1

*   figures in parenthesis indicate T values of components which are not completely resolved

**   as methyl ester methyl glycoside

***   this is only a rough estimate due to incomplete resolution of the majority of the components

and 2,4 - tri - $\underline{O}$ - methyl - $\underline{L}$ - arabinose. Much smaller amounts of 2,3,6 - and 2,3,4 - tri - $\underline{O}$ - methyl - $\underline{D}$ - galactose, 3,4,6 - and 2,4,6 - tri - $\underline{O}$ - methyl - $\underline{D}$ - mannose were found in addition to end-group glucuronic acid and galacturonic acid. Paper chromatography in solvents (e) and (f) also showed the presence of 2 - $\underline{O}$ - methyl - D - galactose.

PREPARATION AND EXAMINATION OF POLYSACCHARIDE I

Preliminary, small-scale experiments established that 0.25M - sodium metaperiodate solution and oxidation time of 72 hours were required for C. hartmannianum gum. Pure gum (23.4 g) was dissolved in distilled water (1274 ml) and 0.5M - sodium metaperiodate solution (1274 ml) was added. Oxidation was carried out in the dark for 72 hours, after which time 8.7 m moles periodate/g polysaccharide had been reduced and 1.7 m moles formic acid/g polysaccharide released. Ethylene glycol (12.7 ml) was added; after dialysis for 2 days, sodium borohydride (6.25 g) was added and the solution left for 30 hours. After dialysis for a further 2 days, the solution was made 1N with respect to sulphuric acid, left for 2 days, dialysed for 2 days, concentrated, filtered and Polysaccharide I obtained as the freeze-dried product (7.8 g, yield 31%).

Polysaccharide I had $[\alpha]_D$ +16.2°. This is markedly different from the value for the untreated polysaccharide (-35°). Hydrolysis with 2N - sulphuric acid followed by chromatography in solvent (g) indicated the absence of any uronic acid. Hydrolysis (1N -) followed by chromatography in solvents (b) and (c) showed the presence of large amounts of galactose and arabinose in addition to

mannose. Both rhamnose and xylose were absent. The ratio gal:mann:ara
was 10:5.4:12.8. Partial acid hydrolysis with 0.5N - sulphuric acid
showed, in addition to galactose, mannose and arabinose, two components
at R_{gal} 0.60 solvent (b), 0.51 solvent (c) (pink spot) and at R_{gal}
0.23 solvent (b), 0.26 solvent (c) (brown spot).

Polysaccharide I (265 mg) was methylated to give a product
(132 mg). (Found: $[\alpha]_D$ -20.8°; OMe 37.1%). A portion of the
product was methanolysed and the mixture of methyl glycosides
examined by g.l.c. The O - methyl sugars identified are shown in
Table V.B,8. Hydrolysis of the methanolysate followed by chromato-
graphy in solvents (e) and (f) also showed the presence of a small
amount of 2 - O - methyl - D - galactose in addition to the methyl
glycosides identified by g.l.c. The main components found were
2,3,4,6 - tetra - , 2,3,6 - and 2,3,4 - tri - and 2,3 - di - O -
methyl - D - galactose, 2,3,5 - tri and 2,4 - di - O - methyl - L -
arabinose and 2,4,6 - tri - O - methyl - D - mannose.

PREPARATION AND EXAMINATION OF POLYSACCHARIDE II

Polysaccharide I (5.0 g) was dissolved in distilled water (135 ml)
and 0.25M - sodium metaperiodate (135 ml) added. Oxidation was carried
out for 72 hours after which time 4.4 m moles periodate/g polysaccharide
had been reduced and 1.8 m moles formic acid/g polysaccharide released.
The solution was treated in the same way as in the Smith Degradation
of C. hartmannianum gum itself. Polysaccharide II was obtained as
the freeze-dried product (1.1 g, yield 22%).

Polysaccharide II had $[\alpha]_D$ +64.3°. Hydrolysis with 1N - sulphuric
acid followed by chromatography in solvents (b) and (c) showed that

TABLE V.B.8.

O – METHYL SUGARS IDENTIFIED IN METHYLATED POLYSACCHARIDE I

Relative retention time (T) of methyl glycosides*		R_g after hydrolysis		O – methyl sugar	Relative amount
Column (1)	Column (2)	solvent (e)	solvent (f)		
0.53,0.65	0.56,0.74	0.96	0.99	2,3,5-tri-O-methyl-L-arabinose	3
0.98	0.99	0.85	0.81	2,3,4-tri-O-methyl-L-arabinose	2
1.85	2.12	0.73	0.81	2,4-di-O-methyl-L-arabinose	10
1.55	1.70	0.90	0.85	2,3,4,6-tetra-O-methyl-D-galactose	5
3.21,3.78	(2.92),4.32	0.73	0.48	2,3,6-tri-O-methyl-D-galactose	4
6.00	6.42	0.73	0.38	2,3,4-tri-O-methyl-D-galactose	3
8.78	9.08	0.51	0.22	2,6-tri-O-methyl-D-galactose	2
11.5,13.6,14.8	12.5,14.8,15.8	0.51	0.15	2,3-tri-O-methyl-D-galactose	4
1.30	1.37	0.90	0.84	2,3,4,6-tetra-O-methyl-D-mannose	1
2.74	(2.92)	0.73	0.69	3,4,6-tri-O-methyl-D-mannose	2
3.38	3.64	0.64	0.69	2,4,6-tri-O-methyl-D-mannose	5
7.72	7.84	0.51	0.22	2,6-tri-O-methyl-D-mannose	2

* figures in parenthesis indicate T values which are not completely resolved

arabinose was present in large amounts, along with galactose and a smaller amount of mannose. Partial acid hydrolysis with 0.5N - sulphuric acid showed the presence of two disaccharides in addition to the monomers, at R_{gal} 0.24 solvent (b), 0.26 solvent (c) (brown spot), and at R_{gal} 0.60 solvent (b), 0.51 solvent (c) (red spot) in approximately equal proportions.

Polysaccharide II was methylated in the normal way, but the methylated polysaccharide did not precipitate in petrol ether (60-80). A repeated methylation gave the same results and so the methylation and subsequent methanolysis were carried out as described for C. erythrophyllum, Polysaccharide II. The O - methyl sugars identified are shown in Table V.B,9. Hydrolysis of the methanolysate followed by chromatography showed the absence of any di - O - methyl sugars or any 2 - O - methyl - D - galactose. This would seem to indicate that polysaccharide II is a single long chain since only chain propagating and chain terminating groups are found. Table V.B,10. shows the sugar content of all the polysaccharides.

TABLE V.B,10.

SUGAR CONTENT OF C. HARTMANNIANUM AND ITS
DEGRADATION PRODUCTS

Polysaccharide	$[\alpha]_D$ degrees	Gal	Ara	Mann	Rha	Xyl	Gal A	Glu A
C. hartmannianum gum	-35	22	43	10	4	6	6	9
Polysaccharide I	+16	36	45	19	-	-	-	-
Polysaccharide II	+64	31	46	23	-	-	-	-

TABLE V.B,9.

O - METHYL SUGARS IDENTIFIED IN METHYLATED POLYSACCHARIDE II

Relative retention time (T) of methyl glycosides		R_g after hydrolysis		O - methyl sugar	Relative amount
Column (1)	Column (2)	solvent (e)	solvent (f)		
0.51,0.64	0.56,0.72	0.98	1.00	2,3,5-tri-O-methyl-L-arabinose	2
0.85	0.98	0.87	0.76	2,3,4-tri-O-methyl-L-arabinose	10
1.78	2.22	0.82	0.76	2,4-di-O-methyl-L-arabinose	5
1.53	1.69	0.92	0.84	2,3,4,6-tetra-O-methyl-D-galactose	7
2.94,3.78	2.96,4.28	0.77	0.49	2,3,6-tri-O-methyl-D-galactose	4
6.12	6.23	0.71	0.38	2,3,4-tri-O-methyl-D-galactose	3
1.30	1.32	0.92	0.84	2,3,4,6-tetra-O-methyl-D-mannose	3
3.38	3.64	0.71	0.68	2,4,6-tri-O-methyl-D-mannose	5

V.B.6.   DISCUSSION

C. hartmannianum gum was found to contain galactose (22%),
arabinose (43%), rhamnose (4%), mannose (10%) and xylose (6%).
Chromatography of the 1N - hydrolysate in solvent (c) showed the
presence of three aldobiuronic acid components the major one being
characterised as 6 - O - (β - D - glucopyranosyluronic acid) - D -
galactose from its R_{gal} value and its hydrolysis products. The
other two components which were found to behave similarly chromato-
graphically and were inseparable in all solvent systems examined
are thought to be of a galactose/4 - O - methyl glucuronic acid and a
mannose/galacturonic acid nature respectively. The mannose/gala-
cturonic acid component would seem to be confirmed by the specific
rotation (-27°) found for the combined components, a value similar
to that of one of the acidic components characterised in Anogeissus
samples, ie:- 2 - O - (β - D - glucopyranosyluronic acid) - D -
mannose, $[\alpha]_D$ = -32°. So it is possible that the mannose/
galacturonic acid component may be 2 - O - (β - D - galactopyranosy-
luronic acid) - D - mannose. This is confirmed by the presence of
3,4,6 - tri - O - methyl - D - mannose on methylation of the gum.
The other component is thought to be 6 - O - (4 - O - methyl -β - D -
glucopyranosyluronic acid) - D - galactose, which would have a very
small positive $[\alpha]_D$ and not contribute much to the $[\alpha]_D$ of the
mixture.

Methylation of C. hartmannianum gum showed the presence of end-
group xylose, rhamnose, glucuronic acid and galacturonic acid as well
as large amounts of end-group arabinose (both in the furanose and

pyranose forms) and galactose. Also present in fairly substantial amounts are 2,4 - and 2,3 - di - $\underline{O}$ - methyl - $\underline{L}$ - arabinose, 2,3,6 - tri - and 2,3,4 - tri - and 2,3 - di - $\underline{O}$ - methyl - $\underline{D}$ - galactose and 3,4,6 - and 2,4,6 - tri - $\underline{O}$ - methyl - $\underline{D}$ - mannose. The presence of 2,6 - di - and 2 - $\underline{O}$ - methyl - $\underline{D}$ - galactose may be due to under-methylation or they may have some structural significance.

Partial acid hydrolysis showed the presence of three biose components. The component at R_{gal} 0.84 solvent (b) 0.87 solvent (c) is expected to be an arabinobiose, and from the presence of 2,4 - di - $\underline{O}$ - methyl - $\underline{L}$ - arabinose in methylated gum, the arabinobiose is expected to be 3 - $\underline{O}$ -β - arabinopyranosyl - $\underline{L}$ - arabinose. This is also expected from its R_{gal} values. The component at $R_{gal} \sim 0.50$, from its R_{gal} value and also spot colouration is thought to contain something linked to arabinose, possibly galactose linked to arabinose. The third component, from its R_{gal} value might possibly be the galactobiose, 6 - $\underline{O}$ -β - $\underline{D}$ - galactopyranosyl - $\underline{D}$ - galactose although the possibility of galactose linked to mannose cannot be ruled out.

A Smith Degradation of C. hartmannianum gum gave Polysaccharide I, which was found to contain only galactose, arabinose and mannose, with arabinose the major component. The $[\alpha]_D$ value of $+16^o$ shows that the structure of Polysaccharide I is considerably different from the gum itself ($[\alpha]_D$ -35°). Methylation of Polysaccharide I showed the main components to be 2,3,4 - tri, and 2,4 - di - $\underline{O}$ - methyl - $\underline{L}$ - arabinose, 2,3,4,6 - tetra -, 2,3,6 - tri - and 2,3,4 - tri and 2,3 - di - $\underline{O}$ - methyl - $\underline{D}$ - galactose and 2,4,6 - tri - $\underline{O}$ - methyl - $\underline{D}$ - mannose.

Partial acid hydrolysis of Polysaccharide I showed that two of the biose components present in the gum itself at $R_{gal} \sim 0.50$ and at $R_{gal} \sim 0.26$ were also present in approximately equal amounts.

Polysaccharide II, obtained on periodate oxidation of Polysaccharide I, also contained galactose, arabinose and mannose. Arabinose was again the major neutral sugar, but the ratio of arabinose:galactose was greater than in Polysaccharide I. This is unusual. Partial acid hydrolysis also showed the presence of the two biose components found in Polysaccharide I.

Methylation of Polysaccharide II showed the main components to be end-group arabinopyranose and galactose residues. End-group arabinofuranose and mannose were also present in addition to 2,4 - di - O - methyl - L - arabinose, 2,3,6 - and 2,3,4 - tri - O - methyl - D - galactose and 2,4,6 - tri - O - methyl - D - mannose. An unusual feature of the methylation was the absence of any di - O - methyl - galactose residues or 2 - O - methyl - D - galactose, which would be indicative of branching points within the polysaccharide chain. The fact that Polysaccharide II shows no branch points but plenty of end-groups may be due to the structure of Polysaccharide II being fragile, with the branches "broken-off" during the methylation procedure, thus giving an abundance of end-groups but with an overall impression of only one long chain.

As in the case of the gum from C. erythrophyllum, no definite structural pattern has emerged from these degradation studies of C. hartmannianum gum. The main features established are:- (1) All uronic acid residues are end-group, linked either 1,6 - to galactose

as in the case of glucuronic acid and its 4 - $\underline{O}$ - methyl analogue or, as is tentatively postulated, linked 1,2 - to mannose in the case of galacturonic acid. (2) There is a substantial amount of end-group arabinofuranose, in addition to 1,3 - linked arabinopyranose side chains linked possibly to galactose in the 4 - or 6 - position. Small chains of 1,5 - linked arabinose may also be present. The pink spot obtained on partial acid hydrolysis of all three polysaccharides may (from its R_{gal} value of ~ 0.50) be 4 - $\underline{O}$ - (α or β) - galactopyranosyl - $\underline{\underline{L}}$ - arabinose, but this requires to be confirmed by a large-scale 0.5N - hydrolysis of any of the fractions. (3) Apart from the presence of 3,4,6 - tri - $\underline{O}$ - methyl - $\underline{\underline{D}}$ - mannose which has been attributed to the presence of galacturonic acid linked 1,2 - to mannose, the main $\underline{O}$ - methyl mannose component found in all methanolysates was 2,4,6 - tri - $\underline{O}$ - methyl - $\underline{\underline{D}}$ - mannose, which might possibly indicate 1,3 - linked mannose chains, possibly also linked 1,4 - to galactose. (4) There are probably 1,6 - linked galactose chains, a fact supported by the presence of 6 - $\underline{O}$ - β - $\underline{\underline{D}}$ - galacto-pyranosyl - $\underline{\underline{D}}$ - galactose in all the partial acid hydrolysates.

On the basis of these findings and postulations a possible structure for C. hartmannianum gum involves a backbone or main chain of 1,6 - linked galactose residues with 1,3 - linked arabinopyranose and 1,3 - linked mannopyranose side chains linked to the main galactose chains at the 4 - position. Certain galactose side chains would be terminated by glucuronic acid and most mannose side chains would be terminated by galacturonic acid.

A possible structural fragment is shown in figure V.B,6. It must be emphasised that this is only a tentative structure; the time

FIGURE V.B.6.

Possible structural fragment of C. hartmannianum gum
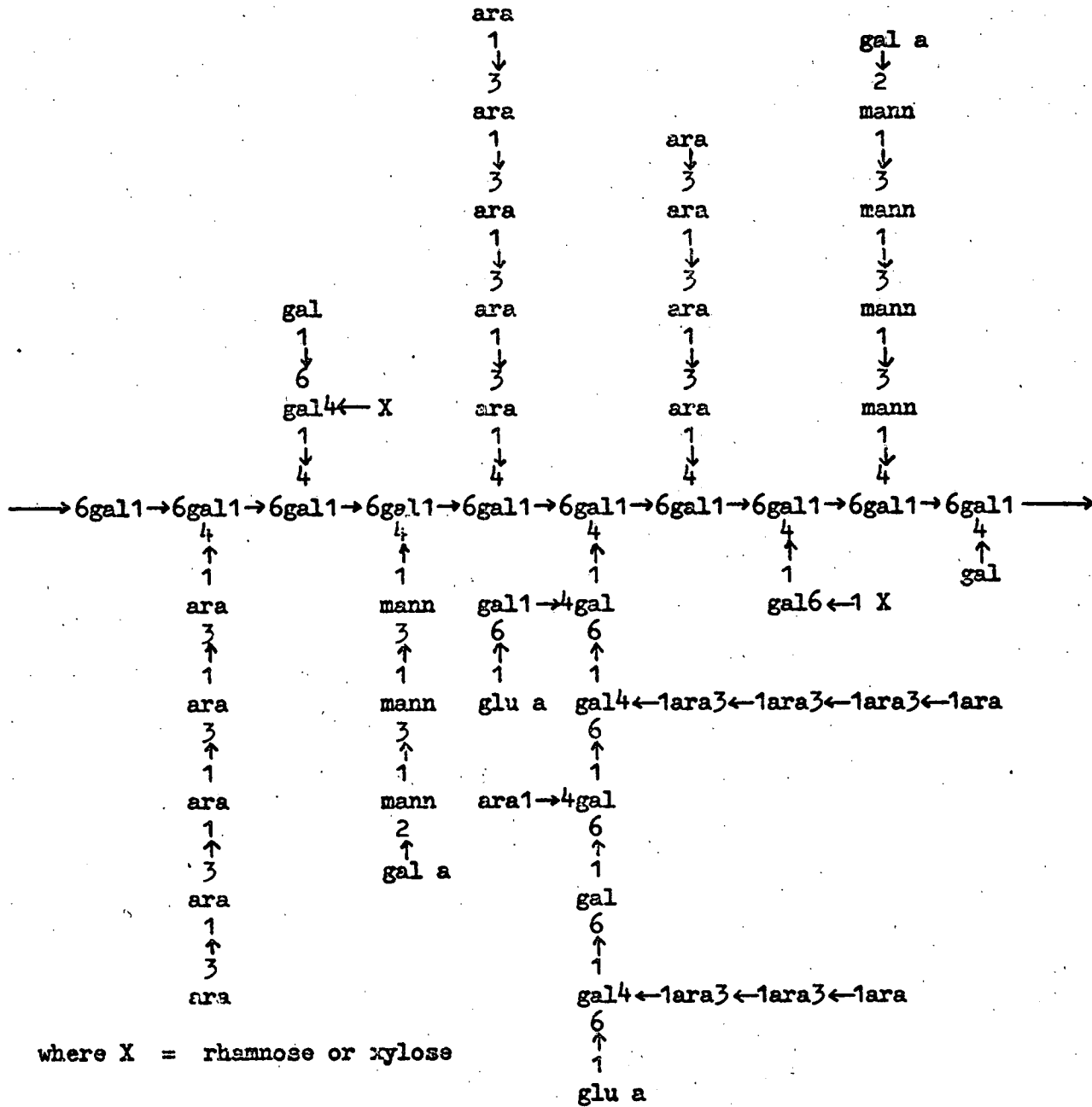
```
                                                    ara
                                                     1
                                                     ↓
                                                     3                                    gal a
                                                    ara                                     2
                                                     1                                       ↓
                                                     ↓                                      mann
                                                     3                        ara            1
                                                    ara                        3             ↓
                                                     1                         ↓             3
                                                     ↓                        ara           mann
                             gal                     3                         1             1
                              1                     ara                        ↓             ↓
                              ↓                      1                         3             3
                              6                      ↓                        ara          mann
                             gal4 ← X                3                         1             1
                              1                     ara                        ↓             ↓
                              ↓                      1                         3             3
                              4                      ↓                        ara          mann
                                                     3                         1             1
                                                    ara                        ↓             ↓
                                                     1                         4             4
                                                     ↓
                                                     4
   → 6gal1 → 6gal1 → 6gal1 → 6gal1 → 6gal1 → 6gal1 → 6gal1 → 6gal1 → 6gal1 → 6gal1 →
              4            4            4            4            4            4
              ↑            ↑            ↑            ↑            ↑            ↑
              1            1            1            1            1           gal
             ara          mann      gal1 → 4gal              gal6 ← 1 X
              3            3          6      6
              ↑            ↑          ↑      ↑
              1            1          1      1
             ara          mann      glu a  gal4 ← 1ara3 ← 1ara3 ← 1ara3 ← 1ara
              3            3                 6
              ↑            ↑                 ↑
              1            1                 1
             ara          mann      ara1 → 4gal
              1            2                 6
              ↑            ↑                 ↑
              3          gal a               1
             ara                            gal
              1                              6
              ↑                              ↑
              3                              1
             ara                  gal4 ← 1ara3 ← 1ara3 ← 1ara
                                             6
         where X = rhamnose or xylose        ↑
                                             1
                                            glu a
```

available did not allow the work involved in a rigorous study to be undertaken. Although much more work must be carried out before a structure can be postulated with confidence, the present exploratory investigation has served much purpose by elucidating some of the features and indicating that the structure involved is unusually complex.

V.B.7.   GENERAL DISCUSSION ON COMBRETUM GUM CHEMISTRY

On the basis of the results found during experimental investigations of C. erythrophyllum and C. hartmannianum gum exudates, a comparison is made between them and also with C. leonense, the structural features of which have been studied by Aspinall and Bhavanandan[14],[15]. The three samples differ extensively but all probably have a backbone of galactose residues linked 1,6 - , because of the presence of 2,3,4 - tri - $\underline{O}$ - methyl - $\underline{D}$ - galactose in all methanolysates. The presence of 2,3 - di - $\underline{O}$ - methyl - $\underline{D}$ - galactose in the methanolysates of all three gum polysaccharides and their degradation products arising from 1,4,6 - linked galactose residues, shows that branch points occur in the galactose backbone (see figure V.B,8). 1,4 - Linked galactose chains are also present.

FIGURE V.B,7.

```
⇐ - - - - - 1 gal 6 ⇐ - - - - - -
                4
                ↑
                I
                I
                I
```
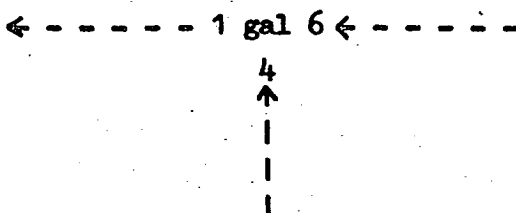
C. erythrophyllum and _C. leonense_ differ markedly from _C. hartmannianum_ in their aldobiuronic acids. All three gum exudates contain $6 - \underline{O} - (\beta - \underline{D} - \text{glucopyranosyluronic acid}) - \underline{D} - \text{galactose}$, which is the most common aldobiuronic acid found in gum chemistry. They are all also thought to contain $6 - \underline{O} - (4 - \underline{O} - \text{methyl} - \beta - \underline{D} - \text{glucopyranosyluronic acid}) - \underline{D} - \text{galactose}$, on the basis of their methoxyl content which does not appear to be associated with a neutral sugar. The difference between the exudates involves the galacturonic acid-containing aldobiuronic acid. Both _C. leonense_ and _C. erythrophyllum_ contain $2 - \underline{O} - (\alpha - \underline{D} - \text{galactopyranosyluronic acid}) - \underline{L} - \text{rhamnose}$, whereas _C. hartmannianum_ apparently contains $2 - \underline{O} - (\beta - \underline{D} - \text{galactopyranosyluronic acid}) - \underline{D} - \text{mannose}$. Furthermore, the majority of the uronic acid residues are intra-chain in _C. leonense_ and _C. erythrophyllum_. In _C. hartmannianum_ the uronic acid all appears as end-group, and is removed by a single Smith Degradation.

The structural significant of rhamnose in _C. hartmannianum_ also differs from that in the other two species. In _C. hartmannianum_ all rhamnose residues are terminal; in _C. erythrophyllum_ and _C. leonense_ only a few rhamnose residues are terminal, the other rhamnose residues occurring deep within the polysaccharide structure (figure V.B,5) or in rhamnose side chains.

The mode of attachment of arabinose side chains also differs in the different exudates. _C. leonense_ contains $3 - \underline{O} - \beta - \underline{D} - \text{galactopyranosyl} - \underline{L} - \text{arabinose}$ indicating arabinose (and possibly also arabinose side chains) attached to the main branched galactose

chains of the structure at the 3 - position. C. erythrophyllum is believed also to contain 3 - O -β - D - galactopyranosyl - L - arabinose, as shown by the presence of 2,4,6 - tri - O - methyl - D - galactose in the methanolysates, although this sugar was not present in sufficient amount for rigorous characterisation. Methanolysis of C. hartmannianum showed no 2,4,6 - tri - O - methyl - D - galactose; hence arabinose and arabinose side chains cannot be joined to the branched galactose chains at the 3 - position. Alternatively, arabinose may be joined to galactose at the 4 - position.

All of the three gum exudates contained large amounts of peripheral arabofuranose residues. C. leonense contained a great deal of 3 - O -β - L - arabinopyranosyl - L - arabinose as did C. hartmannianum. In C. hartmannianum most of the arabinose residues are in the pyranose form as is the case in C. leonense. However in C. erythrophyllum the majority of arabinose residues are in the furanose form. Also C. erythrophyllum tends to differ from the other two exudates in that the relatively smaller amounts of 3 - O -β - L - arabinosyl - L - arabinose are peripheral and removed during the early degradation stages as shown by the absence of any 2,4 - or 2,5 - di - O - methyl - L - arabinose in any of the degradation products.

All three gum exudates show the presence of 2,3 - di - O - methyl - L - arabinose on methanolysis of their methylated products but although it was only present in small amounts in C. leonense and C. hartmannianum, it was the major di - O - methyl arabinose in C. erythrophyllum. It was also present in large amounts in all the degradation products of C. erythrophyllum although it was removed

easily by degradation of the other two exudates. And so whereas
C. leonense and C. hartmannianum have 1,3 - linked arabinobiose side
chains, C. erythrophyllum is expected to contain 1,5 - linked arabinose
side chains. One interesting point is that arabinose residues linked
at the 1 - and 5 - positions should be easily susceptible to periodate-
oxidation. The fact that they persist through the Smith degradations
indicates that they must be protected in some way, and this may be
a steric effect.

C. hartmannianum was found to contain 1,3 - linked mannose side
chains, a feature found in Gum Ghatti[6]. C. erythrophyllum, which
was not found to contain mannose initially, was, after removal of
all uronic acid, found to contain mannose internally in the gum
structure. Since Aspinall and Bhavanandan did not carry out a
Smith degradation study on C. leonense, with subsequent elimination
of uronic acid, it is not known if this is a feature of that gum
exudate also.

Although rigorous structures can not be postulated for gums from
the Combretum genus, this exploratory study has revealed the genetic
complexity of the genus, indicated the complex nature of these gum
exudates, and established their more important structural features.

Other Combretum species must also be studied to check the
postulated division of Combretum gum exudates into three sections
(see Section V.A). Then, perhaps, a better understanding of the
chemistry and structure of Combretum gum exudates will be possible.
It is hoped that the present superficial study will serve to indicate
to future workers the extent of the task that will be involved in
undertaking a rigorous structural investigation.

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3

SECTION VI

ANALYTICAL AND HETEROGENEITY STUDIES OF GUM EXUDATES

FROM ACACIA TORTILIS SUBSPECIES

VI.1. INTRODUCTION

Bentham[1] classified _Acacia heteracantha_ Burch., _A. spirocarpa_ Hochst., and _A. tortilis_ Hayne as closely related species within his Series 4, _Gummiferae_, and he listed _A. perrottetii_ Steud., _A. fasciculata_ Guill. et Perr. non Kunth non R.Br., and _A. raddiana_ Savi as synonyms of _A. tortilis_. Later, two varieties, var. _raddiana_ and var. _pubescens_ A. Chev., were recognised in _A. raddiana_.

Acacia tortilis is a widespread species, complicated genetically, and apparently divisible into a number of more or less distinct geographical races. In Brenan's[*] opinion[2], the following four subspecies can be distinguished:- _A. tortilis_ (Forsk.) Hayne ssp. _tortilis_; _A. tortilis_ (Forsk.) Hayne ssp. _spirocarpa_ (Hochst. ex A. Rich.) Brenan; _A. tortilis_ (Forsk.) Hayne ssp. _heteracantha_ (Burch.) Brenan; and _A. tortilis_ (Forsk.) Hayne ssp. _raddiana_ (Savi) Brenan, which exists as two varieties: var. _raddiana_ and var. _pubescens_ A. Chev. Of these subspecies, _raddiana_ is considered the most distinctive and is still regarded by some workers as a separate species; ssp. _spirocarpa_ and ssp. _heteracantha_ are closely related morphologically, but typical ssp. _tortilis_ is rather isolated from the rest, both morphologically and geographically, being the only one found east of the Red Sea.

Good, authenticated gum specimens from _A. tortilis_ ssp. _heteracantha_, ssp. _spirocarpa_, and ssp. _raddiana_ var. _pubescens_ were obtained, and their chemical composition was investigated to ascertain whether there were significant differences between the subspecies.

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VI.2. ORIGIN OF SPECIMENS

Gum from A. tortilis (Forsk.) Hayne ssp. spirocarpa (Hochst. ex A. Rich.) Brenan was collected by Mr. A.G. Seif-el-Din, Gum Research Officer to the Republic of Sudan, in March 1964; this ssp. is abundant in hard soils in the region $13^\circ45'N$, $30^\circ20'E$. Gum from A. tortilis (Forsk.) Hayne ssp. raddiana (Savi) Brenan var. pubescens A. Chev. was collected as follows: specimen I was obtained in April 1973 in the region $13^\circ45'N$, $30^\circ20'E$, where this ssp. is abundant on loose sandy soils; specimen II was obtained in March 1970 from the main trunk (normally it is the upper branches that are tapped) of a tree at Um Badir, N. Kordofan. (Botanical Vouchers for specimens of gum from A. tortilis ssp. spirocarpa and A. tortilis ssp. raddiana var. pubescens have been authenticated by Mr. J.P.M. Brenan.)

Gum from A. tortilis (Forsk.) Hayne ssp. heteracantha (Burch.) Brenan was collected as follows: specimen I was collected at Salisbury, Rhodesia on 28 February 1971 (botanical voucher Kelly 479 in SRGH); specimens II and III (botanical vouchers G. Pope 426 and 427 in SRGH) were collected in May 1971 at Botswana, near Rakops, Rhodesia ($24^\circ30'E$, $21^\circ4'S$) and sent by Mr. Th. Muller, Curator, Botanical Gardens, Salisbury.

VI.3. PURIFICATION OF SAMPLES

All of the specimens dissolved in cold water overnight to give clear, colourless solutions (ssp. spirocarpa, ssp. heteracantha specimens I and II) or clear, pale yellow solutions (ssp. raddiana var. pubescens specimens I and II, ssp. heteracantha specimen III).

The three specimens of ssp. heteracantha all gave a very slight jelly-type residue which was readily dispersed with alkaline borohydride[3].

The solutions were filtered through muslin to remove insoluble debris, through No. 41 then No. 42 filter papers, and dialysed against running tap water for 2 days (4 days in the case of the borohydride treated samples), after which time the solutions were freeze-dried. Reasonably high yields of the freeze-dried products were obtained in all cases.

VI.4. RESULTS AND DISCUSSION

Hydrolysis with 1N - sulphuric acid for 7.5 hours followed by chromatography in solvents (b) and (c) showed large amounts of arabinose in addition to galactose and small amounts of rhamnose. Chromatography in solvent (c) showed the presence of four spots corresponding to the four aldobiuronic acids: $6 - \underline{O} - (\beta - \underline{\underline{D}} -$ glucopyranosyluronic acid) $- \underline{\underline{D}} -$ galactose, R_{gal} 0.26, $4 - \underline{O} -$ ($\alpha - \underline{\underline{D}} -$ glucopyranosyluronic acid) $- \underline{\underline{D}} -$ galactose, R_{gal} 0.36, $6 - \underline{O} - (4 - \underline{O} -$ methyl $-\beta - \underline{\underline{D}} -$ glucopyranosyluronic acid) $-$ $\underline{\underline{D}} -$ galactose, R_{gal} 0.61, $4 - \underline{O} - (4 - \underline{O} -$ methyl $-\alpha - \underline{\underline{D}} -$ glucopyranosyluronic acid) $- \underline{\underline{D}} -$ galactose, R_{gal} 0.71. Also present in the chromatograms of the heteracantha samples were significant amounts of $4 - \underline{O} -$ methyl glucuronic acid [R_{gal} 1.92 solvent (e); orange/pink spot], which is unusual in a 1N - hydrolysate. Hydrolysis with 2N - sulphuric acid is normally required to break uronosyl linkages. However this is similar to the results found during analytical studies of Acacia species from the Series 1,

<u>Phyllodineae</u> (see Section III).

The identification of four aldobiuronic acids is consistent with the results found for other species of the <u>Gummiferae</u> eg:- <u>A. arabica</u>[4], <u>A. drepanolobium</u>[5] and <u>A. seyal</u>[6]. Subspecies <u>spirocarpa</u> and ssp. <u>raddiana</u> var. pubescens have $6 - \underline{O} - (\beta - \underline{D} - $ glucopyranosyl-uronic acid) $- \underline{D} - $ galactose as their major acidic component with the other components present in much smaller amounts. However ssp. <u>heteracantha</u> shows that the $4 - \underline{O} - $ methyl $- $ aldobiuronic acids are the major components with the two of them present in roughly equal amounts and $6 - \underline{O} - (\beta - \underline{D} - $ glucopyranosyluronic acid) $- \underline{D} - $ galactose being the major of the other two components.

The analytical data for the six specimens studied are shown in Table VI,A. When all the analytical parameters are taken into account, there is little doubt that the three subspecies examined each give a gum exudate that is analytically distinguishable from the others, and from all other <u>Acacia</u> exudates examined to date.

The overall impression is that ssp. <u>raddiana</u> var. <u>pubescens</u> is intermediate between ssp. <u>spirocarpa</u> and ssp. <u>heteracantha</u>, but on the basis of their closely similar viscosities, molecular weights, methoxyl contents and sugar ratios, ssp. <u>raddiana</u> appears to be closer chemically to ssp. <u>spirocarpa</u> than to ssp. <u>heteracantha</u>, in contrast to the morphological relationship between the latter two. Subspecies <u>raddiana</u> is certainly not an entirely distinct species.

The gum from ssp. <u>heteracantha</u> proved to be the most interesting of the three subspecies analytically. It gave clear, colourless or pale yellow solutions, and displayed properties that

TABLE VI.A.

ANALYTICAL DATA FOR PURIFIED GUM
POLYSACCHARIDES FROM ACACIA TORTILIS SUBSPECIES

	Subsp. spirocarpa	Subsp. raddiana var. pubescens		Subsp. heteracantha		
		I	II	I	II	III
Moisture (%)	9.9	10.3	9.6	9.9	11.8	10.7
Ash (%)[a]	1.6	1.9	1.3	1.5	2.0	1.7
Nitrogen (%)[a]	0.46	0.96	1.22	1.51	1.49	1.5
Hence protein (%) (N x 6.25)[a]	2.9	6.0	7.6	9.4	9.3	9.4
Methoxyl (%)[b]	0.58	0.61	0.66	0.96	0.97	1.0
$[\alpha]_D$, in water, (degrees)[b]	+74	+88	+87	+97	+79	+8
$[\alpha]_D$, in 7M urea, (degrees)[b]	+78	+93	+92	+99	+82	+9
Intrinsic viscosity (mlg^{-1})[a]	9.8	9.3	11.2	22.5	16.9	19.4
Molecular weight (MW x 10^4)[a]	25	50	51	210	220	140
Equivalent weight[b]	1590	2040	1940	2440	1730	2100
Hence uronic anhydride (%)[b,c]	11.1	8.6	9.1	7.2	10.1	8.7

Sugar composition after hydrolysis:-

	Subsp. spirocarpa	Subsp. raddiana var. pubescens		Subsp. heteracantha		
		I	II	I	II	III
4 - O - Methylglucuronic acid[d]	3.5	3.7	4.0	5.8	5.8	6.4
Glucuronic acid	7.6	4.9	5.1	1.4	4.3	2.3
Galactose	39	36	37	21	24	24
Arabinose	43	49	46	68	62	64
Rhamnose	7	6	8	4	3	3

Footnotes

a Corrected for moisture content.

b Corrected for moisture and protein content.

c If all acidity arises from uronic acids.

d If all methoxyl groups located in this acid.

would make it of considerable potential commercial interest. It has
the highest ratio of arabinose to galactose recorded so far, the
previous highest being from A. nubica[7]; a high methoxyl content,
surpassed only by A. nilotica[8], A. parramattensis[9] and A. giraffae[8];
a nitrogen content as high as that previously reported for any Acacia,
ie:- A. parramattensis[9]; a molecular weight, which is almost as high
as that recorded previously, ie:- A. arabica[10] (2.3 x 10^{6}) and an
intrinsic viscosity, which, for specimen III, equals the highest
values previously recorded for A. laeta[11] and A. parramattensis[9].

With such a high protein content, and the presence of more than
one component (see later), it appeared that contributions to the
optical rotation in water from tertiary structures were possible.
Rotations in 7M urea were therefore determined, but the values
were only slightly different and always more positive than in water
as was found in a previous study[12] (see Section III).

VI.5. HETEROGENEITY STUDIES

With a view to proceeding to a detailed structural study of the
gum from A. tortilis ssp. heteracantha specimen I, crude gum (68 g)
was dissolved in distilled water (2 l) giving a viscous, clear
solution, which was purified as described previously and obtained
as the freeze-dried product (51 g; 75% yield). The normal tests for
homogeneity were carried out to determine whether the gum from
A. tortilis ssp. heteracantha was heteropolymolecular and analagous
to A. campylacantha[13], A. laeta[11] and A. senegal[14] gums. Interesting
results were found; these prompted the examination of the other

specimens of the subspecies and also of the other subspecies themselves.

Ion exchange chromatography on a DEAE - cellulose column (46 x 1.3 cm), using a sodium chloride gradient (0.0 - 0.5M in 0.02M - acetate buffer), produced one rather diffuse-looking peak and not a sharp symmetric peak as found for Anacardium occidentale (section IV) and Combretum erythrophyllum (section V). The peak "tailed-off" rather noticeably giving the impression of there possibly being a shoulder (see Figure VI.A).

Zone electrophoresis of the polysaccharide was carried out on strips of cellulose acetate on both 0.1M - ammonium carbonate buffer (pH 8.9) and 0.1M - acetate buffer (pH 4.7) and, whereas single bands were obtained with the Combretum and Anacardium samples run at the same time, two distinct thin bands were observed, the less dense band running faster. Similar results were obtained by thin-layer electrophoresis on Phorcslides in 0.1M - ammonium carbonate buffer (pH 8.9) and 0.05M - borate buffer (pH 9.2) at 200 volts for 10 minutes (see Figure VI.B). Thin-layer electro-phoresis of the polysaccharide dyed with Procion Brilliant Red M-2B dye could not be carried out as the dyed polysaccharide was insoluble after freeze-drying.

An ultracentrifuge run was carried out at 44,000 rpm on a 5% solution of polysaccharide in 0.5M - sodium chloride; two distinct peaks were observed (see Figure VI.C). Molecular sieve chromato-graphy of the polysaccharide showed a single peak at the void volume on both Bio-gel A-5m and A-15m columns, however on a Bio-gel A-150m column, a complex two-peak system was observed (see Figure VI.D).

FIGURE VI,A.

ION EXCHANGE CHROMATOGRAPHY ON D.E.A.E. -
CELLULOSE OF SAMPLES OF
A. TORTILIS SSP. HETERACANTHA
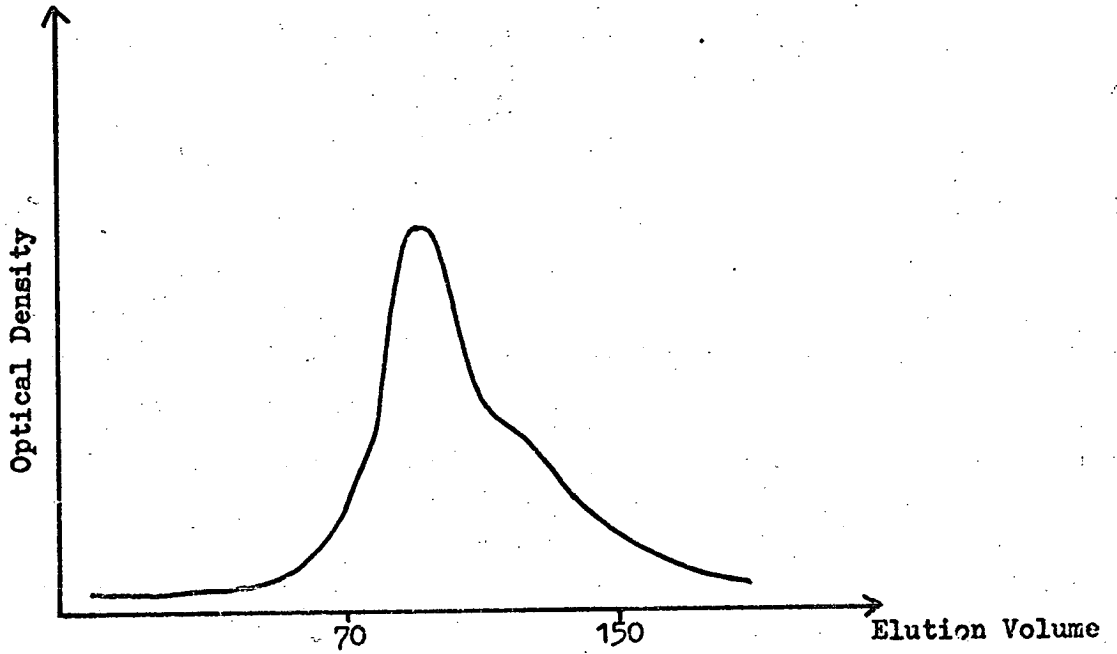
FIGURE VI,B.

THIN LAYER ELECTROPHORESIS OF A. TORTILIS SSP. HETERACANTHA
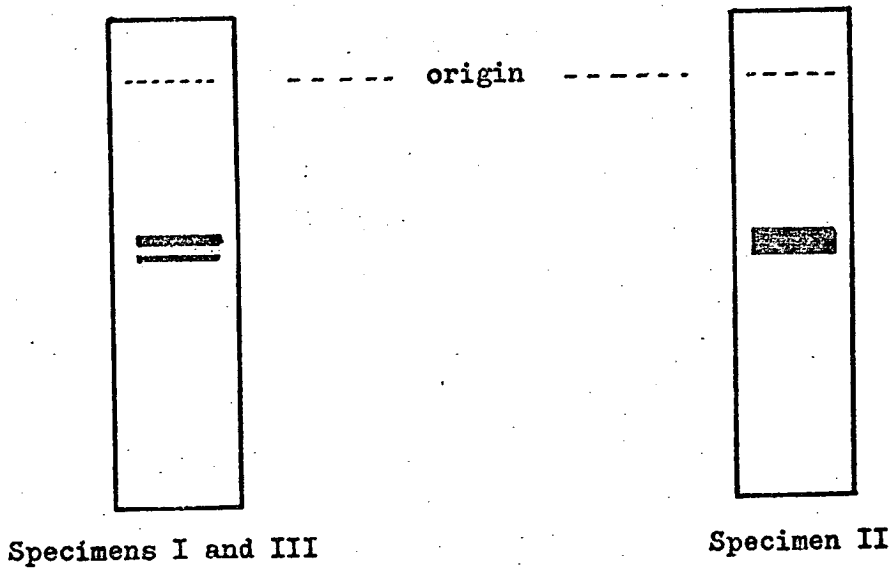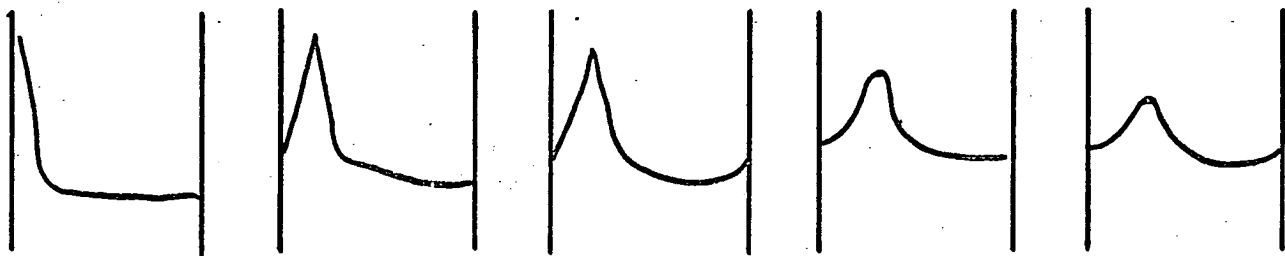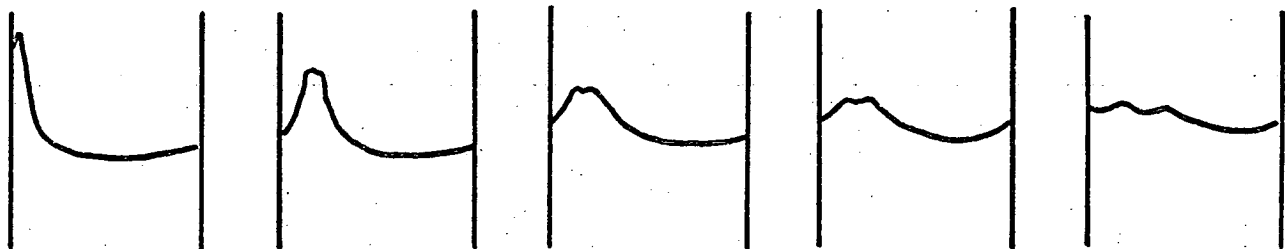ON PHOROSLIDES

Specimens I and III   Specimen II

FIGURE VI,C.

ULTRACENTRIFUGE RUNS OF A. TORTILIS SAMPLES AT 44,000 r.p.m.
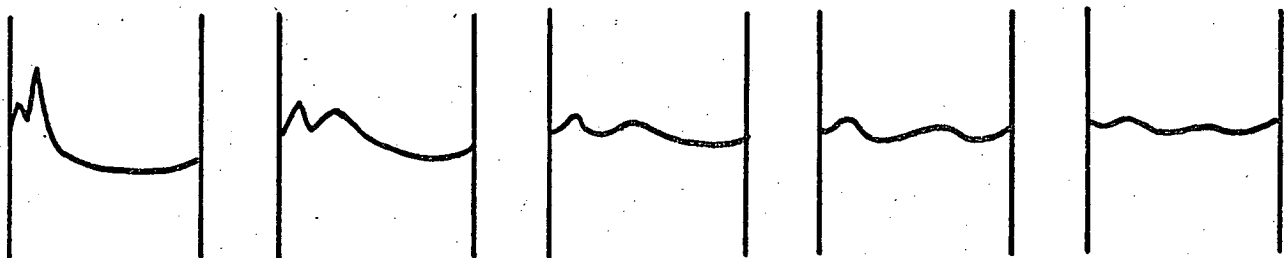
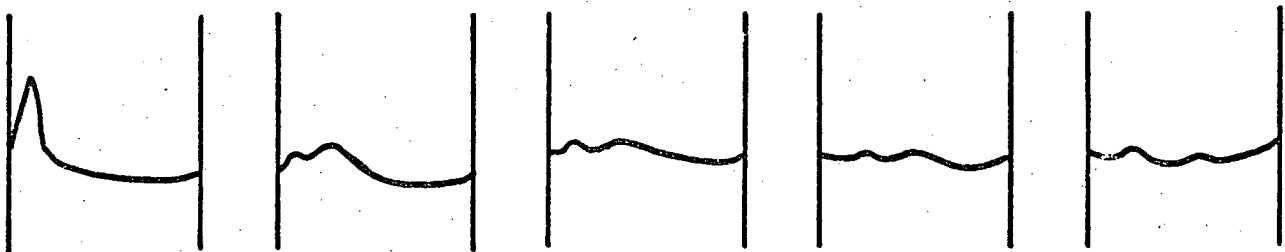### (PHOTOGRAPHS TAKEN AT 8 MINUTE INTERVALS)

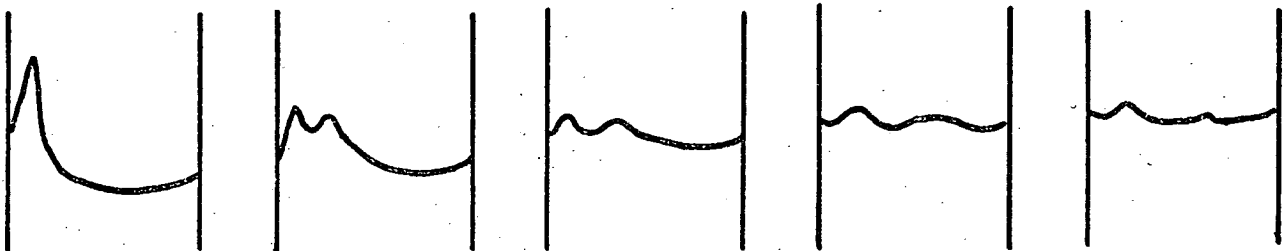ssp. Spirocarpa and ssp. Raddiana var. Pubescens Specimen I

ssp. Raddiana var. Pubescens Specimen II
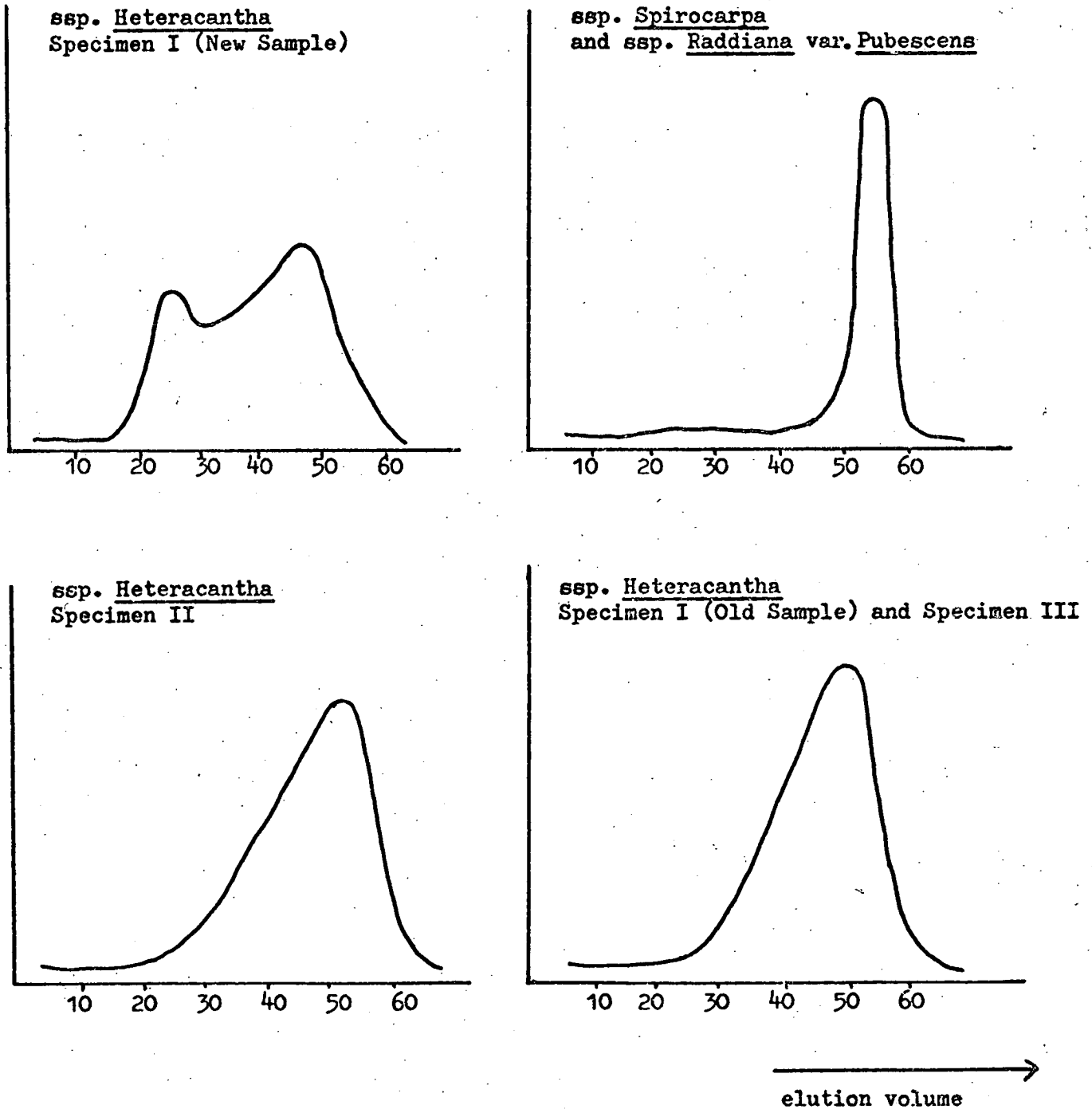
ssp. Heteracantha Specimen I

ssp. Heteracantha Specimen II

ssp. Heteracantha Specimen III

FIGURE VI,D.

MOLECULAR SIEVE CHROMATOGRAPHY OF A. TORTILIS SAMPLES ON A BIO-GEL A-150M COLUMN

ssp. Heteracantha
Specimen I (New Sample)

ssp. Spirocarpa
and ssp. Raddiana var. Pubescens

ssp. Heteracantha
Specimen II

ssp. Heteracantha
Specimen I (Old Sample) and Specimen III

elution volume

Because of the above findings, homogeneity tests were carried out on the other A. heteracantha specimens to see if this heterogeneity was shown by all A. heteracantha samples. Also certain tests were carried out on the other subspecies as a comparison.

Zone and thin-layer electrophoresis of the three A. heteracantha subspecies showed that specimens I and III gave two distinct bands, as for the large-scale sample. However, specimen II gave one broad band covering the same area as the two bands of the other specimens (see Figure VI.B).

Ultracentrifuge runs confirmed two-peak systems for all three samples from A. heteracantha and for ssp. raddiana var. pubescens specimen I (see Figure VI.C). However the polysaccharides from ssp. spirocarpa and ssp. raddiana var. pubescens specimen II showed only one ultracentrifuge peak.

Molecular sieve chromatography on a Bio-gel A-150m column showed one sharp symmetric peak for ssp. spirocarpa and for the two ssp. raddiana samples. However, ssp. heteracantha specimen I (old sample) showed only one peak. The peak was not sharp and was not symmetric. A similar one-peak system was found for the other two heteracantha specimens (see Figure VI.D). The molecular weight, $\overline{M}w$, of the new specimen I was found to be $\sim 4 \times 10^6$, which is double the value found for the previous, larger-scale preparation. Possibly some sub-sampling effect of the non-homogeneous nodular fragments could have occurred, or degradation inadvertantly taken place.

A. tortilis ssp. heteracantha therefore shows the clearest evidence to date for the presence of a heterogeneous system in an

Acacia gum exudate. Clearly, careful attempts to achieve fractiona-
tion or the elimination of some impurity will be an essential pre-
requisite to structural studies.

It would be desirable to study further specimens to ascertain
whether the extent of the differences between the three specimens
studied here are typical of a genetically complex species. Also
it would be of extreme interest to examine gum specimens from ssp.
tortilis and ssp. _raddiana_ var. _raddiana_; efforts to secure
specimens have been initiated.

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APPENDIX

AN OPTICAL ROTATION STUDY OF MIXTURES OF ACACIA SENEGAL
AND ACACIA SEYAL GUM EXUDATES

Small amounts of A. senegal and A. seyal gum were purified in the normal way and obtained as freeze-dried products. Moisture contents were determined for both samples and their specific rotations calculated on a polarimeter. A. senegal had an $[\alpha]_D$ value of -30.2° which was as expected for a good commercial grade of the gum. A. seyal had $[\alpha]_D$ +29.7°. Taking moisture contents into account, weights of each gum were taken so as to give a 1% solution of a gum mixture containing 95% A. senegal, 5% A. seyal. 1% Solutions of other percentage mixtures were made and the optical rotations of all the solutions measured:-

% Senegal	% Seyal	$[\alpha]_D$ (degrees)
100	-	-30.2
95	5	-27.4
90	10	-24.5
85	15	-21.3
80	20	-17.9
75	25	-15.1
70	30	-12.0
65	35	- 8.8
60	40	- 5.9
55	45	- 3.0
50	50	- 0.3
45	55	+ 2.5
40	60	+ 6.2
35	65	+ 8.6
30	70	+12.0
25	75	+14.8
20	80	+17.8
15	85	+21.2
10	90	+23.6
5	95	+26.7
-	100	+29.7

A graph of $[\alpha]_D$ against percentage shows a straight-line correlation, indicating that the $[\alpha]_D$ value of a mixture can be used to give an approximate value for the percentage of each component present.