ANALYTICAL AND STRUCTURAL STUDIES OF *ACACIA* POLYSACCHARIDES

by

M. C. L. GILL B. Sc.

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DECLARATION

I hereby declare that this thesis was composed by myself and that the work therein is my own.

None of the work included in this thesis has been submitted for any other degree or professional qualification.

Some of the analytical data reported in this thesis have already been published (D.M.W. Anderson and M.C.L. Gill, Phytochemistry, 1975, 14, 739) and a manuscript on the chemotaxonomic aspects of the data for Juliflorae spp. was submitted for publication (J. Linnean Society) in September 1978.

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ABSTRACT

An analytical study of eleven Acacia gums from the subseries Juliflorae of the series Phyllodineae showed them to be more proteinaceous, more acidic and more viscous, with higher methoxyl contents and higher molecular weights but with lower proportions of rhamnose and arabinose than the majority of Acacia gums studied so far.

A structural study of the gum from Acacia auriculiformis involving hydrolysis, Smith-degradation and methylation studies, revealed a β-1, 3-linked galactose backbone with side chains of β-1, 6-linked galactose. Glucuronic acid was present as end-groups, linked β-1, 6 to galactose: 4-O-methyl glucuronic acid was present as end-groups, α-1, 4-linked to galactose. Arabinose was present in short, β-1, 3-linked chains, while rhamnose was present as end-group. Proteinaceous material was attached to the molecule by periodate-resistant linkages.

The use of carbon 13-nuclear magnetic resonance spectroscopy for the study of gum exudates was examined. Spectra were obtained for the gum from Acacia auriculiformis and its degradation products, despite their high molecular weight and chemical complexity. The spectra substantiated the findings of the structural study.

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CARBON-13 NUCLEAR MAGNETIC RESONANCE STUDIES ON THE GUM FROM *ACACIA AURICULIFORMIS*.

SECTION I

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Plant gums are complex acidic heteropolysaccharides exuded from the stems of certain tropical and sub-tropical trees found in Africa, Australasia, South America and parts of Asia. Production of the gum is usually preceded by mechanical injury or bacterial infection[1]. The precise mechanism of gum formation is still unknown as is the function of the exudate; it is possible, however, that prevention of water loss, a crucial factor in dry environments, or prevention of invasion by micro-organisms are involved. The exudate usually hardens in the sun, at the site of injury, to form nodules which, in the case of Acacia species, dissolve in water to form neutral or slightly acidic solutions. In the natural state the uronic acid residues present in the gum are wholly or partially neutralised by calcium, magnesium, potassium and sodium ions as well as by micronutrient elements such as iron, copper, strontium, zinc and manganese; the relative proportions of the ions probably vary with the soil type[2].

When the gum nodules dissolve in water they form pale straw to brown coloured solutions, some of which have relatively high intrinsic viscosity. It is this property which has made plant gums commercially important. Since the ancient Egyptians used them for embalming purposes in the seventeenth century B.C., plant gums have found a place in native remedies and in the pharmaceutical preparations of modern man[3].

The present commercial value of plant gums stems from their use in the confectionery industry, in the clarification of wines, as adhesives and thickeners, stabilisers or emulsifiers in a wide variety of foodstuffs. These applications depend also on the lack of flavour and the lack of toxicity of plant gums, a rare combination of properties in a natural product. Although gums do not seem to have any particular nutritional value, galagos or bush babies (galagidae) supplement their predominantly insect diet with gum from Acacia trees[4].

The centre of the gum-producing industry has, for many years, been in Africa; gum has been exported from the Sudan and Egypt for centuries. The main reason for this is that the tree which produces the most acceptable gum, Acacia senegal, is indigenous to these regions. The gum exuded by this tree is exported as 'Gum arabic' although this is frequently adulterated with inferior gum, both accidentally and deliberately. Most of the Gum arabic presently available comes from semi-cultivated gum gardens from which rogue trees have been eradicated, but recent prolonged drought has severely reduced the quantities of gum available from this source. This shortage of gum has prompted a widespread appraisal of the properties of other gums and of the potential of other gum producing regions.

A large number of species of the genus Acacia are indigenous to

Australia, parts of which have the arid climate suitable for gum production. Although many of these Acacia trees are known to produce gum, commercial exploitation on a scale similar to that of 'Gum arabic' has not occurred, partly due to the fact that some of the gum does not meet the rigorous standards of the foodstuffs industry, but also because of ignorance on the part of consumer industries.

Recently an increasing number of Acacia gums of Australian origin have been examined, or are under scrutiny at present; their identities are shown in Table I.1. For many years the genus Acacia has been subdivided according to the classification of Bentham[5], although recently Vassal[6] has improved on the original divisions; Table I.1 shows the species arranged in series, according to Bentham under the subgenera of Vassal. Where possible, the sections and subsections of Vassal, to which the species belong, are indicated by means of the following scheme.

Subgen. Heterophyllum Sect. Heterophyllum Subsect. Spiciferae Hs

Subgen. Heterophyllum Sect. Heterophyllum Subsect. Globiferae Hg

Subgen. Heterophyllum Sect. Uninerva Subsect. Globiferae U

Subgen. Aculeiferum Sect. Aculeiferum Subsect. Globiferae A

Subgen. Aculeiferum Sect. Monacanthea Subsect. Globiferae M

These distinguishing letters are given in square brackets where the

TABLE I.1

Subgen. HETEROPHYLLUM
Series 1. PHYLLODINAE subseries 1-7

tetragonophylla [Hg]	calamifolia [Hg]	uncinata
montana	pruinocarpa	victoriae [U]
aestivalis	bancroftii [U]	dictyophleba
difformis [U]	falcata [U]	jennerae
ligulata [U]	mabellae	microbotrya [U]
murrayana U	penninervis [U]	podalyriifolia [U]
prainii	pycantha [U]	retinoides [U]
rostellifera	rubida [U]	salicina [U]
saligna [U] (syn. cyano-	georginae	harpophylla [Hg]
cyclops [Hg] phylla)	latescens	

(Also beauverdiana Hs - sub-series 7F?)

Subseries 8, Juliflorae

pubifolia [Hs]	acradenia Hs	aneura [Hs]
kempeana [Hs]	coolgardiensis [Hs]	microneura Hs
auriculiformis Hs	leptostachya Hs	stereophylla Hs
torulosa Hs	holosericea Hs	mangium Hs

Series 2. BOTRYOCEPHALAE

dealbata [U]	deanei [U]	decurrens [U]
elata [U](syn. terminalis)	filicifolia U	leucoclada [U]
mearnsii [U]	mollissima [U]	parramattensis U
parvipinnula U	silvestris U	trachyphloia U

Subgen. ACACIA
Series 4. GUMMIFERAE

abyssinica subsp. calophylla	adansonii	arabica
drepanolobium	fistula	farnesiana
gerrardii	erioloba (syn. giraffae)	hebeclada
heteracantha	karroo	kirkii
leucophloea	nebrownii	nilotica
nubica	recifiens	rigidula
	sieberana (var. sieberana, tortilis var. villosa & var. woodii)	

Subgen. ACULEIFERUM
Series 5. VULGARES

berlandieri [M]	campylacantha [A]	catechu [A]
erubescens [A]	fleckii [A]	goetzii [A]
laeta [A]	mellifera [A](subsp.	senegal [A]
sundra [A]	mellifera & subsp. detinens)	

attribution is confident; brackets are omitted where there remains some doubt.

Chemically, gum exudates are the most complex of the polysaccharide group of natural products and, whilst the gums from Acacia species are perhaps not as complex as the gums from other sources, such as the gums Combretum[7], their analysis is a tedious, time-consuming business. For many trade purposes, simple determinations of solubility, % ash, colour, taste, smell and acidity may be sufficient, but for identification of the botanical origin of a particular sample the list of parameters becomes far longer.

The variation in the composition and properties of Acacia gums is considerable, the % nitrogen, for example, ranges from 0.02 to 1.66%; the uronic acid content from 4 to 37%; optical rotation from -66^{o} to $+108^{o}$; molecular weight (Mw) from 47,000 to 3,000,000 and the intrinsic viscosity from 4.2 to 27.7 ml/g[8].

All the Acacia gums examined in detail so far have proved to be highly branched heteropolysaccharides. The neutral sugar components are always D-galactose, L-arabinose and L-rhamnose in varying proportions, although the majority of gums have D-galactose as the major component and L-rhamnose as the minor or trace component. The acidity of Acacia gums arises from the presence of D-glucuronic acid and its 4-O-methyl analogue. Gums also contain proteinaceous

material, the gum from Acacia auriculiformis, for example, examined in Section IV of this thesis, contains 7.12% proteinaceous material and the fact that this material remains while the gum is degraded shows that it is an integral part of the gum molecule.

Most of the Acacia gums examined so far have been shown to contain a backbone of D-galactose, usually β-1, 3 linked.[3] Various structural units are attached to the backbone, these are usually β-1, 6-linked galactose residues or β-1, 3-linked arabinose residues, and the molecule is completed by peripheral residues such as rhamnose and the uronic acids. Any proteinaceous material present is probably chemically linked to the backbone of the molecule.

Within such a structural framework there is obviously scope for a wide range of actual chemical structures and this is mirrored by the wide range of molecular weights and intrinsic viscosities that have been determined for Acacia gums.

One of the objectives of Acacia gum chemistry is chemotaxonomy; more particularly the comparison of the chemical parameters of gums from morphologically similar Acacia trees. Such a study can only be realistic, however, if the botanical origins of gum samples are accurately known, and this depends on the expertise of botanists in the field. The classification of the gums Acacia, on a morphological basis, was first undertaken by Bentham[5] in 1875 and, although

various revisions have been necessary with the discovery of new species, Bentham's main divisions or series are still used.

Bentham's Series I, the Phyllodineae, comprises at least 57 species and it will obviously be a long time before analytical data for a sufficiently large proportion of these species becomes available for a statistically acceptable evaluation to be possible. Data for four species which appear to be typical of this series are given in Table I.2. The gums appear to be characterised by low molecular weight, low acidity, low positive or negative rotations, low rhamnose content and a high ratio of galactose to arabinose.

Of the Acacia gum species studied to date, amongst the most widely differing in chemical composition are A. pycantha Benth. and A. cyanophylla Lindl. which Bentham classified as closely similar botanical species. A recent partial structural study[9] of the exudates of A. difformis, R.T. Baker, A. mabellae Maiden, A. retinoides Schlechtendas and A. rubida, Cunn., and a comparison of the results with those published by earlier workers for A. pynantha and A. cyanophylla has indicated that it is the latter which has features which are atypical of the Phyllodineae.

In recent years, special attention has been given to gum specimens from members of the sub-group Juliflorae. This thesis presents the first data available for a group of these gums (see Section III)

Table I.2

Data For Some Phyllodineae Species of Acacia

	Acacia difformis.	*Acacia falcata.*	*Acacia mabellae.*	*Acacia retinoides.*
Ash, %	1.5	1.8	1.7	2.1
Nitrogen, %	0.28	0.21	0.23	0.48
Methoxyl, %	0.64	0.49	0.41	0.41
Specific Rotation, degrees	-5	+9	+4	+1
Intrinsic Viscosity, ml/g	6.2	5.1	5.8	9.5
Molecular Weight, $Mwx10^3$	47	79	120	730
Equivalent Weight	3420	2290	2870	1770
Hence Uronic acid, %	5	8	6	10
Sugar Composition after Hydrolysis:				
4-O-methyl glucuronic acid	3.5	3	2.5	2.5
Glucuronic acid	1.5	5	3.5	7.5
Galactose	75	85	76	76
Arabinose	19	7	17	12
Rhamnose	1	trace	1	2

and the first structural study on one of their number, A. auriculiformis (see Section IV).

Bentham's Series 2, the Botryocephalae, is comparatively small; only 32 species have been named. The gums from 12 of these species have been analysed[10] and the results indicate that the series may contain two chemically distinct types. Species of type A (Table I.3) differ greatly in composition from gums of the Phyllodineae but the gums from type B species (Table I.4) show strong resemblances to those of the Phyllodineae (Table I.2).

The Gummiferae, Series 4, are a predominantly African group of Acacias and, as such, have received more attention than some Australian gums. The gums in this series are characterised by the highly positive optical rotation and high molecular weight, together with relatively high arabinose content, generally higher than the galactose content, and low proportions of rhamnose. Data for four species in this series are shown in Table I.5.

Bentham's Series 5, the Vulgares, contains the most commercially important Acacia species, Acacia senegal, the tree which produces most of the Gum arabic presently available. This gum has been the subject of more chemical investigations than any other Acacia species; data for this, and two other species, are shown in Table I.6. The main distinguishing features of gums from the species in

Table I. 3

Data For Some Botyrocephalae Species of Acacia Type A

	Acacia deanei.	Acacia paramattensis.	Acacia parvipinnula.	Acacia trachyphloia.
Ash, %	2.4	2.7	2.5	2.2
Nitrogen, %	1.25	1.17	1.01	1.03
Methoxyl, %	0.75	0.84	0.84	1.02
Specific Rotation, degrees	-66.00	-49.00	-54.00	-57.00
Intrinsic Viscosity, ml/g	12.5	15.1	12.00	16.6
Molecular Weight, Mw x 10^3	360	370	340	970
Equivalent Weight	1350	1255	1200	1415
Hence Uronic Acid, %	13.0	14.0	14.7	12.4
Sugar Composition after Hydrolysis:				
4-O-methyl glucuronic acid	4.5	5.0	5.0	6.0
Glucuronic acid	8.5	9.0	9.5	6.5
Galactose	38	38	40	56
Arabinose	43	42	40	27
Rhamnose	6	6	5	5

Table I.4

Data For Some Botyrocephalae Species of Acacia Type B

	Acacia filicifolia.	*Acacia leucoclada.*	*Acacia terminalis.*	*Acacia Silvestris.*
Ash, %	0.9	1.2	1.1	2.3
Nitrogen, %	0.27	0.04	0.55	0.21
Methoxyl, %	0.47	0.65	0.44	1.34
Specific Rotation, degrees	+4	-4	+5	-8
Intrinsic Viscosity, ml/g	4.2	4.4	6.4	6.1
Molecular Weight, $Mwx10^3$	58	130	47	200
Equivalent Weight	4260	3020	3850	1255
Hence Uronic acid, %	4.2	5.8	4.6	14.0
Sugar Composition After Hydrolysis:				
4-O-methyl glucuronic acid	2.8	4.0	2.6	8.0
Glucuronic acid	1.4	1.8	2.0	6.0
Galactose	80	80	82	71
Arabinose	15	12	12	12
Rhamnose	1	2	1	3

Table I.5

Data For Some Gummiferae Species of Acacia

	Acacia drepanolobium.	*Acacia nilotica.*	*Acacia nubica*	*Acacia. seyal.*
Ash, %	2.52	2.48	1.54	2.87
Nitrogen, %	1.11	0.02	0.20	0.14
Methoxyl, %	0.43	0.96	0.96	0.94
Specific Rotation, degrees	+78	+108	+108	+51
Intrinsic Viscosity, ml/g	17.8	9.5	9.5	12.1
Molecular Weight, $Mwx10^3$	0.95	2.2	2.2	0.85
Equivalent Weight	1980	1890	1890	1470
Hence Uronic Acid, %	9	9	9	12
Sugar Composition After Hydrolysis:				
4-O-methyl glucuronic acid	2.5	6	0.5	5.5
Glucuronic acid	6.5	3	6.5	6.5
Galactose	38	44	33	38
Arabinose	52	46	59	46
Rhamnose	1	0.4	1	4

Table I.6

Data For Some Vulgares Species of Acacia

	Acacia campylacantha	*Acacia laeta*	*Acacia senegal*
Ash, %	2.92	n.d.	3.93
Nitrogen, %	0.37	0.65	0.29
Methoxyl, %	0.29	0.35	0.25
Specific Rotation, degrees	-12	-42	-30
Intrinsic viscosity, ml/g	15.8	20.7	13.4
Molecular weight, $Mwx10^3$	312	725	384
Equivalent weight	1900	1250	1100
Hence uronic acid, %	9	14	16
Sugar Composition After Hydrolysis:			
4-O-Methyl glucuronic acid	2	3.5	1.5
Glucuronic acid	7	10.5	14.5
Galactose	54	44	44
Arabinose	29	29	27
Rhamnose	8	13	13

Vulgares appear to be significant negative optical rotations, intermediate molecular weights and the presence of significant proportions of rhamnose, on whose presence in chain-terminal, peripheral positions of the globular-shaped molecule the superior stabilising powers of Gum arabic for oil/water emulsions may depend.

Section V of this thesis reports on the use of natural abundance carbon-13 nuclear magnetic resonance spectroscopy as an analytical tool for the study of gum polysaccharides and their degradation products. Surprisingly good results were obtained despite the high molecular weight of the gum samples.

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SECTION II

GENERAL 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 $\pm$ 0.1 mg.

Dialyses

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

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. Electro-dialysis was continued until a current (applied voltage = 300 volts) ceased to flow.

Reductions in Volume

Reductions in volume were carried out with a rotary evaporator at temperatures below 40^{o}C.

Moisture Contents

Moisture contents were determined by heating to constant weight at 105°C.

Ash Contents

Ash contents were determined by heating to constant weight in a muffle furnace at 550°C.

Carbon, Hydrogen and Nitrogen Contents

Carbon, hydrogen and nitrogen contents were obtained with a Perkin-Elmer 240 Elemental Analyser.

Methoxyl Contents

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

Equivalent Weight Determinations

Equivalent weight determinations on exhaustively dialysed polysaccharides were carried out by direct titration with standard sodium hydroxide solution (ca. 0.01 M).

Quantitative Estimate of Sugars

1. Sugars were separated from hydrolysates by chromatography on previously washed Whatman 3MM

papers. After elution from the paper, sugars were estimated, colourmetrically, by the phenol-sulphuric acid method[3]. The optical density was read on a Unicam SP 1300 spectrophotometer using filter 2. Calibration curves were drawn from known weights of sugars.

2. Sugars were estimated by gas-liquid chromatography of their alditol acetates on a column of 3% ECNSS-M at 190°C[4]. The hydrolysate was reduced with sodium borohydride for 3 hours; excess borohydride was then neutralised with acetic acid and the mixture deionised with Amberlite IR-120(H) resin. After filtration the mixture was taken to dryness and the residue heated under reflux for four hours with a mixture containing acetic anhydride and pyridine (1:1 v/v). The solution was then cooled and injected into the chromatograph.

II.2 PHYSICAL METHODS

Specific Rotations

Specific rotations of aqueous and chloroform solutions were measured using the sodium D-line with a Perkin-Elmer 141 polarimeter at 20±2°C.

Viscosity Determinations

Viscosity determinations were carried out in M-sodium chloride solution in an Ubbelohde suspended-level dilution viscometer at $25.0 \pm 0.1^{o}C$. 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 method 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 solution (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 the 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:-

$$[\eta] = \lim_{c \to o} \eta_{sp}/c = \lim_{c \to o} \frac{t - t_o}{c t_o}$$

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

Light Scattering Measurements

Light scattering measurements were carried out at $27^{o} \pm 0.5^{o}C$ with a SOFICA photogoniodiffusometer Model 4200. Unpolarised green light (546 nm.) was selected from a mercury lamp spectrum with a Wratten Kodak N61 filter. Using the limiting viscosity number as a guideline to the desirable concentration and using M-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 0.45μm. (Millipore Ltd., Bedford, Mass., U.S.A.) with a stainless steel filter holder attached to a 20 ml. syringe. Concentrations of gum solutions were assumed to be unaltered by ultra-filtration[5].

In each concentration the intensity of scattered light at various angles between 30^{o} and 150^{o} was recorded, and corrected scale readings I_{θ} for angle θ were calculated[6] from the equation:-

$$I_{\theta} = \frac{(Isoln. - Isol) \, Sin\,\theta}{I+Cos^{2}\theta}$$

where Isoln. and Isol. are the scale readings for polymer solution and solvent respectively. The reciprocal corrected

scale reading I/I_θ is plotted against $\mathrm{Sin}^2 \theta/2$. Extrapolation of the linear portion of this graph to $\theta = 0$ gives a value for $\left[I/I_\theta\right]_\theta = 0$. The downward curvature of these graphs at low angles is thought to be caused by dust particles suspended in solution[7].

Molecular weights are found from the equation:-

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

where

n_o	=	refractive index of solvent (1.340)
n	=	refractive index of solution
N	=	Avogadro's number (6.023×10^{23})
λ	=	wavelength of incident light (546nm. $= 5.46 \times 10^{-5}$ cm.)
c	=	concentration in g. cm^{-3}.
I_B	=	intensity diffused, selected for standard benzene (0.5)
dn/dc	=	refractive index increment
R	=	Rayleigh constant (16.3×10^{-6})

Using the dn/dc value of 0.146, which is the average value found[8] for a series of Acacia gums, the equation is simplified to:-

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

Infrared Spectroscopy

Infrared spectroscopy was carried out with a Perkin-Elmer P.E. 137 double beam grating spectrophotometer.

Ultracentrifugation

Ultracentrifugation was carried out with a Beckman-Spinco Model E Analytical Ultracentrifuge. Polysaccharide solutions (0.5% in 0.5-M-sodium chloride) were examined at 44,000 rpm. After the ultracentrifuge had attained this speed, the boundary patterns, obtained by a Schlieren optical system, were photographed at 16, 8 or 4 minute intervals.

II.3 CHEMICAL METHODS

Small-Scale Polysaccharide Hydrolyses

Small-scale polysaccharide hydrolyses were carried out with 0.5M-sulphuric acid for 7.5 hours on a boiling water bath, unless otherwise stated. Hydrolysates were neutralised with barium carbonate, filtered, deionised with Amberlite 1R-120(H) resin, and concentrated on a rotary evaporator.

Small-Scale Polysaccharide Methylations

(a) The Haworth method[9]

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 one 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 three hours for each addition. After stirring for twelve hours the reaction mixture was heated at 60°C for 30 minutes with nitrogen bubbling through the solution. After cooling, the reaction mixture was neutralised with 2M-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 was 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 (b.p. 60-80°, 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[10]

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 heated under reflux for 6 hours in the dark in a dry flash fitted with a water condenser and a calcium chloride tube. The mixture was cooled and filtered through sintered glass and the residue was extracted six times with hot chloroform (ca. 50 ml.). The combined filtrate and extracts were reduced in volume and any dissolved silver ions were removed by passing hydrogen sulphide through the solution and refiltering. After concentration to a small volume, the syrup was poured into light petroleum (b.p. 60-80°, ca. 400 ml.) with stirring. After filtration and drying, the precipated methylated polysaccharide was isolated as a white amorphous powder.

Small-Scale Oligosaccharide Methylations[11,12]

The oligosaccharide (0.5-2.0 mg.) was shaken with methyl iodide (0.2 ml.) and silver oxide (0.2 g.) at room

temperature in darkness 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

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 a small volume.

Partially Methylated Alditol Acetates

Partially methylated alditol acetates were prepared by the method of Bjorndal et al[13]. Methylated polysaccharides (10 mg.) were hydrolysed with 0.5-M sulphuric acid on a boiling water bath for 7.5 hours. Hydrolysates were neutralised with barium carbonate, filtered and deionised with Amberlite lR-120(H) resin. The methylated sugars were then reduced with sodium borohydride (ca. 20 mg.) for 2 hours. After treatment with Amberlite lR-120(H) and concentration, boric acid was removed by codistillation

with methanol. The dry product was heated with acetic anhydride/pyridine (1:1, v/v)(2 ml.) for 10 minutes at 100°C and the acetylation mixture was diluted with water, concentrated to dryness on a rotary evaporator and dissolved in chloroform.

Periodate Oxidations of Polysaccharides

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 (200 mg.), with standard sodium arsenite solution (ca. 0.025M) using Thyodene as indicator[14].

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

II.4 CHROMATOGRAPHIC SEPARATIONS

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

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

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

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

(d) butan-2-one, water, ammonia (d:0:88) (200:17:1)

(e) ethanol, hydrochloric acid (0.1M), butan-1-ol (10:5:1)[16]

Before using solvent (e) papers were dipped in 0.3M-sodium dihydrogen orthophosphate solution and air dried. 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° for ca. 3 minutes. R_{gal} values of sugars refer to distance 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.)

Gas-liquid partition chromatography of mixtures of O-methyl sugars[17] was carried with a Pye Series 104 chromatograph fitted with a flame ionisation detector, with nitrogen as the carrier gas, on a column (200 x 0.3 cm.) of 15% (w/w) ethylene glycol adipate polyester on Gas-Chrom Z (45-60 mesh) at 176°C. The nitrogen flow rate was ca. 100 ml./min. Gas-liquid partition chromatography of mixtures of partially methylated alditol acetates[18] was carried out with a Pye Series 104 chromatograph fitted with a flame ionisation detector, with nitrogen as the carrier gas, on a column (200 x 0.3 cm.) of 3% (w/w) ECNSS-M (a copolymer of ethylene glycol succinate polyester and nitrile silicone polymer) on Gas-Chrom Q (100-120 mesh) at 180°C. The nitrogen flow rate was 60 ml./min. Retention times (T) for g.l.c. of methyl glycosides are quoted relative to methyl 2, 3, 4, 6-tetra-O-methyl-β-D-glucopyranoside as standard. Retention times (T) for g.l.c. of partially methylated alditol acetates are quoted relative to 1, 5 di-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-glucitol.

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SECTION III

AN ANALYTICAL STUDY OF *ACACIA* GUM EXUDATES OF THE SERIES *PHYLLODINEAE*, SUBSERIES *JULIFLORAE*

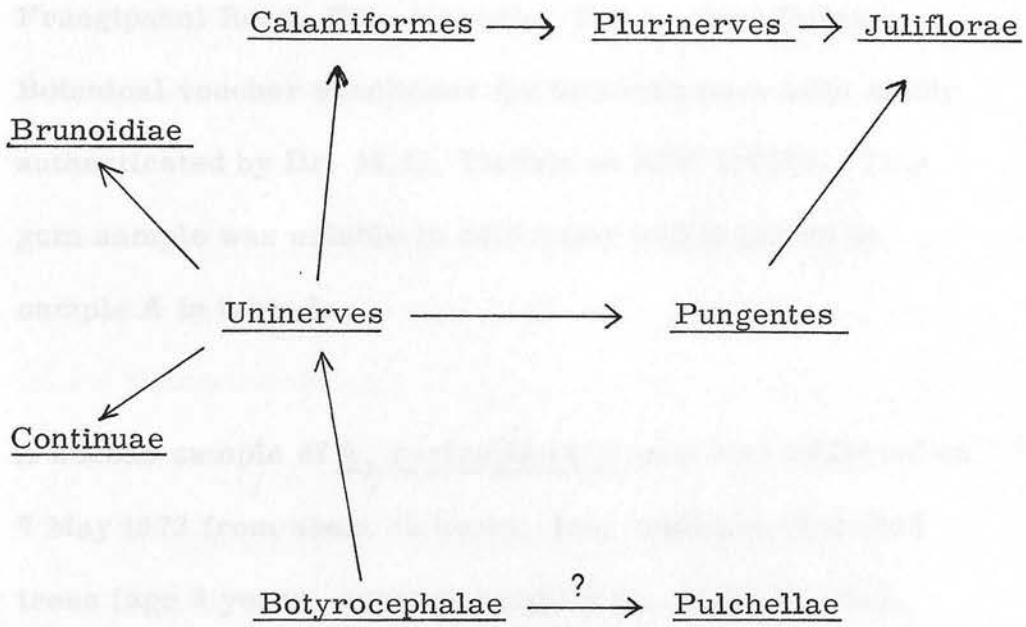
AN ANALYTICAL STUDY OF *ACACIA* GUM EXUDATES OF THE SERIES *PHYLLODINEAE*, SUBSERIES *JULIFLORAE*

III.1 Introduction

The major classification of the genus *Acacia*, by Bentham[1], placed 277 of the then known ca. 600 species in Series I (*Phyllodineae*). More recent research by Tindale[2], however, puts the correct number in the *Phyllodineae* at 570 species: the total number of species known is ca. 900. This large number of species is still best subdivided according to the divisions proposed by Bentham[1], Taubert[3] and Maiden and Betche[4] into eight subseries viz:- *Alatae*; *Continuae*; *Pungentes*; *Calamiformes*; *Brunioidae*; *Uninerves*; *Plurinerves* and *Juliflorae*[5]. Relatively few species of the series *Phyllodineae* have been examined chemically so far, although there have been studies of the distribution of amino acids in some seeds[6] and of the flavonoid content of some heartwoods[7, 8]. To date the gum exudates from only 13 species[9] in the entire series *Phyllodineae* have been studied and most of these have been from subseries 6F (*Uninerves Racemosae*). This thesis presents the first data available for species in subseries 8 (*Juliflorae*) which contains 151 species and is the second largest in the *Phyllodineae*.

Botanically the *Juliflorae* is considered to be a complex

group of Phyllodinous wattles which occur in both tropical and more temperate regions of Australia, Malaysia and the East Indies. Furthermore, Tindale[8] has stated that the Juliflorae are regarded as the most highly evolved of the Acacia species both morphologically and chemically. The following diagram shows possible evolutionary trends in the Acacias[8]:-



The gums studied were from Acacia auriculiformis; A. holosericea; A. mangium; A. pubifolia; A. acradenia; A. aneura; A. kempeana; A. stereophylla; A. microneura; A. coolgardiensis and A. beauverdiana.

In some cases only very small quantities of gum were available and hence some parameters could not be determined.

III.2 Origin of Gum Samples

Gum from Acacia auriculiformis A. Cunn. ex Benth. (Bentham No. 271) was collected by Mr. J.F.U. Zieck on 23 July 1973 from a bushy low-branched tree (about 4 years old, height 8m., dbh. 10 cm. with a smooth to cracked greyish bark with flowers and fruit present) growing on black cracking clay soil of the savannah belt in the office garden of the Forest Products Research Centre, Frangipanni Road, Port Moresby, Papua, New Guinea. Botanical voucher specimens for this tree have been kindly authenticated by Dr. M.D. Tindale as NSW 107339. This gum sample was soluble in cold water and is shown as sample A in table 1.

A second sample of A. auriculiformis gum was collected on 7 May 1973 from about 30 bushy, low, multiple-branched trees (age 3 years, average height 8 m., dbh. 10 cm.), planted closely together to form a hedge. The exudation appeared to have formed on healthy trees that had been attacked by insects or some species of fungus. The water-soluble material from this second sample is shown as sample B in table 1; the water insoluble material present dissolved on the addition of a very small amount of sodium borohydride, and gave sample C after dialysis, filtration and freeze drying.

Gum from Acacia holosericea A. Cunn. ex G. Don (Bentham No. 274) was collected by Mr. J.F.U. Zieck on 23 July 1973 from a crooked, low-forked tree, (about 4 years old, height 8 m., dbh. 11 cm. with a dark coloured smooth to cracked bark and flowers, young and mature fruit present), growing on black, cracking clay soil of the savannah belt in the office garden of the Forest Products Research Centre, Frangipanni Road, Port Moresby. Botanical voucher specimens for this tree have been authenticated by Dr. M.D. Tindale as NSW 107338.

Gum from Acacia mangium Willd. (Bentham No. 275) was collected from a single tree at Ulu Kukut on 16 March 1971 by the Plantation Officer at Sandakan, Sabah, Malaysia.

Gum from Acacia leptostachya Benth. (Bentham No. 256) was collected on 11 August 1969 from a shrub, 2 m. high, growing on acidic volcanic outcrops 39 miles west of Chapter's Towers, Hughenden, Queensland, Australia, by Mr. W.R. Birch of the School of Biological Sciences, University College of Pimlico, Townsville, Queensland. Botanical vouchers were authenticated by Mr. L. Pedley, Research Botanist at Brisbane Botanic Museum and Herbarium as R.C. Correll E. 74.

Gum from Acacia pubifolia Pedley was collected by Dr. M.D. Tindale on 5 January 1969 from a tree 5m. high, with silvery foliage and iron-black bark, on a granite hillside at Wyerba, 4 miles south of Ballendeen, S.E. Queensland. The reference voucher is NSW 102606.

Gum from Acacia kempeana was collected by Mr. J.R. Maconochie, 10 miles S.S.E. of Alice Springs, N.T., Australia. The reference voucher number is NT 37066.

Gum from Acacia acradenia was collected near Alice Springs; the reference voucher number is Latz 5942.

Gum from Acacia aneura was collected 16 miles north of Alice Springs; the reference voucher number is NT 37064.

III.3 EXTRACTION AND PURIFICATION OF GUM SAMPLES

Crude gums (3-4 g. where available) were dissolved in distilled water (2% solution) over 2 days. In the case of A. auriculiformis (sample B), A. mangium, A. leptostachya and A. pubifolia a small amount of sodium borohydride was added to facilitate dissolution. The solutions were filtered through Whatman No. 41 and No. 42 filter papers, dialysed against running tap water for 2 days (4 days in the case of samples heated with borohydride), refiltered, and freeze-dried. The gum from A. aneura gave a dark brown solution; the gums from A. leptostachya and A. pubifolia gave orange-yellow and pale yellow solutions respectively.

III.4 ANALYTICAL COMPARISON

Analytical data for the twelve samples are shown in table III A.B. All the samples were hydrolysed with 0.5-M-sulphuric acid and the hydrolysates were examined by paper chromatography in solvents (a) and (b). The gums all contained galactose, arabinose and rhamnose in common with other Acacia gums so far examined[9], as well as at least two aldobiuronic acids. (R_{gal} 0.2 and 0.67 in solvent (b)) . The aldobiuronic acids in question would appear to be 6-O-(β-D-gluco-pyranosyluronic acid)-D-galactose, a component of most of the Acacia gums so far examined[9], and 4-O-(4-O-

Table III.A.

Analytical Data for Purified Gum Polysaccharides from *Acacia* Species of the Series *Phyllodineae*, Subseries *Juliflorae*

	Acacia auriculiformis.		
	A	B	C
Moisture %	13.3	8.4	12.0
Ash %[a]	4.8	4.5	5.3
Nitrogen %[a]	1.14	1.12	0.92
Protein %[a]	7.12	7.0	5.75
Methoxyl %[b]	1.71	1.90	1.68
$[\alpha]_D$ (degrees)[b]	+18.6	+15.6	+15.8
Intrinsic Viscosity	22.0	22.6	25.0
Molecular Weight $\times 10^5$[a]	19	23	30
Equivalent Weight[b]	590	620	635
Uronic Anhydride %[b, c]	29.7	28.4	27.7
Sugar Composition After Hydrolysis[b]:			
4-*O*-methyl glucuronic acid[d]	10.2	11.4	10.1
Glucuronic acid	19.5	17.0	17.6
Galactose	58	58	59
Arabinose	9	10	8
Rhamnose	3	4	5

Table III.A (Contd.)

Analytical Data for Purified Gum Polysaccharides from Acacia Species of the Series Phyllodineae, Subseries Juliflorae

	Acacia holosericea.	Acacia mangium.	Acacia leptostachya.
Moisture %	9.0	16.2	16.1
Ash %[a]	5.1	5.4	5.8
Nitrogen %[a]	0.28	0.98	0.66
Protein %[a]	1.75	6.12	4.12
Methoxyl %[b]	0.47	1.49	2.24
$[\alpha]_D$ (degrees)[b]	+2.9	+36.4	+53
Intrinsic Viscosity	19.0	27.7	16.7
Molecular Weight x10^5[a]	38	32	13.5
Equivalent Weight[b]	1010	545	475
Uronic Anhydride %[b, c]	17.3	32.2	37.0
Sugar Composition After Hydrolysis:[b]			
4-O-methyl glucuronic acid[d]	2.8	9.0	13.4
Glucuronic acid	14.5	23.2	23.6
Galactose	56	56	54
Arabinose	20	10	7
Rhamnose	6	2	2

Table III B.

Analytical Data for Purified Gum Polysaccharides from *Acacia* Species of the Series *Phyllodineae*, Subseries *Juliflorae*

	Acacia pubifolia.	Acacia acradenia.	Acacia aneura.	Acacia kempeana.
Moisture %	13.4	7.7	10	9.6
Ash %	3.4	4.1	n.d.	4.4
Nitrogen %[a]	1.66	0.14	0.18	2.78
Protein %[a]	10.4	0.84	1.12	17.4
Methoxyl %[b]	1.20	0.89	1.50	2.50
$[\alpha]_D$ (degrees)[b]	-58	-8.5	+5.8	-8.3
Intrinsic Viscosity	25.6	13.7	4.6	14.2
Molecular Weight $\times 10^{-5}$[a]	24.4	9.75	n.d.	3.96
Equivalent Weight[b]	680	1150	765	456
Uronic Anhydride %[b, c]	25.9	15.3	23.0	38.6
Sugar Composition After Hydrolysis:[b]				
4-O-methyl glucuronic acid[d]	7.2	5.3	9.0	15.0
Glucuronic acid	18.7	10.0	14.0	23.6
Galactose	46	50.2	60.3	57.8
Arabinose	25	32	5.8	3.5
Rhamnose	3	1.3	10.8	1.1

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

Table III. B (Contd.)

Analytical Data for Purified Gum Polysaccharides from *Acacia* Species of the Series *Phyllodineae*, Subseries *Juliflorae*

	Acacia stereophylla.	Acacia microneura.	Acacia coolgardiensis.	Acacia beauverdiana
Moisture %	10	10	10	10
Ash %	n.d.	n.d.	n.d.	n.d.
Nitrogen %[a]	0.58	1.01	1.42	0.69
Protein %[a]	3.6	6.31	8.9	4.31
Methoxyl %[b]	0.73	3.40	0.82	0.68
$[\alpha]_D$ (degrees)[b]	-12.5	+10.9	-2.2	-27.1
Intrinsic Viscosity	12.2	8.1	12.3	10.1
Molecular Weight $\times 10^5$[a]	16.1	6	13.8	15.6
Equivalent Weight[b]	761	443	501	624
Uronic Anhydride %[b, c]	23.1	39.7	35.2	28.2
Sugar Composition After Hydrolysis:[b]				
4-O-methyl glucuronic acid[d]	4.4	20.4	4.9	4.1
Glucuronic acid	18.7	19.3	30.2	24.1
Galactose	45.5	54.6	45.3	50.7
Arabinose	20.9	3.6	9.3	8.9
Rhamnose	10.5	2.0	10.2	12.2

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

methyl -α-D-glucopyranosyluronic acid)-D-galactose. Also present in the hydrolysates was 4-O-methyl-D-glucuronic acid (R_{gal} 1.90 solvent (b), orange/pink spot, whose presence in a 0.5M hydrolysate has been reported by Bell[10].

The gums studied are of high molecular weight. Both the phyllodinous and bipinnate Acacias studied previously[9,10,11] have been of much lower molecular weight than the African species studied[12], but the values for A. holosericea and A. mangium considerably exceed the highest value reported previously. In agreement with earlier reports[11] the use of 1% aqueous sodium borohydride[13] does not appear to cause any extensive degradation during the dissolution process; indeed, the previously established tendency[11] for borohydride-solubilized material to be of higher molecular weight than the corresponding water soluble fraction is confirmed here (A. auriculiformis sample C).

In addition to high molecular weight, the Juliflorae species studied give gum solutions of high intrinsic viscosity, with the exception of A. aneura and A. microneura. The value given by A. auriculiformis equals the highest values previously recorded for A. laeta[14], A. paramattensis[14] and A. tortilis subsp. heteracantha[15]; A. mangium (η = 27.7 $mlg.^{-1}$) must now be regarded as the most viscous

of the Acacia gums studied so far.

The methoxyl content of these gums is exceptionally high, with the exception of A. holosericea, and, as the methoxyl groups occur in 4-O-methylglucuronic acid, this is reflected in the high acidity of the samples. The highest value (3.40%), for A. microneura, is considerably higher than the previously reported[18] highest value (for A. giraffae (2.40%)), but this sample contained a considerable amount of bark in admixture and this may have influenced the result. Despite this the values obtained for other samples (A. kempeana, 2.50% and A. leptostachya, 2.24%) must be regarded as among the highest methoxyl contents yet reported for Acacia gums.

The nitrogen content, and hence the protein content, of these gums was above average; A. pubifolia (1.66%) and A. kempeana (2.78%) exceed the previous highest value[11] by a considerable margin. An independent study on the gum from A. auriculiformis (sample A) and A. pubifolia by a French laboratory (Station des Antibiotiques et des Bioconversions, I.N.R.A., Chartres, France) reported that neither gum contained free amino acids and that the majority of the peptides present were in cyclic form. The values reported for amino acid content are shown in table III.C

Table III.C.

Amino Acid Content [a] of Two *Acacia* Gums of the Series *Phyllodineae* subseries *Juliflorae*

Amino Acid	*Acacia Auriculiformis*	*Acacia Pubifolia*
Lysine	5.06	3.07
Histidine	4.09	3.06
Arginine	2.52	2.82
Hydroxyproline	8.05	7.31
Aspartic Acid	12.46	11.58
Threonine	4.82	5.98
Serine	8.39	8.33
Glutamic Acid	7.33	7.13
Proline	7.81	9.78
Glycine	4.06	3.90
Alanine	3.98	3.69
Cystine	-	0.35
Valine	9.47	8.61
Methionine	0.06	0.09
Isoleucine	2.76	4.59
Leucine	8.08	9.76
Tyrosine	3.22	3.29
Phenylalanine	7.87	6.66

a in % per 16g of nitrogen

From Station des Antibiotiques et des Bioconversions, INRA, Chartres, France.

The sugar content of these gums is profoundly influenced by the high acidity they display. For many years A. cyanophylla has been the most acidic Acacia gum known[13] (uronic acid = 24%) but the majority of these gums have values greater than that figure. Values of 39.7% (A. microneura) 38.6% (A. kempeana) and 37.0% (A. leptostachya) are similar to those obtained for members of the genus Combretum[10], while even the lowest value, 15.3% (A. acradenia), is considerably higher than values reported for other Phyllodineae[9].

In all the gums examined, galactose is the major component, usually accounting for 50-60% of the whole gum; these values are considerably lower than those reported for other phyllodinous Acacias[9]. The arabinose contents are more variable, the majority of the gums contain 5-10% of arabinose but A. acradenia (32%), A. pubifolia (25%) and A. holosericea (20%) are notable exceptions. A previous study[9] on gums of the series Phyllodineae also found considerable variation in the arabinose content. Rhamnose, the third neutral sugar component of these gums, is also, generally, the least abundant. In this study, values from 2-6% were typical but A. beauverdiana (12.2%), A. aneura (10.8%), A. stereophylla (10.5%) and A. coolgardiensis (10.2%) are unusually high. In general, the values reported here are

higher than those obtained[9] for other phyllodinous Acacias (typically 1%).

The variations in the sugar composition of the gums are reflected in the wide range of specific rotations obtained, from A. leptostachya ($[\alpha]_D = +58^\circ$) to A. pubifolia $[\alpha]_D = -58^\circ$). Some of the gums have low specific rotations similar to gums from other members of the series Phyllodineae (A. holosericea $[\alpha]_D = +2.9^\circ$)) while others (A. leptostachya ($[\alpha]_D = +58^\circ$)) differ widely.

Although the sample of Juliflorae gums studied here is not large (12 out of a possible 151 species), some parallels are apparent. More striking, however, are the differences between these gums and those of other series within the Phyllodineae (see table III). The exact role of gum exudates in plant physiology is still unknown and, consequently, it is not possible to determine whether a gum with high uronic acid content and high viscosity will perform its task any more efficiently than one with low acidity and viscosity. Tindale[8] has stated that the Juliflorae are regarded as the most highly evolved of the Acacia species, both morphologically and chemically; it is possible, therefore, that their gum exudates, with their relatively high viscosity, high acidity and high protein content are also highly evolved. Until a more

representative sample of gums is analysed, however, this can only be speculation.

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SECTION IV

A STRUCTURAL STUDY OF THE GUM FROM *ACACIA AURICULIFORMIS* A. CUNN. EX BENTH.

A STRUCTURAL STUDY OF THE GUM FROM *ACACIA AURICULIFORMIS* A. CUNN. EX BENTH.

IV.1 Introduction

Structural studies on the gums of *Acacia* species have tended to concentrate on those gums with widespread commercial application, probably because they are easily available in the quantities required for a detailed structural study. This means that the structures of African *Acacia* gums such as *A. senegal*, *A. seyal* and *A. drepanolobium* have been well established[1, 2].

The gums from Australian *Acacias*, by contrast, have received relatively little attention. The Phyllodineae, the largest family of Australian *Acacias*, have been the subject of a recent analytical comparison[2] and methylation analysis. The subseries *Juliflorae*, of which *A. auriculiformis* is a member, has, so far, been completely ignored. This study is the first structural examination of a member of this very interesting group.

IV.2 Origin and Purification of *A. auriculiformis* Gum

Gum nodules from *Acacia auriculiformis* were collected by Mr. J.F.U. Zieck, A. Cunn. ex Benth., on 23 July 1973 from about 30 bushy, low-branched trees planted closely together to form a hedge at the Forest Products Research

Centre, Fort Moresby, Papua, New Guinea. The gum had been compressed into a block; this was broken up and the fragments (95 g.) were dissolved in water (21). The gum solution was decanted off leaving a gel to which more water (21) was added; after a further two days the majority of the gel had dissolved. The gum solutions were combined, filtered and dialysed against running tap water for two days. The polysaccharide (62 g. ; yield 65%) was isolated as the freeze-dried product. Values for the moisture content (8.4%), intrinsic viscosity (22.6 $ml\,g^{-1}$) and specific rotation (+15.6°) were similar to the values observed previously. (See Section III). Ultracentrifugation in 0.5M-sodium chloride at 44, 770 r.p.m. yielded a single symmetric boundary, indicating the gum to be homogeneous.

IV.3 Identification of Neutral Sugars

The gum (12 g.) was hydrolysed with 0.5M-sulphuric acid for 7.5 hours on a boiling water bath. The cooled solution was neutralised, filtered, deionised with Amberlite 1R-120(H) resin 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, paper chromatography in solvents (a) and (b) indicated the presence of galactose,

arabinose and rhamnose; galactose was the major component.

IV.4 Identification of the Aldobiuronic Acids

Elution of the Duolite A-4 column with 5% (v/v) formic acid (500 ml.) yielded the acidic fraction of the hydrolysate. This fraction was concentrated to a syrup and paper chromatography was carried out in solvent (b). Trace amounts of galactose and arabinose were detected together with large amounts of 4-O-methyl-D-glucopyranosyl uronic acid. (R_{gal} 2.3; pink spot with aniline oxalate). Two major spots were also detected (R_{gal} 0.27 and 0.63). In order to establish the identity of these components, chromatography was carried out on Whatman 3MM papers in solvent (b); side strips were cut and the two major acidic fractions eluted. After elution each fraction was concentrated to a syrup and the purity of each was checked by chromatography in solvent (b). The syrup was then transferred to a dry, tared flask and taken to dryness on a rotary evaporator. After drying, in vacuo, 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; M-sulphuric acid (5 ml.) was added and the hydrolysis was carried out for 8 hours to cleave the uronosyl linkages. Fraction 1 had R_{gal} 0.27 solvent (b); it was chromatographically

indistinguishable from 6-O-β-D glucopyranosyluronic acid-D-galactose. After elution the $[\alpha]_D$ value was found to be $+19.8^{o}$. This high value is probably due to traces of galactose impurity. Chromatography in solvent (b) for 48 hours failed to resolve the spot at R_{gal} 0.27 into more than one biuronic acid component. Hydrolysis followed by chromatography in solvents (b) and (e) showed the presence of galactose and glucuronic acid. These results indicate that Fraction 1 is 6-O-β-D-glucopyranosyl uronic acid-D-galactose. This biuronic acid is a common component of the gums of Acacia species.[1.]

Fraction 2 had R_{gal} 0.63 solvent (b). The specific rotation for this component was found to be $+92^{o}$. Hydrolysis, followed by chromatography in solvents (b) and (e) indicated the presence of galactose and 4-O-methyl-glucopyranosyl uronic acid. These results indicate that Fraction 2 is 4-O-(4-O-methyl-α-D-glucopyranosyl-uronic acid)-D-galactose. A portion of the gum (100 mg.) was hydrolysed with M-sulphuric acid and examined by chromatography in solvent (e). Glucopyranosyl uronic acid and its 4-O-methyl analogue were the only uronic acids detected.

IV.5 Partial Acid Hydrolysis of A. auriculiformis Gum

The polysaccharide (5 g.) was hydrolysed with 0.25 M-

sulphuric acid (500 ml.) on a boiling water bath for one hour. The cooled solution was neutralised with barium carbonate, deionised with Amberlite IR-120(H) resin and chromatographed in solvents (a) and (b). Galactose, arabinose, rhamnose and 4-O-methyl-D-glucopyranosyl uronic acid were found present along with two disaccharides. (R_{gal} 0.28 and 0.63 (solvent (b)) . In solvent (a) only one disaccharide was apparent; at R_{gal} 0.34. This suggested that one of the components was acidic. In order to establish the identity of these components, chromatography was carried out on Whatman 3MM papers in solvent (b); side strips were cut and the two disaccharide fractions were eluted.

Fraction 1 had R_{gal} 0.28 solvent (b), 0.34 solvent (a). The specific rotation for this component was $+29^{o}$. Hydrolysis followed by chromatography in solvents (a) and (b) indicated the presence of galactose only. These results suggest that Fraction 1 is 6-O-β-D-galactopyranosyl-D-galactose.

Fraction 2 had R_{gal} 0.63 solvent (b) but no visible spot in solvent (a). The specific rotation was $+92^{o}$. Hydrolysis with M-sulphuric acid followed by chromatography indicated the presence of galactose and 4-O-methyl-D-glucuronic acid. These results suggest that Fraction 2 is 4-O{4O-methyl-α-D-glucopyranosyl uronic acid} D-galactose, the same

biuronic acid which was detected after hydrolysis with 0.5M acid.

IV.6 Preparation of Degraded Gum A

A. auriculiformis gum (11.6 g.) was dissolved in 0.005 M-sulphuric acid (500 ml.) and hydrolysed on a boiling water bath for 96 hours. Aliquots (1 ml.) were removed at intervals of 8 hours and examined by paper chromatography in solvent (a). This indicated the almost immediate release of arabinose and the subsequent release of rhamnose into the solution. After ca. 24 hours a substantial brown precipitate appeared in the solution; previous workers have assumed this to be denatured protein and this was confirmed after filtration and drying (Found: N 8.72%; hence protein 54.5%). After 96 hours the solution was cooled, neutralised with barium carbonate, deionised with Amberlite IR-120(H) resin, concentrated and dialysed against distilled water (2l.) 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 (6.4 g., yield 55%).

The diffusate from the dialysis of Degraded Gum A was concentrated to a syrup and portions were examined by paper chromatography in solvents (a) and (b). Large quantities of arabinose, together with smaller amounts of galactose

and rhamnose were detected, as well as at least four oligosaccharide components. The identity of these components was established by preparative paper chromatography on Whatman 3MM paper in solvent (b); side strips were cut and the fractions eluted.

Fraction 1 had R_{gal} 0.28. The specific rotation was $+32^{o}$. Hydrolysis followed by chromatography in solvents (a) and (b) indicated the presence of galactose only. These results show that Fraction 1 is 6-O-β-D-galactopyranosyl-D-galactose.

Fraction 2 had R_{gal} 0.39. The specific rotation was $+56^{o}$. Hydrolysis followed by chromatography in solvents (a) and (b) indicated the presence of galactose only. These results show that Fraction 2 is 3-O-β-D-galactopyranosyl-D-galactose.

Fraction 3 had R_{gal} 0.63. The specific rotation was $+106^{o}$. Hydrolysis followed by chromatography in solvents (a) and (b) indicated the presence of 4-O-methyl glucuronic acid and galactose. A methoxyl determination gave MeO = 6.75% indicating a content of ca. 40% of 4-O-methyl glucuronic acid. These results suggest that the component is 4-O-(4-O-methyl-α-D-glucopyranosyl-uronic acid)-D-galactose.

Fraction 4 had R_{gal} 1.15 (pink spot). The specific rotation was $+92^{o}$. Hydrolysis followed by chromatography in solvents (a) and (b) indicated the presence of arabinose only. These results suggest that Fraction 4 is 3-O-β-L-arabinopyranosyl-L-arabinose.

IV.7 Examination of Degraded Gum A

Degraded Gum A (20 mg.) was hydrolysed with 0.5M-sulphuric acid for 7.5 hours on a boiling water bath. After neutralisation, deionisation and concentration the hydrolysate was examined by paper chromatography in solvents (a) and (b). Galactose and arabinose were present along with components having the chromatographic mobilities of 6-O-β-D-glucopyranosyluronicacid-D-galactose and 4-O-(4-O-methyl-α-D-glucopyranosyluronicacid)-D-galactose. (R_{gal} 0.27 and 0.63 respectively solvent (b)). Degraded Gum A had $[\alpha]_{D}+24.9^{o}$, while the molecular weight, determined by light scattering, was 7.3×10^{4}. The equivalent weight, found by titration of the free gum acid prepared by electrodialysis was 670, indicating a uronic acid content of 26.4%. Partial acid hydrolysis of Degraded Gum A, with 0.25M-sulphuric acid for one hour on a boiling water bath, followed by chromatography in solvents (a) and (b), revealed the presence of 6-O-β-D-galactopyranosyl-D-galactose; major component (R_{gal} 0.26 solvent (b), 0.30

solvent (a) and minor component 3-O-β-D-galactopyranosyl-D-galactose, (R_{gal} 0.34 solvent (a) in 0.42 solvent (b)). Degraded Gum A (241 mg.) was methylated to give a product (147 mg.). (Found: $[\alpha]_D$ -8°, OMe 39.9%). A portion of this product was methanolysed and examined by g.l.c. The O-methyl sugars identified are shown in Table IV.1. A further portion was hydrolysed with 0.5-M-sulphuric acid, neutralised, deionised with Amberlite IR-120(H) resin and converted into alditol acetates. The mixture of partially methylated alditol acetates was examined by g.l.c.; the derivatives found are shown in Table IV.2. A portion of the hydrolysed, methylated Degraded Gum A was examined by paper chromatography in solvent (c). The results are shown in Table IV.3.

IV.8 Smith Degradation of Degraded Gum A

Degraded Gum A (3.2 g.) was dissolved in water (75 ml.) and 0.25 M-sodium periodate (75 ml.) was added. After 96 hours in darkness at room temperature 12.37 m moles periodate/g polysaccharide had been reduced and 3.6 m moles formic acid/g polysaccharide had been released. The reaction was stopped by the addition of ethane-1,2-diol (2 ml.) and the solution was dialysed against running tap water for 2 days. Sodium borohydride (1 g.) was added and, after 30 hours at room temperature, the solution was dialysed for a further two days. The solution was made 0.5M with respect to sulphuric acid and the polyalcohol was hydrolysed for 48 hours at room temp. then dialysed for 2 days. Degraded Gum B was

isolated as the freeze-dried product (800 mg., yield 25%).

IV.9 Examination of Degraded Gum B

Degraded Gum B was hydrolysed with 0.5M-sulphuric acid. Chromatography in solvents (a) and (b) indicated the presence of galactose and traces of arabinose. Partial acid hydrolysis with 0.25M-sulphuric acid indicated that 6-O-β-D-galactopyranosyl-D-galactose and 3-O-β-D-galactopyranosyl-D-galactose were present. The nitrogen content was found to be 1.12% while the equivalent weight of 3320 indicated a uronic acid content of 5.3%.

IV.10 Methylation of A. auriculiformis Gum

The gum (463 mg.) was methylated to give a product (301 mg.) (Found: $[\alpha]_D$ -10°, OMe 39.8%). Methanolysis of a portion of the methylated product gave the methyl glycosides identified by g.l.c. shown in Table IV.1. A further portion of the methylated product was hydrolysed with 0.5M-sulphuric acid and the partially methylated sugars were converted into their alditol acetates. The partially methylated alditol acetates were examined by g.l.c. and are shown in Table IV.1. A portion of the hydrolysate from above was examined by paper chromatography in solvent (c); the O-methyl sugars identified are shown in Table IV.1.

Table IV.1.

O-Methyl Sugars Identified in Methylated Acacia Auriculiformis Gum

Relative Retention Time (T) of Methyl Glycosides	Relative Retention Time (T) of Partially Methylated Alditol Acetates	R(g) After Hydrolysis (Solvent (C))	O-Methyl Sugars Identified	Relative Amounts (%)
0.47	0.48	1.01	2, 3, 4-tri-O-methyl-L-rhamnose	4
0.54, 0.68	0.56	0.98	2, 3, 5-tri-O-methyl-L-arabinose	19
1.42, 1.82	1.06	0.82	2, 5-di-O-methyl-L-arabinose	3
1.67	1.25	0.88	2, 3, 4, 6-tetra-O-methyl-D-galactose	12
3.60, (4.00)	2.10	0.72	2, 3, 6-tri-O-methyl-D-galactose)	13
3.81, (4.00)	2.30	0.72	2, 4, 6-tri-O-methyl-D-galactose)	
6.03	3.37	0.72	2, 3, 4-tri-O-methyl-D-galactose	8
15.4, 16.8	6.00	0.52	2, 4-di-O-methyl-D-galactose	20
2.33, 2.98	-	-	2, 3, 4-tri-O-methyl-D-glucuronic acid	14
7.15, 8.30	-	-	2, 3-di-O-methyl-D-glucuronic acid	6
-	7.50	-	2-O-methyl-D-galactose	trace

Table IV.2.

O-Methyl Sugars Identified in Methylated Degraded Gum A

Relative Retention Time (T) of Methyl Glycosides	Relative Retention Time (T) of Partially Methylated Alditol Acetates	Rg After Hydrolysis Solvent (c)	O-Methyl Sugar Identified	Relative Amount (%)
0.43	0.48	1.01	2,3,4-tri-O-methyl-L-rhamnose	trace
0.51, 0.63	0.51	0.98	2,3,5-tri-O-methyl-L-arabinose	11
1.47	1.16	0.88	2,3,4,6-tetra-O-methyl-D-galactose	24
3.01, (3.54)	1.98	0.72	2,3,6-tri-O-methyl-D-galactose)	10
3.27, (3.54)	2.11	0.72	2,4,6-tri-O-methyl-D-galactose)	
5.75	2.97	0.72	2,3,4-tri-O-methyl-D-galactose	26
12.9, 14.1	5.52	0.52	2,4-di-O-methyl-D-galactose	24
2.11, 2.66	-	-	2,3,4-tri-O-methyl-D-glucuronic acid	5
-	6.90	0.32	2-O-methyl-D-galactose	trace

IV.11 Preparation and Examination of Polysaccharide 1

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

A. auriculiformis gum (35.3 g.) was dissolved in water (900 ml.) and 0.25M-sodium metaperiodate solution (900 ml.) was added. Oxidation was carried out in darkness at room temperature and the reaction was followed by measuring the release of formic acid with time. After 48 hours, 12.15 m moles periodate/g polysaccharide had been reduced and 3.2 mmoles formic acid had been released. The reaction was stopped by the addition of ethane -1, 2-diol (20 ml.) and the solution was dialysed against running tap water for 2 days. Sodium borohydride (12.0 g.) was added and the mixture was kept at room temperature for 30 hours, then dialysed for 2 days. The solution was made 0.5M with respect to sulphuric acid, by addition of 2M acid, and the polyalcohol was hydrolysed for 48 hours at room temperature. After dialysis against running tap water for 2 days, Polysaccharide 1 was isolated as the freeze-dried product (14.6 g. yield 41.3%). Polysaccharide 1 had $[\alpha]_D$ -9.2° and molecular weight 2.5×10^5 (light scattering). The nitrogen content was 2.3%; hence the protein content = 14.4%.

Hydrolysis with M-sulphuric acid followed by chromatography in solvents (a), (b) and (e) showed the presence of galactose, arabinose and glucuronic acid. The ratio galactose:arabinose was 10:1.65. Hydrolysis with 0.5M-sulphuric acid followed by chromatography in solvent (a) indicated small amounts of 6-O-β-D-glucopyranosyluronic acid-D-galactose.

A sample of Polysaccharide 1 was electrodialysed; the equivalent weight was 1830 indicating a uronic acid content of 9.6%. On this basis, the sugar ratios were calculated and are shown in Table IV.6.

Partial acid hydrolysis of Polysaccharide 1 with 0.25M-sulphuric acid followed by chromatography in solvents (a) and (b) indicated the presence of 6-O-β-D-galactopyranosyl-D-galactose (R_{gal} 0.23 solvent (b), 0.20 solvent (a)) as the major component with 3-O-β-D-galactopyranosyl-D-galactose (R_{gal} 0.55 solvent (a), 0.34 solvent (b) as the minor component.

Polysaccharide 1 (405 mg.) was methylated to give a product (320 mg.)(Found $[\alpha]_D$ -20.5°, OMe, 39.5%). A portion of the product was methanolysed and the mixture of methyl glycosides was examined by g.l.c. The O-methyl sugars identified are shown in Table IV.3. A further portion of the

methylated product was hydrolysed with 0.5M-sulphuric acid and the partially methylated sugars were converted to their alditol acetates. The mixture of partially methylated alditol acetates was examined by g.l.c.; the results are shown in Table IV.3. A portion of the hydrolysate was examined by paper chromatography in solvent (c). The O-methyl sugars identified are shown in Table IV.3.

The major components were 2, 3, 6-tri-O-methyl-D-galactose, 2, 3, 4, -tri-O-methyl-D-galactose and 2, 3, 4, 6-tetra-O-methyl-D-galactose. Both arabinose and glucuronic acid were present as end-group only.

IV.12 Preparation and Examination of Polysaccharide II

Polysaccharide I (10.1 g) was oxidised in darkness for 72 hours, after which time 8.2 m moles periodate /g polysaccharide had been reduced and 1.6 m moles formic acid /g polysaccharide released. The reaction was stopped by the addition of ethane-1, 2-diol (5.2 ml.) and the solution was dialysed against running tap water for 2 days. Sodium borohydride (3.5 g) was added and the mixture kept at room temperature for 30 hours, then dialysed for 2 days. The solution was made 0.5M with respect to sulphuric acid and the polyalcohol was hydrolysed for 48 hours at room temperature. After dialysis against running tap water for

Table IV.3.

O-Methyl Sugars Identified in Methylated Polysaccharide I

Relative Retention Time (T) of Methyl Glycosides	Relative Retention Time (T) of Partially Methylated Alditol Acetates	R(g) After Hydrolysis Solvent (C)	O-Methyl Sugar Identified	Relative Amount (%)
0.51, 0.64	0.52	0.98	2, 3, 5-tri-O-methyl-L-arabinose	10
1.48	1.25	0.88	2, 3, 4. 6-tetra-O-methyl-D-galactose	22.1
3.33, 3.64	2.16	0.72	2, 4, 6-tri-O-methyl-D-galactose	29.6
5.90	3.24	0.72	2, 3, 4-tri-O-methyl-D-galactose	11.3
13.6, 14.9	5.90	0.52	2, 4-di-O-methyl-D-galactose	19.6
2.12, 2.67	-	-	2, 3, 4-tri-O-methyl-D-glucuronic acid	7.5
-	7.39	-	2-O-methyl-D-galactose	trace

2 days Polysaccharide II was isolated as the freeze dried product (2.8 g., yield 27%). Polysaccharide II had $[\alpha]_D$-17.3° and a nitrogen content of 2.86%, hence a protein content of 17.8%. Hydrolysis with M-sulphuric acid followed by chromatography in solvent (e) showed the presence of small quantities of glucuronic acid. Hydrolysis with 0.5M-sulphuric acid followed by chromatography in solvents (a) and (b) showed that Polysaccharide II contained large amounts of galactose and small quantities of arabinose. (Gal:Ara - 10:1.34). Polysaccharide II (245 mg.) was methylated to give a product (140 mg.)($[\alpha]_D$-12.3°, OMe 39.1%). A portion of the product was methanolysed and the mixture of methyl glycosides was examined by g.l.c. The O-methyl sugars identified are shown in Table IV.4. A further portion of the methylated product was hydrolysed with 0.5M-sulphuric acid and the partially methylated sugars were converted to their alditol acetates. The mixture of partially methylated alditol acetates was examined by g.l.c. The derivatives identified are shown in Table IV.4. A portion of the hydrolysate was examined by paper chromatography in solvent (c); the O-methyl sugars identified are shown in Table IV.4. The main components were 2,4,6-tri-O-methyl-D-galactose and 2,3,4,6-tetra-O-methyl-D-galactose together with smaller quantities of 2,3,4-tri-O-methyl-D-galactose and 2,4-di-O-methyl-D-galactose. Partial acid hydrolysis of Polysaccharide II

Table IV.4.

O-Methyl Sugars Identified from Methylated Polysaccharide II

Relative Retention Time (T) of Methyl Glycosides	Relative Retention Time (T) of Partially Methylated Alditol Acetates	R(g) After Hydrolysis Solvent (C)	O-Methyl Sugars Identified	Relative Amounts (%)
0.58, 0.70	0.45	0.98	2, 3, 5-tri-O-methyl-L-arabinose	6
1.72	1.20	0.88	2, 3, 4, 6-tetra-O-methyl-D-galactose	31
3.88, 4.27	2.22	0.72	2, 4, 6-tri-O-methyl-D-galactose	56
6.90	3.48	0.72	2, 3, 4-tri-O-methyl-D-galactose	7
15.0, 16.5	6.02	0.52	2, 4-di-O-methyl-D-galactose	trace
-	7.60	-	2-O-methyl-D-galactose	trace

with 0.25M-sulphuric acid followed by chromatography in solvents (a) and (b) showed the presence of 6-O-β-D-galactopyranosyl-D-galactose (R_{gal} 0.35 solvent (a), 0.25 solvent (b)) and 3-O-β-D-galactopyranosyl-D-galactose (R_{gal} 0.45 solvent (a), 0.34 solvent (b)), as the major component.

IV.13 Preparation of Polysaccharide III

Polysaccharide II (1.5 g.) was dissolved in water (40 ml.) and 0.25M-sodium metaperiodate (40 ml.) was added. After 72 hours, 2.5 m moles formic acid/g polysaccharide had been released. The reaction was stopped by the addition of ethane-1,2-diol (1 ml.) and the solution was dialysed against running tap water for 2 days. Sodium borohydride (0.5 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 room temperature. After dialysis against running tap water for 2 days, Polysaccharide III was isolated as the freeze-dried product, (322 mg., yield 21.5%). Polysaccharide III had $[\alpha]_D$ -60°, a nitrogen content of 6.70%, and hence a protein content of 42%. Polysaccharide III was hydrolysed with 0.5M-sulphuric acid and the hydrolysate examined by paper chromatography in solvents (a) and (b). Galactose was the major component and

traces of arabinose were also present, (Gal:Ara = 10:0.36). A small quantity of Polysaccharide III was methylated. A portion of the methylated product was methanolysed and the mixture of O-methyl glycosides examined by g.l.c. The methyl glycosides identified are shown in Table IV.5. A further portion of the methylated product was hydrolysed with 0.5M-sulphuric acid and the partially methylated sugars were converted to their alditol acetates. The mixture of partially methylated alditol acetates was examined by g.l.c. The results are shown in Table IV.5. A portion of the hydrolysate was examined by paper chromatography in solvent (c). The O-methyl sugars identified are shown in Table IV.5. The main components were 2, 4, 6-tri-O-methyl-D-galactose and 2, 3, 4, 6-tetra-O-methyl-D-galactose. Traces of 2, 3, 4-tri-O-methyl-D-galactose were also present. These results show that Polysaccharide III consists mainly of 1→3 linked galactose residues, although the presence of so much protein indicates a complex structure.

IV.14 Preparation of Polysaccharide IV

A very small quantity of Polysaccharide III was converted to Polysaccharide IV by the usual Smith degradation procedure. The quantity of Polysaccharide IV recovered was not sufficient for analyses other than a nitrogen content and the data of sugar ratios. The nitrogen content was 10.0%, indicating

Table IV.5.

O-Methyl Sugars Identified in Methylated Polysaccharide III

Relative Retention Time (T) of Methyl Glycosides	Relative Retention Time (T) of Partially Methylated Alditol Acetates	O-Methyl Sugars Identified	Relative Amounts (%)
1.65	1.23	2, 3, 4, 6-tetra-O-methyl-D-galactose	40
3.71, 4.07	2.29	2, 4, 6-tri-O-methyl-D-galactose	60
6.6	3.35	2, 3, 4-tri-O-methyl-D-galactose	trace

a protein content of 62.5%.

IV.15 Amino Acid Content of A. auriculiformis Gum[a]

Lysine	5.06	Glycine	4.06
Histidine	4.09	Alanine	3.98
Arginine	2.52	Cystine	-
Hydroxyproline	8.05	Valine	9.47
Aspartic Acid	12.46	Methionine	0.06
Threonine	4.82	Isoleucine	2.76
Serine	8.39	Leucine	8.08
Glutamic Acid	7.33	Tyrosine	3.22
Proline	7.81	Phenylalanine	7.87

a in % per 16g of nitrogen

(From Station des Antibiotiques et des Bioconversions I.N.R.A. Chartres, France).

Table IV.6.

Sugar Content of Acacia Auriculiformis and Its Degradation Products

Polysaccharide	$[\alpha]_D$ degrees	Protein (%)	Gal.	Ara.	Rha.	Glu A.	4-O-Me-GluA
A. auriculiformis Gum	+15.6	7.0	58	10	4	17	11.4
Polysaccharide I	- 9.2	14.4	78.5	11.6	-	9.6	-
Polysaccharide II	-17.3	17.8	88	12	-	-	-
Polysaccharide III	-66	41.8	97	3	-	-	-
Polysaccharide IV	n.d.	59.4	100	-	-	-	-
Degraded Gum A	+25	4.6	70	3.6	-	12.6	13.8
Degraded Gum B	- 4.1	7.0	91	3.1	-	5.3	-

n.d. - not determined

IV.16 Discussion

A. auriculiformis gum contains galactose (58%), arabinose (10%), rhamnose (4%) and uronic acid (28%). The uronic acid content consists of glucuronic acid, 4-O-methyl-glucuronic acid and glucurono-6,3-lactone.

Hydrolysis with 0.5M-sulphuric acid followed by chromatography in solvent (b) indicated the presence of two aldobiuronic acids (R_{gal} 0.27 and 0.63). The major component (R_{gal} 0.27) was chromatographically indistinguishable from 6-O-β-D-glucopyranosyl uronic acid-D-galactose. Its $[\alpha]_D$ value and hydrolysis experiments indicated that 6-O-β-D-glucopyranosyl uronic acid-D-galactose was indeed present, although the slightly high value obtained for the specific rotation indicates the presence of an impurity, possibly another biuronic acid or galactose. Lengthy preparative paper chromatography was, however, unable to resolve the mixture. The component at R_{gal} 0.63 was shown, by hydrolysis studies, to contain 4-O-methyl-D-glucopyranosyl uronic acid and galactose and its specific rotation indicated an α linkage. This indicates that the component is 4-O-(4-O-methyl-α-D-glucopyranosyl uronic acid)-D-galactose, although a small galactose impurity was almost certainly present. Considerable amounts of 4-O-methyl glucuronic acid (R_{gal} 2.3, pink spot with aniline oxalate) were also liberated

by the hydrolysis with 0.5M-sulphuric acid. It is unusual for such a hydrolysis to cleave uronosyl linkages although this phenomenon has been observed previously for Acacia gums of the series Phyllodineae.[5]

Partial acid hydrolysis of the gum followed by paper chromatography indicated the presence of the disaccharides 6-O-β-D-galactopyranosyl-D-galactose (major component) and 3-O-β-D-galactopyranosyl-D-galactose (minor component); $[\alpha]_D$ values and hydrolysis studies confirmed the identities of these components. A further spot at R_{gal} 0.63 (solvent (a) only) was identical with the component found after 0.5M-hydrolysis, namely 4-O{4-O-methyl-α-D-glucopyranosyl uronic acid}-D-galactose. The presence of this aldobiuronic acid in a partial acid hydrolysate indicates an extremely labile linkage from the galactose residue to the remainder of the gum.

A. auriculiformis gum was methylated. Methanolysis, followed by examination by g.l.c. showed the presence of large quantities of 2,4-di-O-methyl-D-galactose together with smaller amounts of 2,3,4-tri-O-methyl-, 2,4,6-tri-O-methyl- and 2,3,6-tri-O-methyl-D-galactose. Arabinose was present both as 2,3,5-tri-O-methyl-L-arabinose and as 2,5-di-O-methyl-L-arabinose, while glucuronic acid was

present as 2, 3, 4-tri-O-methyl-and 2, 3-di-O-methyl-D-glucuronic acid. Rhamnose was present as 2, 3, 4-tri-O-methyl-rhamnose. A small quantity of 2-O-methyl-D-galactose was detected as the alditol acetate; this probably arises from undermethylation.

This methylation study indicates a highly branched structure with a combination of 1, 6-, 1, 4-and 1, 3-linkages between galactose units. The arabinose present is 1, 3-linked while the rhamnose is all present as end-group. The glucuronic acid is predominantly end-group although a small quantity is intra-chain. It would appear that a certain amount of glucuronic acid was lost during methylation; the proportion detected by g.l.c. is less than that found by electrodialysis of the gum. If this is the case, the amount of 2, 3, 4, 6-tetra-O-methyl-D-galactose present would be increased. Hydrolysis of A. auriculiformis gum with 0.005M-sulphuric acid on a boiling water bath for 96 hours gave Degraded Gum A. The distilled water dialysate from the preparation of Degraded Gum A was concentrated and examined by paper chromatography. Large amounts of arabinose were detected together with smaller amounts of rhamnose and galactose. Four disaccharide components were also present (R_{gal} 0.28, 0.39, 0.63, 1.15; solvent (b)). These were identified as 6-O-β-D-galactopyranosyl-D-galactose, 3-O-β-D-

galactopyranosyl-D-galactose, 4-O-{4-O-methyl-α-D-glucopyranosyl uronic acid}-D-galactose and 3-O-β-L-arabinopyranosyl-L-arabinose. The presence of the aldobiuronic acid was confirmed by preparative paper chromatography, hydrolysis and methoxyl content. It is remarkable that this acid is released by such a mild hydrolysis and it indicates the existence of a very labile linkage between this aldobiuronic acid and the remainder of the gum molecule.

Degraded Gum A contained galactose, arabinose, glucuronic acid, 4-O-methyl-glucuronic acid and glucurono-6,3-lactone. The rhamnose had all been removed. Partial acid hydrolysis showed that 6-O-β-D-galactopyranosyl-D-galactose was the major biose present; there were, however, small quantities of 3-O-β-D-galactopyranosyl-D-galactose.

Methylation of Degraded Gum A indicated the presence of end-group galactose, arabinose and uronic acid and the presence of large amounts of 2, 3, 4-tri-O-methyl-D-galactose confirmed the discovery of 6-O-β-D-galactopyranosyl-D-galactose in the partial acid hydrolysate. Large quantities of 2, 3-di-O-methyl-D-galactose reveal the highly branched nature of the gum structure. It is interesting to note the disappearance of 2, 3-di-O-methyl-D-glucuronic acid after a mild acid hydrolysis. It is possible that the rhamnose is 1, 4-linked to

this acid in the gum; such a structure has been demonstrated for other Acacia gums,[5] and this would leave only end-group glucuronic acid.

A. auriculiformis gum was Smith-degraded to give Polysaccharide I which contained galactose, arabinose and uronic acid. Both the rhamnose and 4-O-methyl-glucuronic acid have been removed; this suggests that these components are both present as end-group, while a dramatic reduction in total uronic acid content suggests that the majority of the glucuronic acid is end-group. A sharp increase in nitrogen content is an interesting feature of Polysaccharide I; the value found (2.3%) indicates a protein content of 14.4%. Unfortunately, previous workers do not appear to have recorded nitrogen contents for Smith degradation products, so comparisons cannot be made.

Methylation of Polysaccharide I followed by methanolysis showed large amounts of galactose end-groups with smaller amounts of arabinose and glucuronic acid as end-groups. The other main components were 2,4,6-tri-O-methyl-D-galactose and 2,4-di-O-methyl-D-galactose. The relative amount of 2,3,4-tri-O-methyl-D-galactose had diminished considerably, as one would expect, since -1,6-linkages are periodate-vulnerable. A small quantity of 2-O-methyl-D-

galactose was detected as the alditol acetate; again probably due to undermethylation.

It is interesting to note that the nitrogen content of the methylated gum was very low (0.43%), indicating a certain amount of breakdown. In view of the conditions used in the Haworth methylation procedure, this is hardly surprising. Polysaccharide I was Smith-degraded to give Polysaccharide II which contained galactose and small amounts of arabinose. The percentage yield of Polysaccharide II was very low (27%) suggesting an increase in vulnerability after removal of certain components in the first Smith-degradation. It is possible that certain -1, 6-linked residues were protected by arabinose, linked at the 3 position; removal of this arabinose by the first Smith-degradation allowed periodate attack on the -1, 6-linked galactose residues by the second Smith-degradation.

The trend in increasing nitrogen content was maintained; Polysaccharide II had 2.86% nitrogen indicating a protein content of 17.8%. Polysaccharide II was methylated and g.l.c. examination of the methanolysate showed the main components to be 2, 4, 6-tri-O-methyl-D-galactose and 2, 3, 4, 6-tetra-O-methyl-D-galactose. Once again a decrease in the ratio of -1, 6- to -1, 3-linkages was observed, due to the periodate vulnerability of the -1, 6-linkage.

Polysaccharide II was Smith-degraded to give Polysaccharide III which contained galactose and traces of arabinose. The yield was similar to that of Polysaccharide II, i.e. 21.5%. A further increase in nitrogen content was noted, the figure for Polysaccharide III being 6.7%, indicating a protein content of 41.8%.

Methylation of Polysaccharide III showed large amounts of 2,4,6-tri-O-methyl-D-galactose but only traces of 2,3,4-tri-O-methyl-D-galactose. This indicates that 3 Smith-degradations are sufficient to remove all -1,6-linked substituents.

A very small quantity of Polysaccharide III was Smith-degraded to give Polysaccharide IV. The yield was sufficient for a nitrogen determination only. The nitrogen content was 10.0%, indicating a protein content of 62.5%. This continued increase in protein content through 4 Smith-degradations points to the existence of a periodate resistant linkage from the polysaccharide to the protein or polypeptide.

The main structural features to arise from the present work are:-

1. A. auriculiformis gum contains chains of β -1,3

linked galactose residues. These chains are probably relatively short since the ratio of 2, 3, 4, 6-tetra-O-methyl-D-galactose to 2, 4, 6-tri-O-methyl-D-galactose in Polysaccharide III is about 1:2.

2. To these chains are attached numerous side chains consisting of β-1, 6 linked galactose residues. This is shown by the relatively large amounts of 2, 3, 4-tri-O-methyl-D-galactose in methylated A. auriculiformis gum and also by the fact that the disaccharide 6-O-β-D-galactopyranosyl-D-galactose was the major component on partial acid hydrolysis.

3. Arabinose is present in short chains, 3 units long at the most, β-1, 3 linked, and probably in the pyranose form.

4. An integral part of the molecule consists of protein or polypeptide, possibly cyclic. This is attached to the polysaccharide by a glycosidic linkage which is periodate-resistant.

5. The uronic acid is situated on the fringes of the gum molecule. The 6-O-β-D-glucopyranosyl uronic

acid-D-galactose is probably linked directly, via other galactose residues, while the 4-O{4-O-methyl-α-D-glucopyranosyl uronic acid}D-galactose must be linked via some labile linkage, e.g. arabinose. This would explain the release of the aldobiuronic acid in hydrolysis with 0.005M-acid.

6. The rhamnose is all present as end-group and is thus situated on the fringes of the molecule. The point of attachment is probably -1,4 to glucuronic acid; this would explain the presence of small amounts of 2,3-di-O-methyl-D-glucuronic acid in the methanolysate of methylated A. auriculiformis gum and its absence in methylated Degraded Gum A and Polysaccharide I. This linkage has been detected in previous studies of Acacia gums.[6]

These results indicate a structure basically similar to that suggested for other Acacia gums. The role of the polypeptide in the gum is not known and the nitrogen content of this gum is by no means the highest in Acacia species. Considerable further research will be needed to elucidate the nature and function of the polypeptide.

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SECTION V

CARBON - 13 NUCLEAR MAGNETIC RESONANCE STUDIES ON THE GUM FROM ACACIA AURICULIFORMIS

CARBON - 13 NUCLEAR MAGNETIC RESONANCE STUDIES ON THE GUM FROM ACACIA AURICULIFORMIS

V.1 Introduction

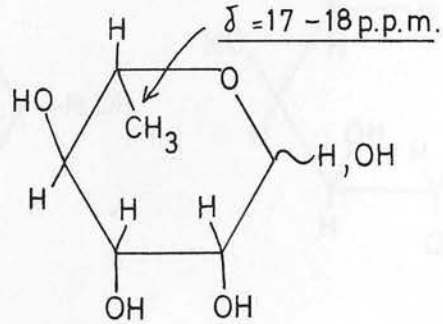
In recent years there has been an increasing number of studies[1-5] in which natural abundance ^{13}C n.m.r. spectroscopy has been used to assist in the structural determination of polysaccharides. Much of the preliminary research was concerned with recording spectra of monosaccharides, substituted monosaccharides and oligosaccharides[1], and subsequent assignation of carbon atoms to signals. In this way models have been built up on which signal assignments in the spectra of polysaccharides may be based. The preliminary work on larger molecules was restricted to homopolysaccharides, e.g. dextrans[3] and yeast mannans[4], the chemical structures for which had already been established. Such ^{13}C-n.m.r. studies were able to confirm the composition and sequence of a glucan containing mixed linkages[2]; to assign anomeric configurations in a water soluble dextran containing various glycosidic linkages[3] and to examine yeast mannans and related oligosaccharides[4].

More recently, studies of heteropolysaccharides have begun with the assignment of signals to disaccharides as models for subsequent spectra of immuno-polysaccharides[5]; the spectra of various chondroitin sulphate molecules have been

recorded[6]. These studies have all shown that valuable structural information can be obtained from ^{13}C-n.m.r. spectroscopy, despite the complexity of the polysaccharide concerned.

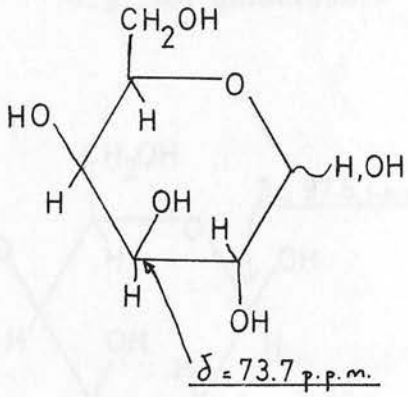
This study presents a series of natural abundance ^{13}C-n.m.r. spectra of the gum from Acacia auriculiformis and its degradation products. An attempt has been made to assign signals on the basis of the structural study described in Section IV and the results of previous workers[(1-6)]. Signal assignment in the spectra of polysaccharides is considerably simplified by the fact that ^{13}C-n.m.r. resonances fall into certain well defined regions, regardless of the identity of the sugar involved[7]. The sugars known to be present in Acacia auriculiformis gum are galactose, arabinose, rhamnose, glucuronic acid and 4-O-methyl-glucuronic acid and the regions in which carbon atoms in these sugars resonate are as follows:-

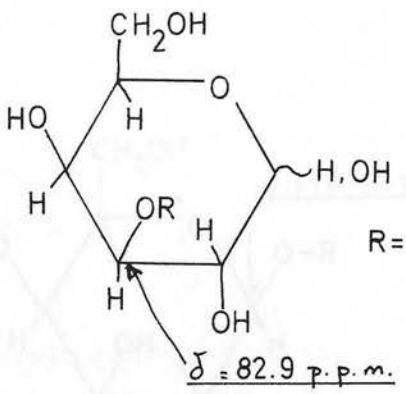
(a) C6 of rhamnose absorbs at high field δ =17-18 p.p.m.



(b) Hydroxylated carbons of the sugar ring absorb between δ =65 p.p.m. and δ =76 p.p.m. Substitution, either by an alkyl group or by another sugar, causes the signal to be shifted downfield by 8-11 p.p.m.

e.g. for galactose:-

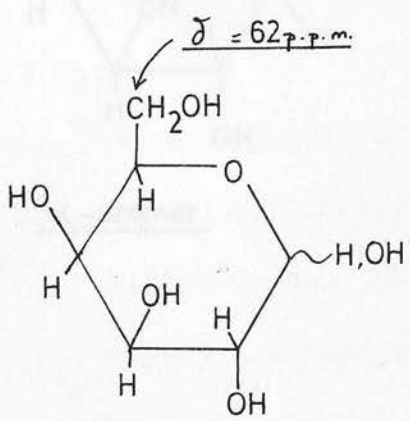


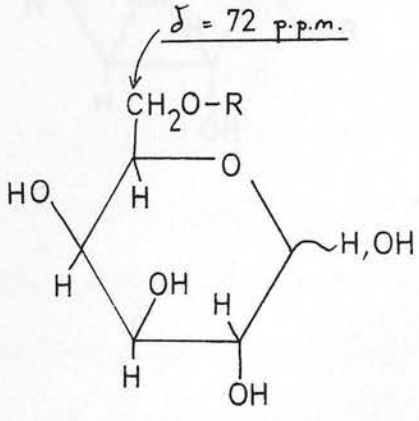


R = alkyl or sugar.

(c) Hydroxylated exocyclic carbons e.g. C6 of galactose absorb at higher field than carbons in the ring, typically δ =62 p.p.m. Substitution, either by an alkyl group or by another sugar, causes the signal to be shifted downfield by about 10 p.p.m.

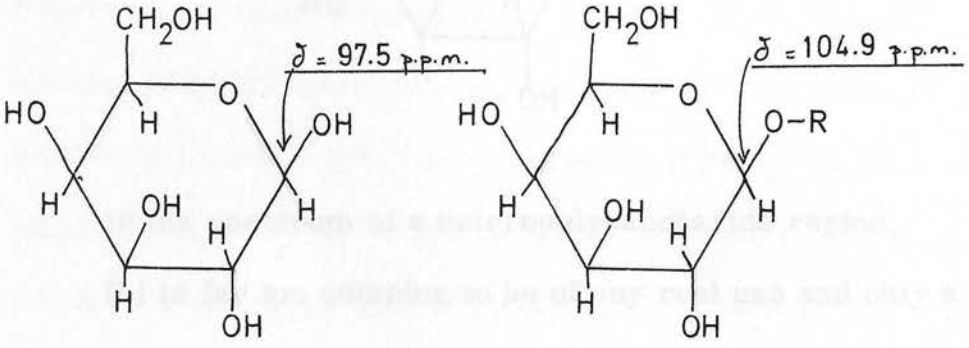
e.g. for galactose:-



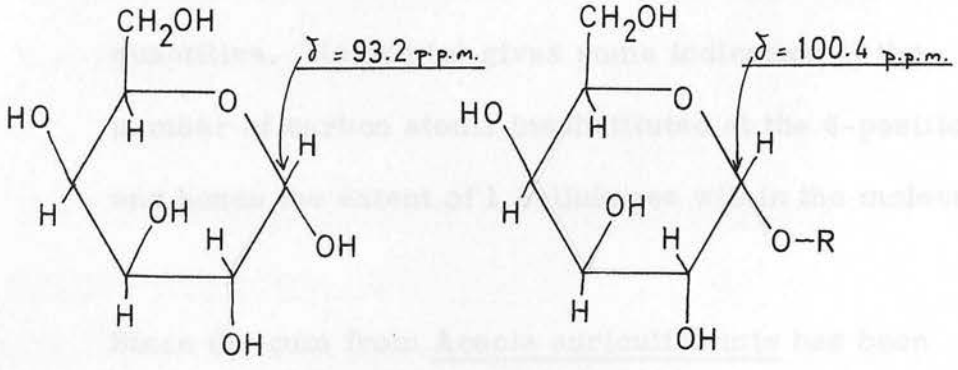


(d) Anomeric carbons absorb at δ =90-98 p.p.m. depending on the configuration. Formation of a glycosidic linkage, however, causes a downfield shift in the resonance of the anomeric carbon of about 7 p.p.m.

e.g. for galactose:-



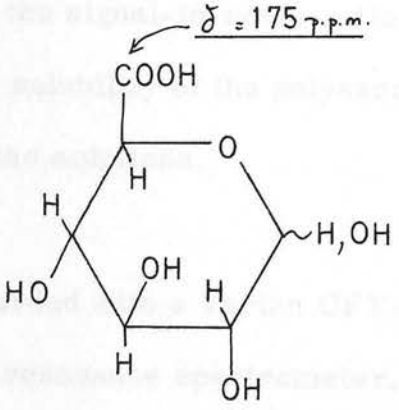
β - anomer.



α - anomer.

(e) Carbonyl carbons, e.g. from uronic acid, absorb at low field δ =175 p.p.m.

e.g. for glucuronic acid:-



In the spectrum of a heteropolysaccharide region (b) is far too complex to be of any real use and only a limited amount of information can be obtained. Region (d), however, can provide interesting information about the nature of the glycosidic linkages in the polysaccharide and, possibly, their relative quantities. Region (c) gives some indication of the number of carbon atoms insubstituted at the 6-position and hence the extent of 1, 6-linkages within the molecule.

Since the gum from Acacia auriculiformis has been shown (Section IV) to be basically a galactan, it is not surprising that ^{13}C-n.m.r. spectra of the gum and its degradation products show considerable similarity to the spectra of galactose and its derivatives.

V.2 Experimental

The polysaccharide (100-200 mg.) was dissolved in deuterium oxide (2 ml.). Attempts to make more concentrated solutions and thus improve the signal-to-noise ratio were frustrated by the relatively low solubility of the polysaccharides and by the high viscosity of the solutions.

Spectra were recorded with a Varian CFT-20 Carbon-13 nuclear magnetic resonance spectrometer. Data points were accumulated overnight at a temperature of 36°C, a spin rate of 22 r.p.s. and with complete proton decoupling. The number of transients varied from 150,000 to 280,000 depending on the quantity of material available. The spectral width was 4000 Hz (200 p.p.m.) and the spectra were calibrated by the addition of small amounts of 1,4-dioxan to the samples. The four equivalent carbon atoms resonate at 1348 Hz (67.39 p.p.m.).

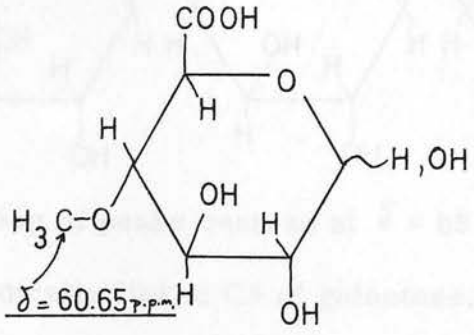
V.3 Natural Abundance ^{13}C-n.m.r. Spectrum of Acacia auriculiformis Gum

The ^{13}C-n.m.r. spectrum of A. auriculiformis gum is extremely complex; since the gum is of high molecular weight, however, and contains five different sugar residues, this is not surprising. The regions defined earlier (Section V.1) are clearly visible and allow a certain amount of signal

assignment as follows:-

(a) A single peak at δ = 17.36 p.p.m. is due to C6 of rhamnose.

(b) A prominent peak at δ = 60.65 p.p.m. is probably due to the methoxyl group present in 4-O-methyl-D-glucuronic acid. This peak is too far upfield to be due to C6 of galactose and, in fact, disappears in later spectra after degradation.

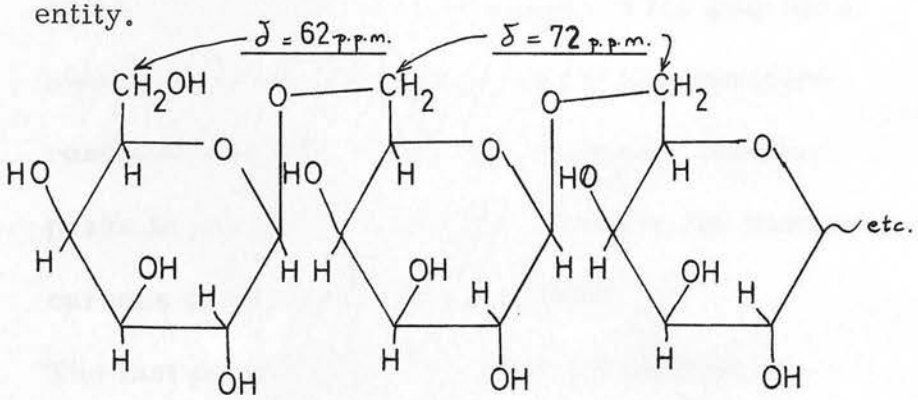


(c) A smaller peak at δ = 62.03 p.p.m. is probably due to hydroxylated C6 of galactose. This is the resonance found by other workers[7] for this atom and in the spectra of the degradation products it increases in size relative to other resonances.

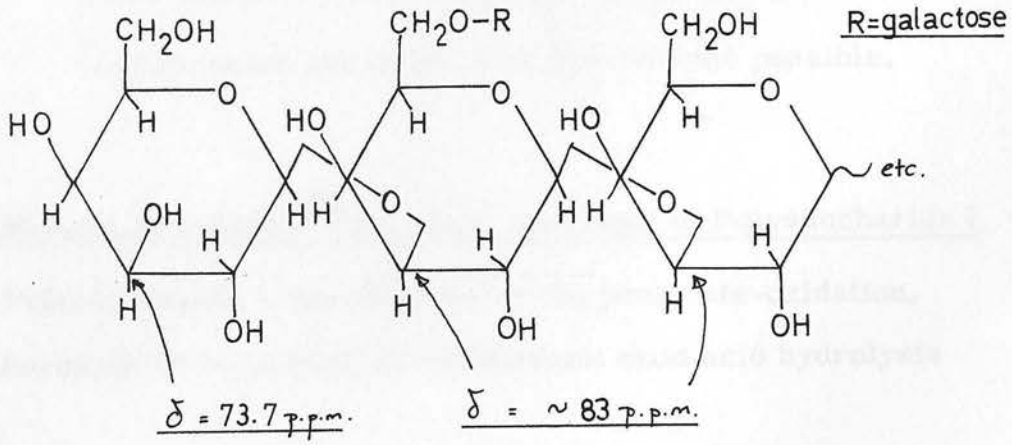
(d) The large peak at δ = 67.39 p.p.m. is due to the added calibration compound, 1,4-dioxan.

(e) The resonances of the hydroxylated ring carbon atoms consist of many peaks between δ = 67 p.p.m. and δ = 77 p.p.m. This region is far too complex to be of any practical significance, with the possible

exception of the largest peak at δ = 72.8 p.p.m. This resonance is almost certainly due to glycosidically linked C6 of galactose, shifted downfield by 10 p.p.m. relative to the unsubstituted C6 of galactose. This resonance gradually disappears in subsequent spectra and may, therefore, be due to a periodate-vulnerable entity.



(f) The group of peaks centred at δ = 83 p.p.m. is due to glycosidically linked C3 of galactose. Since A. auriculiformis gum has been shown to contain β-1, 3-linked galactose residues (Section IV) the presence of these resonances is expected. The multiplicity of peaks in this region indicates several different environments within the molecule.



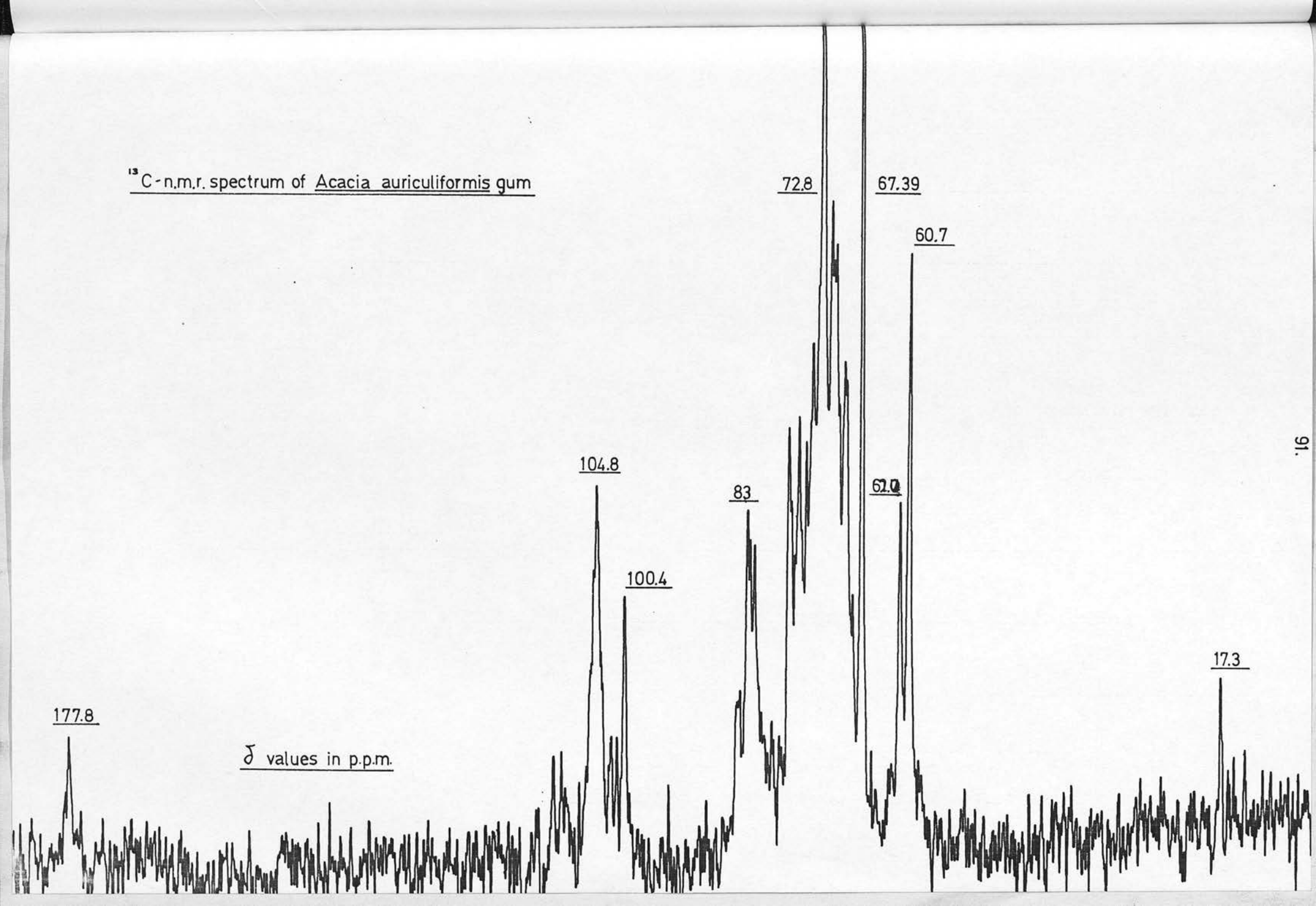
(g) The resonances of the anomeric carbons lie in the region δ = 100-105 p.p.m. A prominent peak at 100.4 is probably due to an α-linkage whose presence has been shown in Section IV . The group of peaks centred on δ =104.3 p.p.m. is almost certainly due to β-linked C1 of galactose. The majority of the glycosidic linkages in the gum have been shown to be β (see Section IV) and therefore resonances in this region are expected. Smaller peaks in the same region may be due to the anomeric carbons of the other sugars present.

(h) The last peaks in this spectrum are centred at δ = 177 p.p.m. and are almost certainly due to carbonyl groups in the uronic acid residues.

Thus, although the ^{13}C-n.m.r. spectrum of A. auriculiformis gum is very complex, some structural information can be deduced from it, and after other complex polysaccharides of this type have been studied by this technique, other structural assignments and deductions may become possible.

V.4 Natural Abundance ^{13}C-n.m.r. Spectrum of Polysaccharide I

Polysaccharide I was obtained by the periodate-oxidation, borohydride-reduction and subsequent mild acid hydrolysis


^{13}C-n.m.r. spectrum of Acacia auriculiformis gum
72.8
67.39
60.7
104.8
83
61.0
100.4
17.3
177.8
δ values in p.p.m.

of A. auriculiformis gum (Section IV). The resulting polysaccharide was much simpler structurally, containing galactose (78.5%), arabinose (11.6%) and glucuronic acid (9.6%). The rhamnose and 4-O-methyl glucuronic acid had been eliminated. The ^{13}C-n.m.r. spectrum is considerably simpler, as is to be expected; peak assignment is therefore a more realistic operation.

(a) The peak at δ = 61.8 p.p.m. is due to hydroxylated C6 of galactose. It is interesting to note that the peak which was at δ = 60.6 p.p.m. in Spectrum I has disappeared; the methoxyl content of polysaccharide I was found to be negligible. The origin of the peak at δ = 63.1 p.p.m. is unknown, but it disappears in later spectra and could, therefore, be due to hydroxylated C5 of arabinose.

(b) The large peak at δ = 67.39 p.p.m. is due to the added calibration compound, 1,4-dioxan.

(c) The resonances in the region due to hydroxylated ring carbons δ = 67-77 p.p.m. have become considerably more simplified. The most prominent, at δ = 69.4, and 75.9 p.p.m., are probably due to the hydroxylated C2 and C5 of galactose.

(d) The region attributed to C3 of galactose, glycosidically linked at δ = 83.2 p.p.m. has also been considerably

simplified. Presumably removal of periodate-vulnerable peripheral residues has considerably reduced the number of different environments.

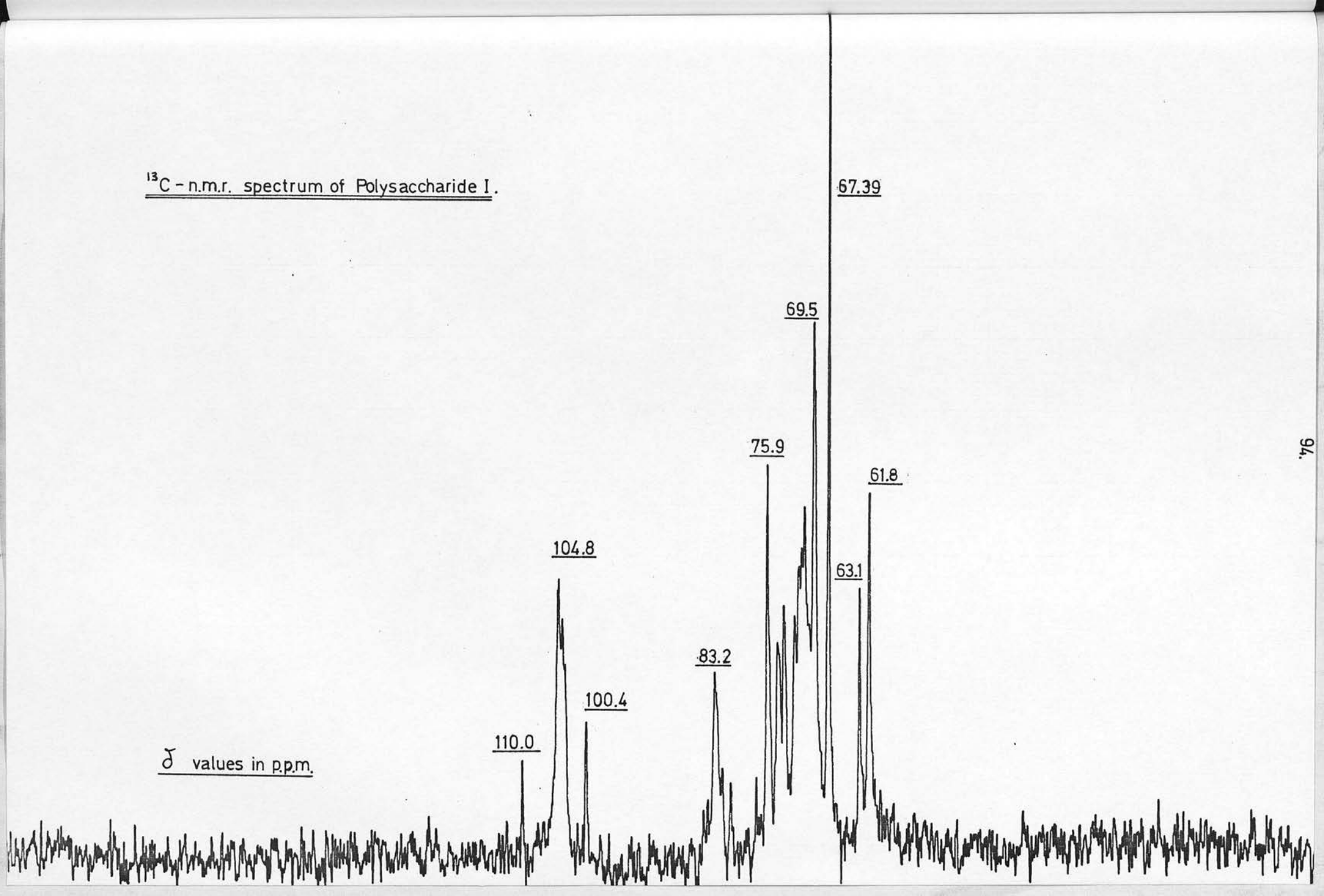
(e) The number of peaks in the region of the spectrum given by anomeric carbon atoms (δ = 100-105 p.p.m.) has decreased slightly. The vast majority of the resonances appear to come from β-linked galactose (104-105 p.p.m.); this is consistent with earlier findings. The smaller peak at δ = 101 p.p.m. may be due to an α-linkage or to arabinose as it disappears in the spectra of degradation products.

(f) The origin of the peak at δ = 110 p.p.m., which disappears in the spectra of degradation products, is unknown.

The ^{13}C-n.m.r. spectrum of Polysaccharide I, although complicated, reflects the considerably chemical simplification undergone by the parent A. auriculiformis gum.

V. Natural Abundance ^{13}C-n.m.r. Spectrum of Polysaccharide II

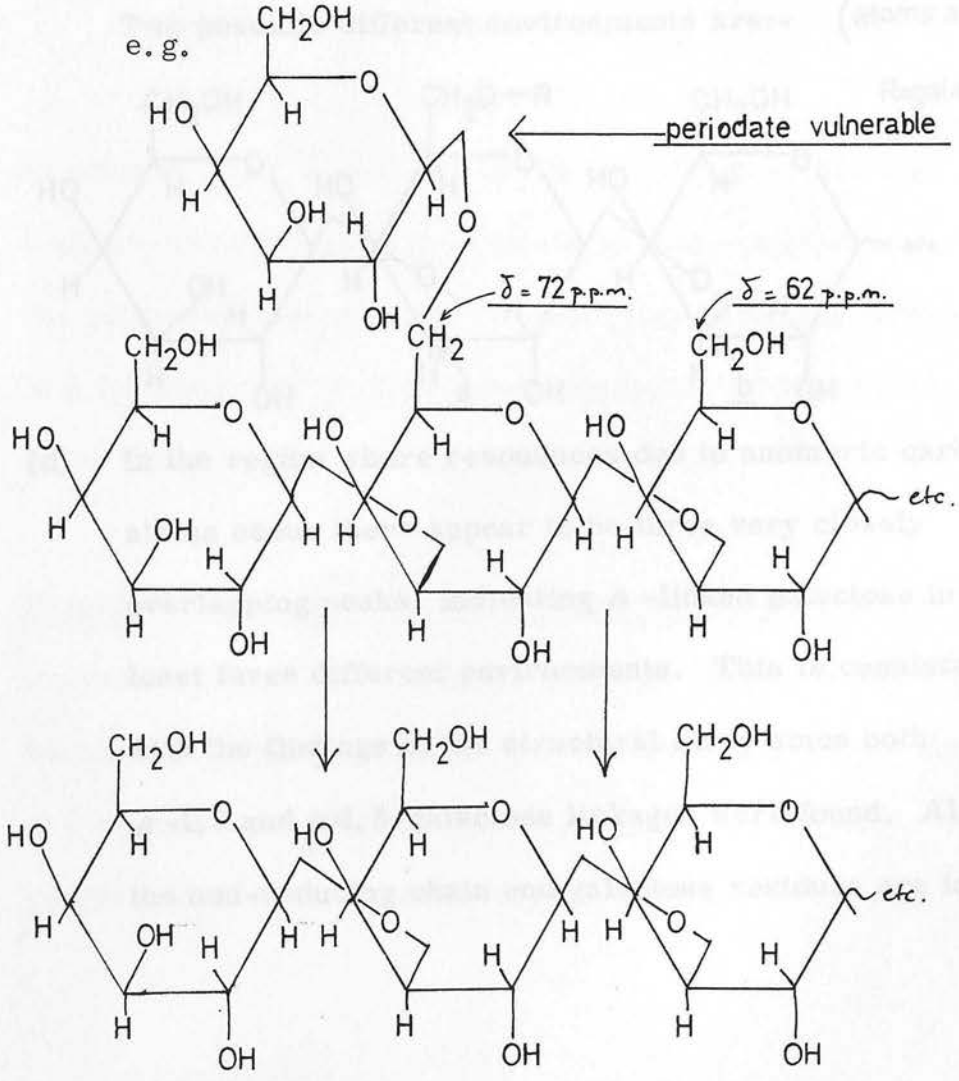
Polysaccharide II, obtained by the periodate-oxidation, borohydride-reduction and subsequent mild acid hydrolysis of Polysaccharide I (see Section IV), was shown to contain galactose (88%), arabinose (12%) and uronic acid (trace). Since the polysaccharide has undergone two periodate


^{13}C - n.m.r. spectrum of Polysaccharide I.
67.39
69.5
75.9
61.8
104.8
63.1
83.2
100.4
110.0
δ values in p.p.m.

oxidations a large number of vulnerable residues in the original gum have been removed and the ^{13}C-n.m.r. spectrum is considerably simpler.

(a) The peak due to hydroxylated C6 of galactose at δ = 61.8 p.p.m. has increased in size relative to the other peaks. This indicates that a periodate-vulnerable residue is attached to the C6 of galactose in A. auriculiformis gum. Continued periodate-oxidation removes the vulnerable residue leaving hydroxylated C6s.

e.g.

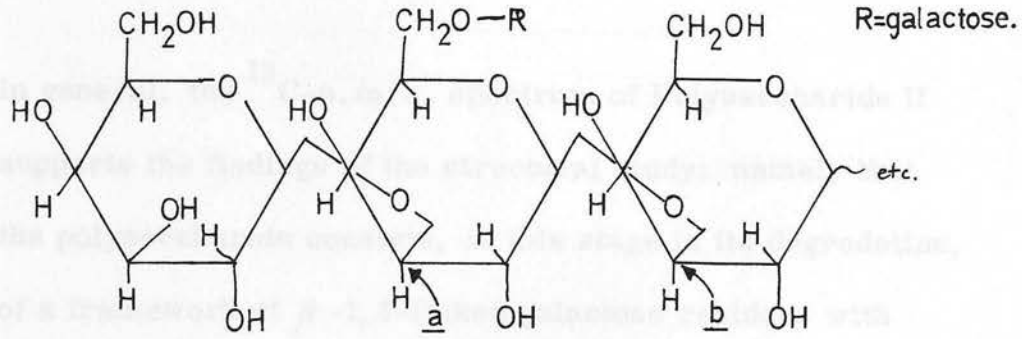


The diagram shows how the relative number of hydroxylated C6s increases with periodate oxidation.

(b) The region due to hydroxylated carbon atoms of the sugar ring (δ = 67-77 p.p.m.) is basically unchanged, although there are minor differences in relative peak sizes.

(c) The resonances due to glycosidically linked C3 of galactose at δ = 82.3 p.p.m. have become considerably simplified, with only two or three overlapping peaks present. This indicates a corresponding reduction in the number of environments available to the C3 atom.

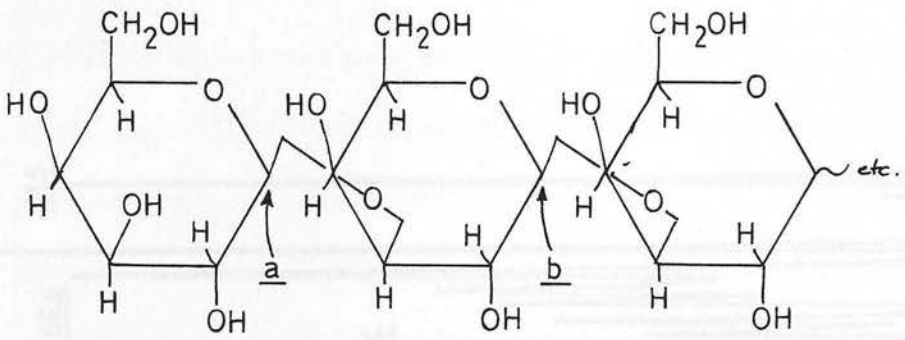
Two possible different environments are:- (atoms a & b)



(d) In the region where resonances due to anomeric carbon atoms occur there appear to be three very closely overlapping peaks, indicating β-linked galactose in at least three different environments. This is consistent with the findings of the structural study since both β-1, 3 and β-1, 6-galactose linkages were found. Also the non-reducing chain end galactose residues are in

marginally different environments and therefore the chemical shifts of their anomeric carbon atoms are likely to be different from those of intra-chain galactose residues.

e.g. (atoms a & b)

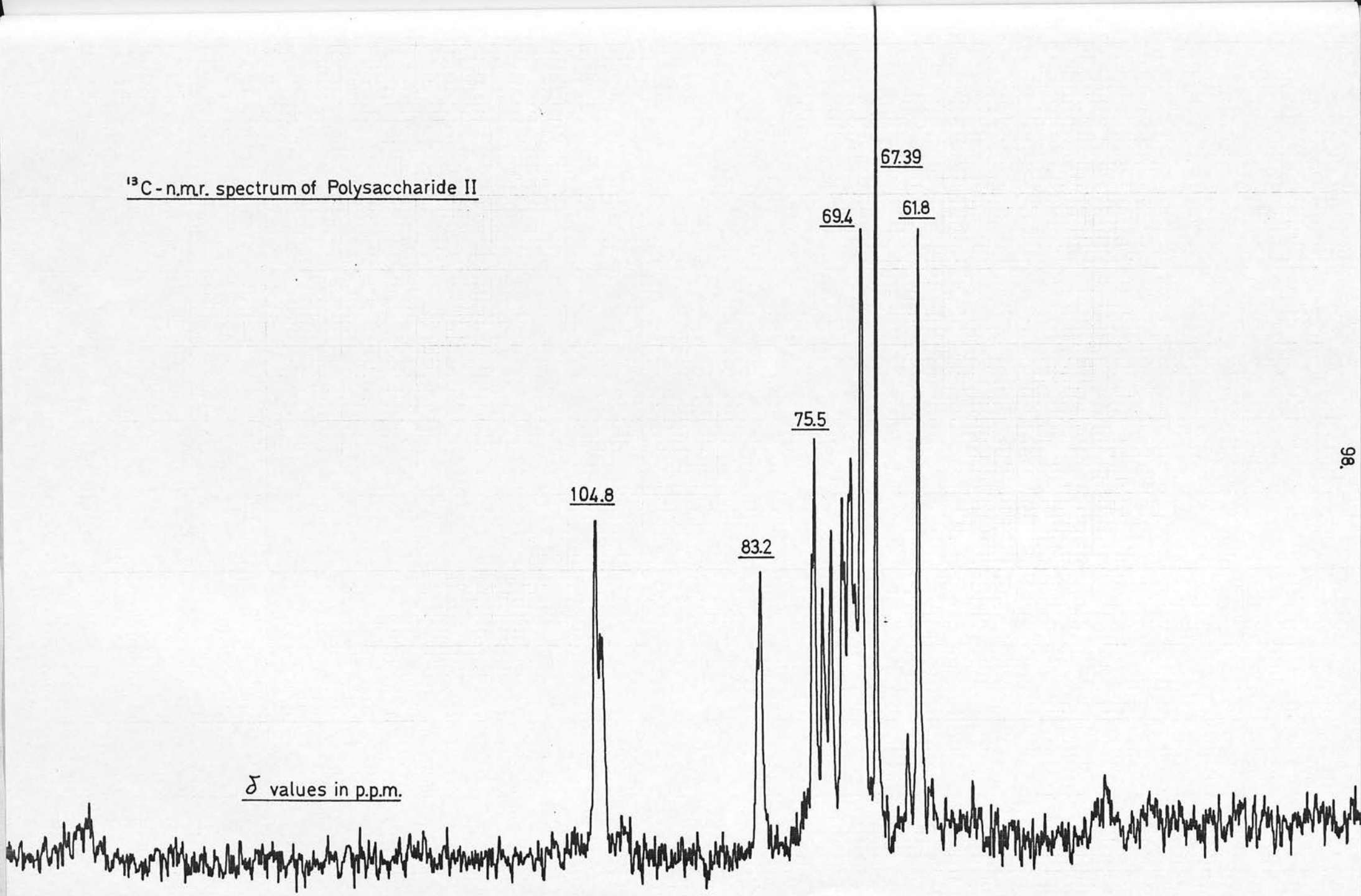


The actual difference, in terms of chemical shift, however, is likely to be very small.

In general, the ^{13}C-n.m.r. spectrum of Polysaccharide II supports the findings of the structural study; namely that the polysaccharide consists, at this stage in its degradation, of a framework of β-1, 3-linked galactose residues with some substitution at the C6 of galactose.

V.7 Natural Abundance ^{13}C-n.m.r. Spectrum of Polysaccharide III

Polysaccharide III was obtained by the periodate oxidation, borohydride reduction and subsequent mild acid hydrolysis of Polysaccharide II (See Section IV). The resulting polysaccharide was shown to contain galactose (97%) and


^{13}C - n.m.r. spectrum of Polysaccharide II
67.39
69.4
61.8
75.5
104.8
83.2
δ values in p.p.m.
98.

arabinose (3%). A methylation study indicated the presence of only trace amounts of 6-O- β -linked galactan.

At first sight the ^{13}C-n.m.r. spectrum appears to be more complicated than its predecessors, surprising considering the sugar composition of the polysaccharide. In fact the spectrum shows not only resonances of the galactan component of the molecule but also contributions from the polypeptide part (41.8%). Analysis of this spectrum must, therefore, be divided into two operations, firstly assignment of galactan peaks and secondly a tentative assignment of the amino acid resonances.

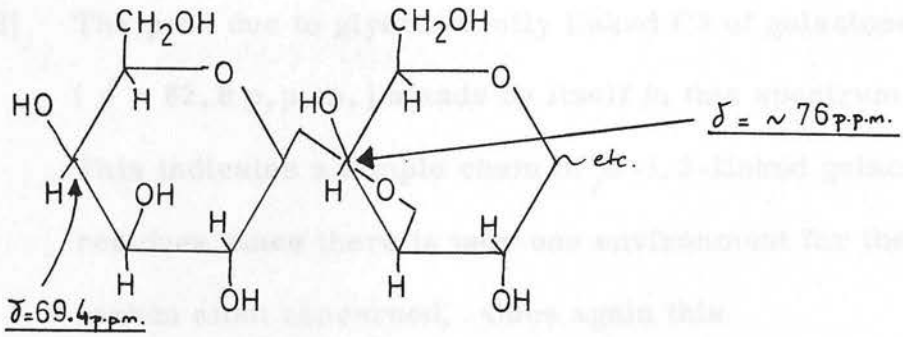
(a) The large peak at δ = 61.8 p.p.m. is due to hydroxylated C6 of galactose. This peak has increased in size relative to the others throughout the degradation and this is consistent with continual removal of periodate vulnerable side chains from the periodate resistant galactan core.

(b) The large peak at δ = 67.39 p.p.m. is due to the added calibration compound, 1,4-dioxan.

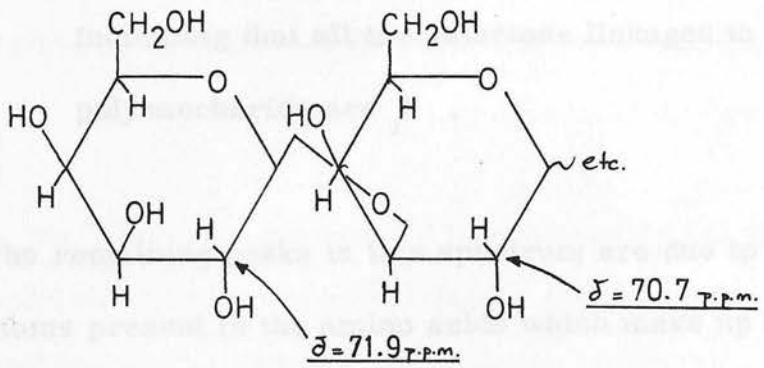
(c) The resonances of the hydroxylated carbon atoms of the sugar rings (δ = 67-77 p.p.m.) are much clearer in this spectrum than in those discussed previously. It is interesting to note that the three main peaks in

this region are doublets whose separation is very small. Assignments for these peaks are as follows:-

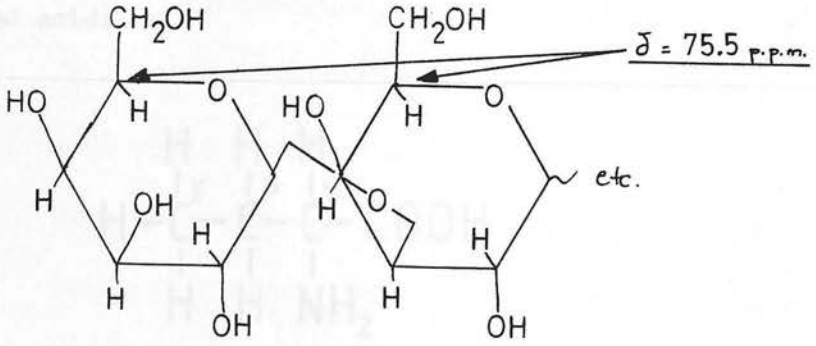
δ = 69.4 p.p.m. C4 of galactose at non-reducing chain end, substitution at C3 shifts this resonance downfield by about 7 p.p.m.



δ = 70.7 p.p.m. C2 of galactose when the C3 position is substituted. C2 of non-reducing chain end galactose resonates at slightly lower field, possibly the resonance at δ = 71.9 p.p.m.



δ = 75.5 p.p.m. C5 of galactose in any part of the molecule. This resonance is relatively unaffected by substitution except in the 6 position.

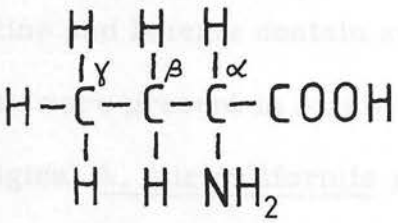


(d) The peak due to glycosidically linked C3 of galactose (δ = 82.8 p.p.m.) stands by itself in this spectrum. This indicates a simple chain of β -1, 3-linked galactose residues since there is only one environment for the carbon atom concerned. Once again this interpretation is in agreement with the results from the methylation study (see Section IV).

(c) The resonances of the anomeric carbon atoms are very similar to those in the previous spectrum, indicating that all the galactose linkages in the polysaccharide are β .

The remaining peaks in this spectrum are due to the carbon atoms present in the amino acids which make up the protein or polypeptide part of the molecule. The positions of the peaks are characteristic of amino acid carbon atoms. When assigning peaks to individual carbon atoms it is helpful, once again, to divide the spectrum into regions. Previous workers[7] have allocated spectral regions as follows:-

For an amino acid:



(i) C_γ and methyl carbon atoms = 12.5-27.5 p.p.m.

(ii) C_β = 27.5-42.5 p.p.m.

(iii) C_α = 52.5-62.5 p.p.m.

(iv) Aromatic and heteroaromatic carbon atoms = 122.5-142.5 p.p.m.

(v) Carbonyl carbon atoms = 168.5-173.5 p.p.m.

Since all normal amino acids are α-amino acids and since, by definition, all must contain carbonyl groups, it is not surprising to find regions (iii) and (v) predominating.

In the ^{13}C-n.m.r. spectrum of Polysaccharide III the regions described above are clearly visible and are easily distinguishable from the carbohydrate resonances. It would be virtually impossible to assign any of these peaks to individual amino acids without recourse to lengthy studies with model compounds, but some conclusions can be drawn:-

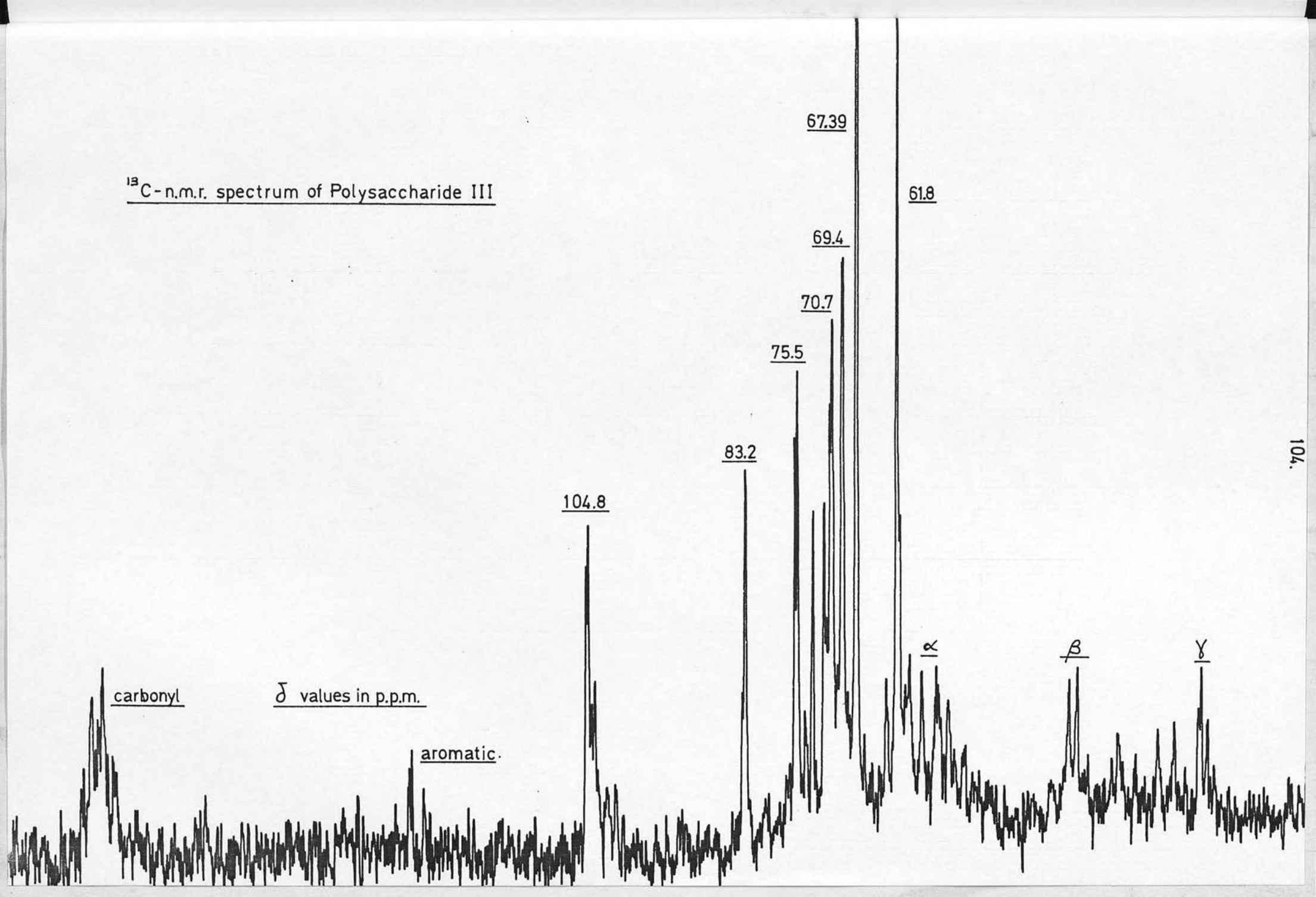
1. There is a sizeable contribution from at least one γ or methyl carbon atom (δ = 19.2 p.p.m.). Both valine and leucine contain such carbon atoms and both were present in significant quantities in the original A. auriculiformis gum (see Section IV).

2. Two prominent peaks (δ = 36.5 p.p.m. and δ = 37.7 p.p.m.) are probably due to β -carbon atoms, many amino acids, however, contain β carbon atoms.

3. A series of peaks (δ = 54-64 p.p.m.) are almost certainly due to α -carbon atoms; since each component amino acid contains such a carbon atom however, no assignment can be made.

4. A small group of resonances (δ = 129 p.p.m.) are due to aromatic carbon atoms. In the original gum substantial quantities of phenylalanine and smaller quantities of histidine and tyrosine were detected.

5. The group of resonances between δ = 170 p.p.m. and δ = 175 p.p.m. are due to the carbonyl carbon atoms of the peptide bonds which hold the polypeptide together. In theory, a count of the number of resonances should give the approximate number of different peptide linkages and hence some idea of the number of amino acids; in practice, however, such an analysis would be very difficult to achieve.


^{13}C-n.m.r. spectrum of Polysaccharide III
67.39
61.8
69.4
70.7
75.5
83.2
104.8
104.
α
β
γ
carbonyl
δ values in p.p.m.
aromatic

Despite the apparent complexity of the ^{13}C-n.m.r. spectrum of Polysaccharide III a considerable amount of information can be gleaned from it and it is particularly interesting from the point of view of further studies on similar peptidoglycan molecules such as degraded plant gums.

V.8 Conclusion

Although the natural abundance ^{13}C-n.m.r. spectra of heteropolysaccharides such as plant gums are very complex, some information about the structure is available. The gum from Acacia auriculiformis has been shown to be very complex structurally, it also has a high molecular weight and a relatively high viscosity. That such a gum should be chosen for a feasibility study is unfortunate but it shows that spectra are obtainable even for the most complex natural products. The majority of plant gums hitherto studied are of much lower molecular weight and of much lower viscosity and therefore far more concentrated solutions can be made; this in turn should lead to less noisy spectra and hence greater ease of interpretation. The technique could be applied to quick analysis of a plant gum to avoid the fairly lengthy procedures of hydrolysis, paper chromatography and sugar ratio determination; information such as approximate rhamnose content, uronic acid content and methoxyl content should be readily available.

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THE COMPOSITION OF *ACACIA* GUM EXUDATES FROM SPECIES OF THE SUBSERIES *JULIFLORAE**

D. M. W. ANDERSON and M. C. L. GILL
Department of Chemistry, The University, Edinburgh EH9 3JJ, Scotland

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Key Word Index—*Acacia* spp.; Leguminosae; gum exudates; polysaccharides; chemotaxonomy.

Abstract—An analytical study has been made of gum specimens from *Acacia auriculiformis* (two specimens), *A. holosericea*, *A. mangium*, *A. leptostachya* and *A. pubifolia*, which belong to subseries Juliflorae of the Series Phyllodineae. These gums appear to be more proteinaceous, more acidic and more viscous, with higher methoxyl contents and higher molecular weights but with lower proportions of rhamnose and arabinose, than the majority of *Acacia* gums studied so far.

INTRODUCTION

Although Bentham [2] placed 277 Australian species in his Series I (Phyllodineae) of the genus *Acacia*, Tindale [3] now considers the correct number to be at least 570. This large number of species is still best subdivided, according to the divisions proposed by Bentham [2], Taubert [4] and Maiden and Betche [5] into eight subseries viz. Alatae; Continuae; Pungentes; Calamiformes; Brunioideae; Uninerves; Plurinerves; and Juliflorae [6].

Relatively few species of the Series Phyllodineae have been examined chemically so far, although there have been studies of the distribution of amino acids [7] in some seeds (including one species in the Juliflorae), and of the flavonoid content of some heartwoods [8,9] (including 15 species in the Juliflorae). To date, the gum exudates from only 13 species [10] in the entire Series Phyllodineae have been studied; of these, one is in Bentham's subseries 4C, eleven species are in subseries 6F, and one in subseries 7F. This paper presents the first data available for species in subseries 8 (Juliflorae), which contains 151 species and is the second largest subseries in the Phyllodineae. Botanically, the Juliflorae is considered [3] to be a most complex group of Phyllodinous wattles, which occur in both tropical and more temperate regions of Australia, Malaysia and the East Indies.

RESULTS AND DISCUSSION

The analytical data obtained for the five species studied (seven specimens) are shown in Table 1. Although generalizations cannot be drawn for the characteristic properties of gums from the Juliflorae (151 species) in terms of the few species studied here, it is clear that it is quite distinct from the other subseries of phyllodinous *Acacias* studied so far.

The gums studied are of high MW. Both the phyllodinous and bipinnate *Acacias* studied previously [10,11] have been of much lower MW than the African species studied (for some typical values see ref. 12), but the values for *Acacia holosericea* ($3{\cdot}8 \times 10^6$) and *A. mangium* ($3{\cdot}2 \times 10^6$) exceed considerably the highest value (*A. adansonii*, *A. arabica*, $2{\cdot}3 \times 10^6$) reported previously. In agreement with earlier reports [11]; the use of 1% aq. sodium borohydride [13] does not appear to cause any extensive degradation during the dissolution process; indeed, the previously established tendency [11] for borohydride-solubilized material to

* Part 47 in the Series "Studies of Uronic Acid Materials". For Part 46 see ref. 1.

Table 1. Analytical data for purified gum polysaccharides from *Acacia* species of the subseries *Juliflorae*

	A. auriculiformis A	B	C	*A. holosericea*	*A. mangium*	*A. leptostachya*	*A. pubifolia*
Moisture (%)	13·3	8·4	12·0	9·0	16·2	16·1	13·4
Ash (%)*	4·8	4·5	5·3	5·1	5·4	5·8	3·4
Nitrogen (%)*	1·14	1·12	0·92	0·28	0·98	0·66	1·66
Hence protein (%) (N × 6·25)*	7·12	7·0	5·75	1·75	6·12	4·12	10·4
Methoxyl (%)†	1·71	1·90	1·68	0·47	1·49	2·24	1·20
$[\alpha]_D$, In water (degrees)†	+18·6	+15·6	+15·8	+2·9	+36·4	+58	−58
Intrinsic viscosity $[\eta]$ (ml g^{-1})*	22·0	22·6	25·0	19·0	27·7	16·7	25·6
Molecular weight (MW × 10^6)	1·9	2·3	3·0	3·8	3·2	1·35	2·44
Equivalent weight†	590	620	635	1010	545	475	680
Hence uronic anhydride (%)‡	29·7	28·4	27·7	17·3	32·2	37·0	25·9
Sugar composition after hydrolysis							
4-*O*-Methylglucuronic acid§	10·2	11·4	10·1	2·8	9·0	13·4	7·2
Glucuronic acid	19·5	17·0	17·6	14·5	23·2	23·6	18·7
Galactose	58	58	59	56	56	54	46
Arabinose	9	10	8	20	10	7	25
Rhamnose	3	4	5	6	2	2	3

* Corrected for moisture content.
† Corrected for moisture and protein content.
‡ If all acidity arises from uronic acids.
§ If all methoxyl groups located in this acid.

be of higher MW than the corresponding water-soluble fraction is confirmed here (*A. auriculiformis* sample C).

In addition to high MW, the Juliflorae species studied give gum solutions of high intrinsic viscosity. The value given by *Acacia auriculiformis* equals the highest values reported previously (cf. *A. laeta* [14], *A. parramattensis* [11], and *A. tortilis* subsp. *heteracantha* [15]. *A. mangium* [$(\eta) = 27·7$ ml/g] must now be regarded as the most viscous of the *Acacia* gums studied so far.

The methoxyl contents reported here are also high. The value for *A. leptostachya* (2·24%) closely approaches the highest value reported [16] to date (*A. giraffae*, 2·40%) and with the exception of *A. holosericea*, the methoxyl contents of the other species studied here all exceed the values for *A. nilotica* [17] and *A. parramattensis* [11] which, previously, came second only to *A. giraffae*.

With the exception of *A. holosericea* and *A. leptostachya* gums the nitrogen contents of the Juliflorae species studied tend to be high, with *A. pubifolia* (1·66%) now exceeding the highest value reported [11] previously (*A. parramattensis*, 1·55%). Attention must also be drawn to the unusually high acidity of the Juliflorae species studied. With the exception of *A. holosericea*, the other species studied here have considerably higher uronic acid contents than *A. cyanophylla* (uronic acid 24%), which, for nearly 20 years, has been the most acidic *Acacia* gum known [18].

Although the tendencies toward low rhamnose content and high galactose/arabinose ratios typical of the other subseries of phyllodinous wattles [10] are easily recognizable, it is already apparent that the botanical complexity of the Juliflorae is reflected in the wide ranges of values shown in their various analytical parameters for their gums.

EXPERIMENTAL

Origin of gum specimens. Gum from *Acacia auriculiformis* A. Cunn. ex Benth. (Bentham No. 271) was collected by Mr. J. F. U. Zieck on 23 July 1973 from a bushy low-branched tree (about 4-yr-old, height 8 m, dbh 10 cm, with a smooth to cracked greyish bark, with flowers and fruit present) growing on black cracking clay soil of the Savannah belt in the office garden of the Forest Products Research Centre, Frangipanni Road, Port Moresby, Papua, New Guinea. Botanical voucher specimens from this tree have been kindly authenticated by Dr. M. D. Tindale as NSW 107339. This gum sample was soluble in cold water, and is shown as sample A in Table 1. A second sample of *A. auriculiformis* gum was collected on 7 May 1973 from the same location as sample A, but as a bulk sample from about 30 bushy low multiple-branched trees (age 3 yr, average height 8 m, dbh 10 cm) planted closely together to form a hedge. The exudation appeared to have formed on unhealthy trees that had probably been attacked by insects or some species of fungus. The water-soluble material from this second sample is shown as sample B in Table 1; the water-insoluble material present dissolved on the addition of a very small amount of $NaBH_4$ [13] and gave sample C after dialysis, filtration, and freeze-drying. Gum from *Acacia holosericea* A. Cunn. ex G.

Don (Bentham No. 274) was collected by Mr. J. F. U. Zieck on 23 July 1973 from a crooked, low-forked tree (about 4-yr-old, height 8 m, dbh 11 cm, with a dark-coloured smooth to cracked bark, and flowers, young and mature fruit present) growing on black cracking clay soil of the Savannah belt in the office garden of the Forest Products Research Centre, Frangipanni Road, Port Moresby. Botanical voucher specimens from this tree have been authenticated by Dr. M. D. Tindale as NSW 107338. Gum from *Acacia mangium* Willd. (Bentham No. 275) was collected from a single tree at Ulu Kukut on 16 March 1971 by the Plantation Officer at Sandakan, Sabah, Malaysia. Gum from *A. leptostachya* Benth. (Bentham No. 256) was collected on 11 Aug 1969 from a shrub, 2 m high, growing on acidic volcanic outcrops 39 miles west of Chapter's Towers, Hughenden, Queensland, by Mr. W. R. Birch of the School of Biological Sciences, University College of Pimlico, Townsville, Queensland: botanical vouchers were authenticated by Mr. L. Pedley, Research Botanist at Brisbane Botanic Museum and Herbarium, as R.C. Correll E74. Gum from *Acacia pubifolia* Pedley was collected by Dr. M. Tindale on 5 Jan 1969 from a tree, 5 m high, with silvery foliage and black iron-bark, on a granite hillside at Wyberba, 4 miles south of Ballandeen, S.E. Queensland: the reference voucher is NSW 102606.

Preparation of samples for analysis. Acacia auriculiformis gum sample A and *A. holosericea* gum dissolved slowly in cold H_2O to give clear, colorless solutions. After dialysis against tap H_2O for 24 hr and then against distilled H_2O for 2 × 24 hr, the gum solutions were filtered through Whatman No. 41, then No. 1, and finally No. 42 papers, and freeze-dried.

Acacia auriculiformis gum sample B contained some water-insoluble material; this dispersed on the addition of a small amount of $NaBH_4$, and the material recovered after dialysis and freeze-drying is shown as sample C in Table 1. *Acacia mangium*, *A. leptostachya* and *A. pubifolia* gums required the addition of traces of dil. alkali and $NaBH_4$ [13] to effect complete dissolution; although these gums were partially soluble in cold water, the amounts of gum available were very small and it was decided not to examine the water-soluble and water-insoluble fractions separately. After recovery as the freeze-dried solid, *A. mangium* gum gave a clear, colorless solution; the solutions from *A. leptostachya* and *A. pubifolia* gums were respectively orange–yellow and pale yellow in colour.

Analytical methods. The standard analytical methods used have been described [10].

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