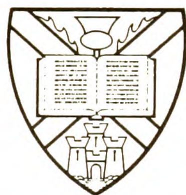

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ANALYTICAL, STRUCTURAL AND METABOLIC STUDIES OF PLANT GUM EXUDATES

by

J.G.K. FARQUHAR B.Sc., Dip.Ed.

Thesis presented for the degree of Doctor of Philosophy

University of Edinburgh

October 1980

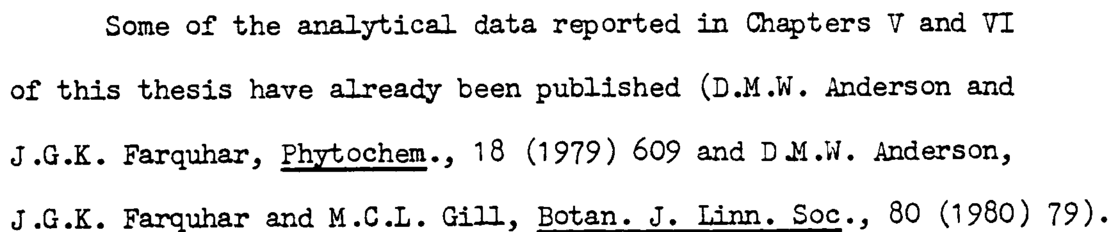
TO MY MOTHER AND MAURICE

AND TO THE MEMORY OF MY FATHER

DECLARATION

I hereby declare that this thesis was composed by myself and that the work reported 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 Chapters V and VI of this thesis have already been published (D.M.W. Anderson and J.G.K. Farquhar, Phytochem., 18 (1979) 609 and D.M.W. Anderson, J.G.K. Farquhar and M.C.L. Gill, Botan. J. Linn. Soc., 80 (1980) 79).



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ABSTRACT

A review of the literature on studies of the metabolism and possible toxicity of gum arabic when ingested or externally adsorbed by laboratory animals and man revealed a distinct lack of reliable knowledge and of modern studies of the assumed safety of this widely used food additive and cosmetic and pharmaceutical ingredient.

Analysis of 7 Prosopis gum exudates, including commercial mesquite gum, showed these gums, apart from the anomalous P. juliflora, to be chemically similar (though less viscous) to other plant gums. The results supported a taxonomic revision of this complex genus.

Fifteen gum exudates of the Series Phyllodineae (Sub-series Juliflorae), Gummiferae and Vulgares in the genus Acacia have been analysed. The Juliflorae samples were more proteinaceous, more viscous and more acidic with higher methoxyl and lower rhamnose contents than most other Acacia gums studied to date and the range of several parameters was greater than expected within a single Sub-series. A reappraisal of Bentham's Juliflorae classification is offered as a result. The Gummiferae and Vulgares samples were also more acidic with a higher methoxyl content than previously found for these Series. Outstanding features were a nitrogen content of 9.4% in A. hebeclada gum and a glucose content of 12% in A. erubescens gum, the first reported occurrence of glucose as a component of an Acacia gum exudate.

An analytical study of a bulk sample of powdered commercial gum arabic carried out repetitively at intervals over a period of two years, revealed an increase in acidity, laevorotation and a loss of methoxyl content with time. Data for the changes in composition of such complex natural products when stored have not

previously been available.

Hydrolysis, methylation and Smith-degradation studies of the gum from Acacia deanei sub-species paucijuga showed a highly branched 3,4 - and 4,6 - disubstituted galactan framework of (1 $\longrightarrow$ 4) - , (1 $\longrightarrow$ 3) - and (1 $\longrightarrow$ 6) - linked galactose chains. Extremely long side chains of (1 $\longrightarrow$ 2) - and (1 $\longrightarrow$ 3) - linked arabinofuranose units were attached to this "core" and terminated by non-reducing arabinofuranose, arabinopyranose, galactose and glucuronic acid residues. The laevorotatory gum contained proteinaceous material attached by periodate-resistant linkages and, unexpectedly, an α - (1 $\longrightarrow$ 4) - linked aldobiuronic acid.

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CHAPTER I

GENERAL INTRODUCTION

It is probably true to say that the carbohydrate group of natural products has proved to be one of the most interesting fields of organic chemistry. Carbohydrates, in providing the structural framework of all plants, the food reserves of plants and animals and the metabolites of lipids and amino acids, are the ultimate source of most of man's food, clothing and shelter and hence his life. From their study, the formulation of stereochemical principles, the use of protective groups, the development of specific functional group reagents and advances in macromolecular chemistry have been some of the great contributions made by carbohydrate chemists to the science.

Plant gum exudates are the most complex of the polysaccharide group and their study has presented the most formidable problems in carbohydrate chemistry (1). Plant gums are complex acidic hetero-polysaccharides exuded from the stems of certain tropical and sub-tropical trees and shrubs found in Africa, Australia, India South America and parts of Asia. Exudation usually follows mechanical injury or bacterial infestation of the bark (2), but the precise mechanism of gum formation is still not fully understood; bacterial action, enzymic conversion of starch or hemicelluloses and direct synthesis have been mentioned as possible explanations (2). Functionally, plant gums seal off wounds against further attack and also form a protection against tissue dehydration.

The gums occur as partially neutralised mixed salts of complex polysaccharide acids containing hexose, pentose, methyl pentose and uronic acid residues linked together in a complex, highly branched manner. About one hundred plant gums from a variety

of botanical genera have now been studied and the neutral sugars most frequently found are D - galactose, L - arabinose and L - rhamnose, with D - xylose and D - mannose also present in certain species, e.g. from the genera Combretum and Grevillea (3,4). D - glucose has so far only been detected in the gum from Anacardium occidentale (3,5); its first noted presence in an Acacia gum exudate is reported in Chapter VI of this thesis.

The acidity of plant gums arises most frequently from the presence of D - glucuronic acid and its 4 - O - methyl derivative, but some genera also contain D - galacturonic acid (2). The fact that so many different sugar components are present and that each is involved in more than one type of linkage makes these natural products among the most complicated chemical systems ever studied. The uronic acid content has been found to vary from species to species and even within samples of the same species (6). Acacia pycnantha gum (7,8) has the lowest uronic acid content (3.3%) found so far in any genus, while typical values fall in the range 10 - 15%. Some Combretum and Acacia species have been found to contain over 30% uronic acid (3,4,9); several Acacia gums with similar high values are reported in Chapters V and VI of this thesis.

The molecular weights of Acacia gums are typically within the range 5×10^4 to 3×10^6 (10), although recent work on Combretum, Grevillea and Parkia species has shown that some gums have molecular weights considerably higher (3,4).

A small proportion (under 5%) of proteinaceous material is also present in most gums, although Neem gum (Azadirachta indica) has been found to contain up to 40% protein (11). The gum from

Acacia dictyophleba has, very recently, been reported as containing ca. 47% protein (4) and this thesis reports further Acacia species containing over 40% protein (Chapters V and VI). Evidence available to date seems to imply that there is chemical bonding between protein and polysaccharide; attempts to isolate the proteinaceous material without causing extensive degradation to the polysaccharide have been unsuccessful (11, 12).

Exudate gums are generally readily soluble in water, yielding viscous solutions. The intrinsic viscosity of Acacia gums is typically within the range 4 to 28 ml/g (10), although several samples are reported in this thesis (Chapter V) with viscosities of up to 69 ml/g. Recent work on Grevillea (4) and Combretum (3) gums has yielded intrinsic viscosities of up to 575 ml/g.

The ability of gums to dissolve readily to give viscous solutions makes them very useful commercially (2, 13, 14). The ancient Egyptians used gums as paint thickeners and for embalming; today, gums are widely used as binding, emulsifying, stabilising and thickening agents in the food, confectionery, pharmaceutical and cosmetic industries and in drinks, printing inks and preparations, glazes, sizes, adhesives, foundry sands, explosives, etc. Among the gums used in the food industry, gum arabic is perhaps the one most encountered. This gum, also known as gum acacia, is mainly the exudate from the species Acacia senegal (syn. verek); good quality samples are practically colourless, odourless and flavourless and completely dissolve in water to give a stabilising solution of a useful viscosity ($[\eta]$ ca. 20 ml/g), a rare combination of desirable properties in a natural product.

The essential property of a food additive is of course a total lack of toxicity and because gum arabic has been used for centuries

without any apparent ill effects, it has long been assumed to be safe. In the light, however, of increasingly responsible attitudes towards food additives and since their types and uses are steadily growing, the safety of gum arabic and other gums as food additives is becoming a matter of concern (15 - 17). Ideally, an additive and its metabolites, if any, should be shown to be non-toxic, non-carcinogenic and non-allergenic both when ingested in small quantities and after long term exposure.

Chapter II of this thesis presents a specific literature review of the metabolism, toxicity and allergenicity of gum arabic as used as a food additive and in cosmetic, industrial and pharmaceutical preparations. This review revealed a distinct lack of reliable knowledge and research into the metabolism and toxicity of the gum and consequently a comprehensive and well controlled feeding study with laboratory animals is currently being undertaken in this laboratory.

This study presented the unique demand for an accurate chemical analysis of a bulk quantity of gum arabic and this task is reported in Chapter VII along with a study of changes in its composition during a long period of storage. This showed an interesting increase in the acidity of the gum over the period (two years), a change which may possibly be due to aerial or enzymic oxidation.

The analytical parameters used to characterise gums are ash, protein and methoxyl contents, specific rotation, intrinsic viscosity, molecular and equivalent weights, uronic anhydride content and sugar composition after hydrolysis. These parameters taken overall establish a form of "fingerprint", which is characteristic of each particular gum. It is now evident that the combination of parameters of a gum from any genus (_Acacia_, _Albizia_, _Araucaria_, _Combretum_, _Grevillea_,

Parkia, Prosopis, etc.) gives one of the most sensitive ways of establishing the identity of a species (14).

Consequently, a great deal of analytical work has been done to obtain data to support chemotaxonomy, especially in the genus Acacia (14, 18-21), the most studied and commercially most important gum-bearing genus.

Chapter IV of this thesis reports an analytical study of seven gums from the American Prosopis genus, which includes the widely available mesquite gum. Botanical classification of this genus is particularly complex and often disputed and a review of this is presented and discussed on the basis of the chemical data obtained.

Bentham, in 1875, classified the genus Acacia into six Series (22), a classification which has largely been supported by chemical analysis to date. Recent work, however, on several gums from Series 1, Phyllodineae, has shown the Series to be much more complex than previously thought (9, 23) and Chapter V of this thesis presents an analytical study of six previously unexamined samples from the Phyllodineae, Sub-series Juliflorae and provides further evidence of this complexity. Chapter VI contains an analysis of nine further exudates from species in Bentham's Series 4, Gummiferae and Series 5, Vulgares. This study detected glucose in an Acacia gum for the first time and revealed several gums with extremely high protein contents.

Detailed structural studies of Acacia gums have so far centred around Bentham's Series 4 and 5 (18); Chapter VIII consists of a partial structural study of the gum from Acacia deanei subspecies paucijuga which is in Bentham's Series 2, Botryocephalae. The structural features found differed in several respects from previous gums (18), further highlighting the benefits of chemical analysis to the taxonomist.

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CHAPTER II

A LITERATURE REVIEW OF STUDIES OF THE METABOLISM AND POSSIBLE TOXICITY OF GUM ARABIC WHEN INGESTED BY LABORATORY ANIMALS AND MAN

II. 1. PHYSIOLOGY

1a. Digestibility

Although sparse, a number of reports have been published on investigations into the toxicity of gum arabic, but there is debate and uncertainty as to whether the gum molecule is digested (1). There has been a long controversy whether gum arabic is a food or not; conclusive evidence that the intact gum molecule is absorbed from the gastro-intestinal tract is lacking.

The information known pre-1930 has been discussed by Amberson (2): although these trials were poorly controlled and of little worth, they suggest that the gum is not absorbed nor digested by mammals, herbivorous animals being an exception. An interesting report is mentioned however, of gum arabic being claimed as an acceptable emergency ration in desert areas; "above one thousand persons, belonging to an Abyssinian caravan, were supported for two months by gum arabic alone".

A single study in 1941 (3) attempted to determine the digestibility of the gum, but was of totally insufficient duration. Two groups of ten young rats (average weight 140g) were fed 34% or 3.3g gum arabic in a single meal; seventy-two hours later their liver glycogen levels were not higher than those of the control group. It was concluded that orally administered gum arabic was excreted unchanged without enzymic degradation.

In 1949, an interesting study by Booth et al. was carried out on guinea-pigs to investigate the importance of bulk in purified diets (4). A basal diet was known to produce an average weight increase of 1.8g per day compared with 6.9g per day from a commercial diet. Various hemicelluloses and gums (gum arabic, gum mesquite and gum

tragacanth) were tried as supplements and of these, gum arabic at a level of 15% consistently gave the best results of 5.1g per day. The remaining growth delay was shown to be due to a lack of minerals. The growth-promoting effect of the gum arabic was investigated and it was concluded that the intact gum molecule was essential, probably due to a "bulk" effect on the lower intestinal tract. It was also suggested that the gum may affect the type of bacteria in the lower digestive tract, possibly favouring those which were themselves beneficial to the animal(Wichmann et al. (5) noted later, in 1954, that intestinal synthesis of vitamin B_{12} was promoted in gum arabic fed guinea-pigs, but no details were available). It was noticed that the level of protein in the diet could be reduced in the gum-fed animals. No discussion was given on the actual digestibility of the gum. The neutralised acid-hydrolysate of the gum was ineffective as a supplement. Twenty animals were fed the gum-containing diet, over a period of six weeks. Roine et al., also in 1949, observed similar findings (6).

Further investigations in the fifties and sixties failed to establish clearly the fate of ingested gum arabic. In another study on the dietary requirements of guinea-pigs, O'Dell et al. in 1957 (7) confirmed the growth-promoting ability of gum arabic found by Booth et al. in 1949 (4). They did not accept that this was due to a "bulk" action, but suggested that the mineral content of the gum was responsible. Their investigations concerned balance studies of calcium, magnesium, potassium, sodium and phosphorus in the presence of 15% gum arabic. They claimed that the gum was 90% digested, but no explanation of this figure was given. They mentioned that guinea-pigs fed gum arabic diets produced small and scanty faecal pellets. The experiments were only carried out for ten days, with six groups of three or four animals.

The same year, Hove and Herndon (8) demonstrated that gum arabic was apparently utilised to some extent by rabbits, though only four animals were used. The rabbits (average weight 400 - 500g) were given a basal diet plus 20% gum arabic for forty days. A growth response of 16.7g per day was obtained, which was better than that from the basal diet but less than the accepted normal rate (35g per day). No other information was given. Shue _et al._ in 1962 (9) attempted to measure the caloric value of gum arabic in rats. A diet including 16% gum arabic was given to weanling rats such that consumption of gum arabic was 1g per rat per day. They stated that the caloric value of the gum was 75% of that of sucrose, as judged by weight gain, and that the gum was 80% digested, but no discussion was given on numbers of animals, feeding duration or of the results. A similar study in rats was presented by Booth _et al._ in 1963 (10). A group of six rats ingested a diet containing 15% gum arabic and mean weight gains and food efficiency (gain/food intake) after sixty-two days were normal. They reported 71% digestibility of gum arabic, based on (total intake of gum minus increase in faecal dry weight)/ total intake of gum. Similar results were obtained with 6% gum guar. The digestibility however, was only measured over a seven day period in five rats ingesting a control diet plus 0.75g gum arabic per rat per day. Another study in 1965 (11), of which again only an abstract is available, reported similar findings. Using a method involving restricted food and caloric intake, gum arabic was fed to groups of 10 weanling male rats for 7 days at levels of 0.5 and 2.0g per day. The gum was shown to have caloric values of 131% and 110% respectively of corn starch, but no explanation was given.

These studies on animals have therefore yielded information which clearly can only be tentative. The work reported has in general

involved insufficient numbers of animals and for much too short durations. No mention was made in any of the reports of checks on the gum samples' homogeneity or chemical composition. The results from guinea-pigs must be interpreted with caution, as the digestive tract of these herbivorous animals exhibits a large functioning caecum.

Only one study, in 1931, was traced on man (12), in which twenty-two infants from one to fifteen months old were fed 15 - 20g gum arabic per day as treatment for oedema. No evidence for absorption of the intact gum molecule was found - no urinary pentose excretion was observed and there was significant excretion of gum arabic in faeces. No other details or discussion were included.

This uncertainty about the digestibility of gum arabic in animals and man has continued in the literature of the last few years.

In 1977, a short review of the digestion and absorption of carbohydrates (13a) mentioned that disaccharides and oligosaccharides have not been observed to pass through the intestinal membrane to any significant degree and accepted the claim by previous workers that gum arabic was assimilated and used for energy. The review pointed out that, in view of the known structural features of gum arabic (proposed by Smith in 1940 (14) and more fully characterised by Anderson et al. (15) in 1966), it is unlikely that any of the enzymes normally associated with gastric and duodenal secretions could cause significant degradation. Also considered unlikely was acidic hydrolysis in the stomach, but this claim is questionable as gum arabic is readily hydrolysed with 0.01N hydrochloric acid. Degradation by microflora in the gut was suggested as being responsible for decomposition of the gum, although it was pointed out that this was surprising as gums with simpler structures are less degraded (13a). In contrast,

a review of gums and hydrocolloids published in 1976 (16) assumed that low molecular weight fractions of hydrocolloids such as carrageenans and pectins were assimilated and that most vegetable gums would behave in the same way. No evidence was presented for this claim. In 1974, the U.S. Food and Drug Administration Select Committee on Generally Recognised as Safe (GRAS) Substances concluded, "It would appear that gum arabic is capable of being digested to simple sugars in herbivores, and to some extent in omnivores such as Man. After absorption, the digestion products are available for oxidation. Conclusive evidence indicating that the intact gum arabic molecule is absorbed under normal conditions is lacking" (17).

The digestion and utilisation of gum arabic has therefore not been sufficiently investigated; additional work is required to resolve the uncertainty and characterise the metabolism of the ingested gum.

1b. Toxicity

Reports of acute or long-term toxicity studies of orally ingested gum arabic could not be traced. This indicates the need for further well-planned feeding studies, particularly into any toxicity from long-term assimilation of the gum or its metabolites. Similarly, toxicity data from short-term feeding studies are sparse; the information traced has been summarised below. In the case of the rat, guinea-pig and rabbit, studies designed specifically to determine the toxicity of gum arabic were not traced but information was gleaned indirectly from the digestibility studies reported in part 1a.

i. Rat:

A group of six rats ingested a diet containing 15% gum arabic for sixty-two days and the only unusual action of the gum was a

cathartic effect (10). This was reported to produce bulky, sticky, stringy faeces, presumably due to the excretion of undigested hydrophilic residue. Haematological findings and organ weights were normal. No significant response was mentioned in a study (11) in which two groups of weanling male rats ingested a diet containing 0.5g or 2.0g gum arabic per day for seven days. An additional trial with a diet containing 29% gum arabic caused serious diarrhoea on the second day but this was almost normal on the seventh day. Caecal dilation was also observed with this diet.

ii. Guinea-pig:

Very little toxicological information is available. Booth et al. (4) reported that diarrhoea was frequently observed in a group of twenty animals ingesting a diet containing 15% gum arabic. Apart from that and the growth delay already discussed, toxic effects or serious deficiencies were not observed, even after eight months feeding. The other study discussed by O'Dell et al. (7), also using 15% gum arabic diets, gives no toxicological information other than that the animals produced small and scanty faecal pellets, attributed to the high digestibility of the gum.

iii. Rabbit:

In a digestibility study (8) deleterious effects were not noted in a group of four rabbits after ingesting a diet containing 20% gum arabic for forty days.

iv. Chicken:

Vohra and Kratzer (18) in 1964 studied the effect of various polysaccharides on chicken growth over a period of twenty-one days. The number of birds involved was not given. They reported that gum

arabic, at a level of 2% in an adequate control diet, did not result in any appreciable growth delay. This was considered unusual in that 2% guar gum, 2% carob bean gum, and 2% gum tragacanth produced "definite" growth depressions of 25 - 30%. Predictions about the growth inhibitory properties of the polysaccharides from their chemical composition were not possible, but the presence of methoxyl groups in pectin was shown to be essential for growth inhibition. Hydrolysed guar gum did not cause growth delays. The degree of branching in the polysaccharide structure was also mentioned as possibly being important in growth inhibition.

v. Pigeon:

In 1934, Lecoq (19) reported that pigeons fed 20g per day of a diet containing 66% gum arabic developed an "alimentary disequilibrium". This led to symptoms akin to polyneuritis and death after fifteen to twenty-five days. It was concluded that the "disequilibrium" was due to galactose from the gum, as galactose itself produced the same effect when fed at high levels. The effect of the gum was not decreased when large supplements of yeast, i.e. B vitamins, etc., were fed. Birds fed a diet containing 35% gum arabic survived for thirty to sixty days; this was increased to over six months when supplemented with small amounts of yeast.

It must of course be emphasized that the digestive tract of the pigeon and the chicken is totally different from that of the rat and man and that 66% of gum arabic in any diet is an extremely high level.

vi. Cultured Mammalian Cells:

A carefully planned study of carbohydrate utilisation by certain cell lines (20) showed in 1972 that arabic acid is toxic to CHO-K1

(Chinese hamster ovary cells) and to LM (TK) cells (mouse L - cells lacking thymidine kinase). D - glucuronic acid and D - glucuronolactone gave over 50% inhibition of growth in the presence of glucose. D - gluconic acid, D - galacturonic acid, D - galactose and L - arabinose did not permit growth when present as the sole carbohydrate source. D - galacturonic acid and D - galactose did permit growth when present with pyruvate.

vii. Galactosaemia:

No report of galactosaemia initiated by ingestion of any natural galactose-containing polymer (e.g. gum arabic or other natural polymers) was found.

1c. Embryogenicity and Teratogenicity

A paper published in 1964 (21) revealed that all five of a group of five-day incubated hen eggs in which the yolk sac was injected with 0.1 ml of 1% aq. gum arabic (a dose of 1mg) failed to hatch. Injections of the same volume of water had no effect. No explanation of this effect was advanced.

A comprehensive teratologic evaluation of gum arabic in various animals was reported in 1972 by the U.S. Food and Drug Administration (22).

Starting from day six of gestation, doses of gum arabic as a suspension in anhydrous corn oil (1ml per kg body weight) were administered daily by oral intubation for ten consecutive days to pregnant mice and rats. Four dose levels were used: 16, 75, 350 and 1600 mg/kg with nineteen mice per level and twenty-four rats per level. Groups of nineteen hamsters received the same dose levels for five days and four groups of an average of thirteen rabbits received 8, 37, 173 and 800 mg/kg for thirteen days. In all cases,

except the two higher dose levels in rabbits, no clear effects on nidation, maternal or foetal survival were shown. The number and type of abnormalities seen in foetal soft or skeletal tissues did not differ from controls. However, in the rabbits dosed at 173 and 800 mg/kg, maternal toxicity ensued and three and six of the animals respectively in the two groups died. Death was preceded by severe diarrhoea containing blood, urinary incontinence and anorexia. The surviving does and their offspring were normal. It was concluded that gum arabic was not a teratogen in the rabbit under the test conditions employed.

1d. Mutagenicity and Carcinogenicity

The U.S. Food and Drug Administration also sponsored an extensive study in 1972 on the mutagenic effects of gum arabic (23). This was conducted as a host-mediated assay, a cytogenetic assay and as a dominant lethal gene test. In the host-mediated assay, gum arabic did not produce any measurable mutagenic response or alteration in the recombination frequency for *Saccharomyces cerevisiae* in either the assay or the associated *in vitro* tests. In the cytogenetic assay, a slight adverse effect on the metaphase chromosomes of rats fed gum arabic at concentrations of 5.0g/kg and 2.5g/kg was produced after six hours. A similar effect also occurred in *in vitro* tissue culture tests on human embryonic lung cells at all concentrations. In general, the effect represented chromosomal breaks rather than recombinations and occurred within six hours after treatment. In the dominant lethal gene test, the responses were not consistent with any mutagenic effects of gum arabic in rats.

Reports of carcinogenicity attributable to orally ingested gum arabic were not found.

II. 2. HYPERSENSITIVITY

2a. Introduction

The allergic reactions of individuals to various foods, dusts, pollens and hairs are well known. Reports of sensitivity to gum arabic and to other gums appeared from 1933 on and included cases of asthma, other respiratory and gastro-intestinal symptoms and urticaria. These were elicited by contact, inhalation or ingestion of factory dusts and sprays, and various foods, pharmaceuticals and toiletries (24-38). Although the numbers of individuals affected can only be estimated, available data do not suggest an incidence of reactions sufficiently greater than those from other foods (38). This is reflected in the decision as to a recommended "Acceptable Daily Intake" by the World Health Organisation/ U.N. Food and Agricultural Organisation Joint Expert Committee on Food Additives, it being stressed by them that "no approval would be given for the use of a substance causing serious or widespread hypersensitivity reactions". A much firmer immunological basis and comprehensive statistical survey would be required, other than subjective case reports, to substantiate the claim that food additives are indeed responsible for allergic responses in man. Previous to this, a large number of papers were published concerning reactions following the intra-venous use of gum arabic (39 - 78) and a summary of this work is included below.

2b. Summary of Intra-venous Research

In the period during and after the First World War, gum acacia solution was extensively used intra-venously in cases of haemorrhage and shock as a blood substitute (39, 40). Although repeatedly demonstrated to be therapeutically useful, some cases of severe

toxicity in humans were reported where there was some question as to the preparation or bacteriological purity of the gum solution (41 - 45). This resulted in considerable debate; investigations were made into the action and metabolism of the gum, mainly in dogs, guinea-pigs and rabbits (44 - 78). The information gained is summarised as follows. Solution of acacia is a mild antigen and under certain conditions, anaphylactic sensitivity to it may develop. Rabbits do not in general exhibit any symptoms from its repeated injection (44). It is possible to cause anaphylaxis from acacia solution in guinea-pigs properly prepared by suitably designed experiments along standard procedures (44, 53 - 55). No mention was found of the now proven protein content of acacia gum (ca. 1 - 5%); the dangers of i.v. proteinaceous injections are well known.

The gum has been shown not to be biochemically inert following i.v. injection, but to be deposited in liver, spleen and kidney (causing organ enlargement) and in lung, lymph node and bone marrow (69). The general opinion reached was that this deposition leads to marked liver damage and impairment of function, detectable grossly and microscopically, e.g. diminution of plasma protein (46, 47, 69). Elimination of these deposits was found to be slow (50), acacia being detected even after three years in one case (74). Loss of acacia in the urine was found to be only slight (73, 75). Typical pathology resulting from i.v. injection of gum acacia is given by Studdiford (45) and features mainly foam-cell accumulation, haemorrhagic diathesis and cell fatty degeneration and atrophy.

It has also been shown that acacia is not inert in the bloodstream. It brings about a disturbance of the blood colloidal equilibrium (haemoclasia or colloidoclasia), which presumably is

fundamentally concerned with the functional disturbances of allergic phenomena in general. That is, its effect is a physico-chemical alteration of cell surface properties. A compilation of detailed haematological findings has been published (78).

It must be emphasized that much of this work took place pre-1930 when gum acacia was not adequately characterised by modern analytical techniques, and details of the origins of the specimens used were not given.

2c. Environmentally Contacted Gum Arabic

The first report of sensitivity to environmental acacia appears to be that of Spielman and Baldwin (25) who described a case of acacia sensitivity in a plaster moulder employed in a candy factory. He developed vasomotor rhinitis and bronchial asthma after working for six months in the plant. Direct tests produced a marked reaction to the factory dust containing crude acacia and also to purified acacia. Passive transfer tests were likewise positive.

A similar source of contact with acacia prevails in the printing trade. The gum is used in solution with dextrin, alcohol and water as a drying, or offset, spray for printed material. The spray fills the workrooms with a fine mist, which is unavoidably inhaled unless masks are worn (26). A number of cases of sensitivity to such sprays were reported, beginning with Levin (27) in 1939. His patient developed asthma a few months after a gum arabic spray was introduced in his work and he was subsequently shown to be sensitive to the spray mist and dust. Following this report, Feinberg and Schoenkerman (28) reported another case of bronchial asthma in a printer which was attributed to acacia. Gum arabic and karaya gum both gave positive skin reactions. They also reported a case of sensitivity to acacia

from furniture preparations used by a furniture dealer.

Similar such reports increased in frequency with Bohner et al. (29) in 1941 presenting ten cases of asthma in printers, with exposure periods ranging from two weeks to one year. Other cases were reported by King (30) and by Sprague (31), with the latter case having the interesting feature that four years of exposure to the gum spray were required for sensitisation.

However, the most comprehensive study of asthma in printers (32) gave the results of a unique study within a printing firm which required their workers to attend for examination if they thought they were affected by the gum spray. Asthma was demonstrated in thirty-two workers and a further fourteen printers had symptoms of sensitisation to the spray. The average duration of exposure before asthma developed was almost five years, but two and a half years for six printers who had a past or family history of allergy. In another firm, 19% of the printers had asthma and a further 30% had early symptoms of asthma due to sensitisation to the spray.

Allergic reactions from ingestion of the vegetable gums are known, the most carefully documented series of studies being that of Gelfand (24) in 1949. Ten patients were treated for symptoms including vasomotor rhinitis, gastro-intestinal symptoms, epigastric distress, nausea, belching, flatulence, urticaria, allergic cough, angioedema, diarrhoea, blocked and running nose, sneezing and bronchial asthma. Direct skin tests with gum arabic, gum tragacanth and karaya gum, chemical trials and elimination and positive serological findings confirmed the gums as the causative agents. Elimination of the irritant foods obviated the symptoms. The foods involved were, for example, salad dressings, various sauces, cream cheeses, confectionery, pie fillings and ice cream, all of which were known to contain gum

arabic, gum tragacanth or karaya gum. Cross-sensitivity between the gums was demonstrated. This is apparently the only report in the literature of an undesirable reaction to orally ingested acacia in man.

Allergies from other sources of the other vegetable gums have been well documented - from karaya gum in hair-waving lotions, dental powders, laxatives, emulsified mineral oils, gelatins, diabetic foods and tooth pastes (33 - 36) and from gum tragacanth in tablets (37) and in factory dust (26). This latter report noted that gum tragacanth was a particularly powerful allergen, capable of causing extremely severe reactions.

II. 3. BIOCHEMICAL ASPECTS

3a. Introduction

Although gum polysaccharides may be more or less chemically inert, being hydrocolloids their unusual physical properties render them capable of exerting noticeable effects during their passage *in vivo.* Their use as food additives is of course the result of their particular physical properties (see Chapter I). This part of the review presents a short discussion of the biochemistry of gum arabic, its effect on water, mineral and dietary balance and its action on active ingredients of drugs and on enzymes.

3b. Orally Ingested Gum Arabic

The controversy as to whether gum arabic is a food has previously been mentioned - see page 8. Digestibility has been reported in the guinea-pig with contradicting reports for the rat, while in man, excretion of unmetabolised gum has been reported (4, 7 - 12). No studies on actual gum metabolites or breakdown products have been found, which may point to total digestion and utilisation or to total non-absorption.

3c. Effects on Enzymes, Diet and Drugs

i. Interference with enzymes:

The anti-enzymic activity of some of the hydrocolloids is well known (16) and includes the anti-peptic properties of carrageenan, mucoitin sulphate and chondroitin sulphate.

A recent paper by Bachmann *et al.* (79) has investigated the effects on the function of rat heart and liver mitochondria and on liver mixed function oxidases of various commonly used suspending media and thickening agents. Gum arabic, gum tragacanth, methylcellulose and carboxymethylcellulose were given by gavage as solutions in doses

of 0.2 ml/100g body weight twice daily for four weeks. The dose levels of gum arabic were 40, 80 and 400 mg/kg per day, the number of animals in each group not being given. The primary effect on heart and liver mitochondrial function was a dose-dependent uncoupling of oxidative phosphorylation. The lowest dose led to a significant change in both organs after two weeks, the middle dose in two days for liver, seven days for heart, while the highest dose produced marked uncoupling in both organs after two days. Heart mitochondria partially recovered after one week and liver mitochondria recovered slowly as the experiment progressed. Gum arabic also led to a progressive inhibition of the biphenylhydroxylase system in the hepatic microsomal fraction, the highest dose giving rise to fifty per cent inhibition after only two doses. The middle dose caused twenty per cent inhibition after six days, the lowest dose having no effect. Continuing treatment led to an inhibition of mixed-function oxidases of seventy per cent after four weeks. These effects were considered to be unexpected, but this was not explained. They were assumed to be caused by breakdown products of the gum.

A much earler study by Kodera (80) in 1928 investigated the influence of gum arabic and starch on the cleavage of acetylcholine in blood. The degree of hydrolysis in human serum was found to vary with the degree of envelope formation by gum arabic molecules around albumin particules.

The effect of gum arabic on in vitro ester hydrolysis was also studied by Fodor (81) in 1946. It was found that gum arabic, as an emulsifier, accelerated the cleavage of olive oil by glycerol extracted pancreatin. The effect was dependent on the mode of sample preparation and not on the concentration of gum arabic. It was without

effect on the cleavage of monobutyrin by the same enzyme. It strongly inhibited the saponification of esters of simple alcohols (such as ethyl butyrate) by this enzyme. It also strongly inhibited the cleavage of these simple esters by beef liver juice and by glycerol extracts of honeybee worker maggots. Two separate types of enzymes were thought to be involved: a lipase, which cleaves glycerides, and was not inhibited and an esterase, which cleaves simple esters and was inhibited. The actual inhibitory mechanism was not discussed.

ii. Repercussions on dietary utilisation and intestinal function:

The colloidal character of plant hydrocolloids, such as gum arabic, makes them collect and retain a large proportion of water. Thus, leaving aside their actual metabolism if any, they still do not behave as totally inert substances, but have a tendency to modify the nature and consequences of digestion mechanisms. An excellent discussion by Adrian (16) has shown that water, nitrogen, fat, mineral, vitamin and carbohydrate metabolism is affected by hydrocolloids, including plant gums and mucilages, pectins, alginans and carrageenans, but gum arabic is not mentioned specifically in this paper. General principles of the effects of hydrocolloids are discussed and include increased water intake, increased faecal excretion of water (and with this, a suggested loss of hydrosoluble nutrients such as minerals, vitamins and nitrogenous products), decreased intestinal assimilation of water and regulation of intestinal function, such as prevention of constipation. The considerable research already carried out into intestinal ulceration in herbivores as a consequence of carrageenan assimilation is also discussed. A small number of other studies have been done which include gum arabic and these are discussed

below.

O'Dell et al.(7), in a comprehensive study on diet composition and mineral balance in guinea-pigs, noted that animals fed 15% gum arabic showed a 10% higher absorption of all cations than with diets containing 15% celluflour as the bulk. This was the study in which 90% digestibility of the gum was claimed (see page 9) and this was assumed to be the reason for the observed higher cation absorption.

A short review published recently on carbohydrates and mineral nutrition (13b) mentions that there is no unifying concept that can adequately explain or predict the effects of carbohydrates on mineral absorption under all conditions. The substitution of fibre (assumed indigestible carbohydrates such as cellulose, hemicelluloses, pectins and gums) for other carbohydrates was said to generally result in decreased utilisation of the trace elements iron, zinc and copper. The effects of increased fibre on calcium utilisation were reported as inconsistent, but as mostly indicating depressed calcium utilisation. Again, unfortunately, this review did not include gum arabic, a clear case for justifying continued research.

Guar gum, carrageenan, carboxymethylcellulose and particularly pectins are known for their hypocholesterolemic effect, that is, lowering of serum cholesterol levels. The mechanism has been strongly indicated to involve binding of bile acids, thereby preventing their reabsorption from the intestine (13c). This increases bile acid excretion and therefore cholesterol synthesis is reduced. Gum arabic has been investigated for this property, but results have been inconsistent.

Tsai et al. in 1976 (82) investigated the effect of various dietary fibres on serum and tissue cholesterol levels in rats. Three

trials were carried out with gum arabic on unspecified numbers of animals: 1) using a casein-sucrose diet containing 15% soybean oil, 0.5% cholesterol and 7% gum for thirty-two days, 2) using a skimmed milk - wheat flour diet with or without 0.5% cholesterol and with 7% gum for thirty-five days, and 3) as with trial 2) but with 0.2% cholesterol and 5% gum for sixteen weeks. The effect of gum arabic was not consistent. Except at fifty-six days in trial 3) when a slight increase was observed, it had no effect on serum cholesterol levels. Liver cholesterol levels were increased in trials 1) and 2) (cholesterol free diet), unchanged in trial 3) and reduced in trial 2) (cholesterol supplemented diet). The discrepancy was assumed due to "dietary interaction" and future investigation of this was promised. This inconsistency supports the results of Kiriyama et al. (83) in 1969, who observed a "moderate" plasma cholesterol depression in a group of five rats fed 5% gum arabic in a hypercholesterolemic (1%) diet for five days. They emphasised the need for confirmation.

Kelley and Tsai (84) restudied the phenomenon of cholesterol absorption, synthesis and turnover in rats and reported as follows:-

a) Absorption study. Gum arabic (along with pectin and agar) reduced the dietary ^{14}C-cholesterol absorption by 17% and reduced the level of absorbed ^{14}C-cholesterol in liver, though only pectin reduced the radioactivity of ^{14}C-cholesterol in serum. The diets contained 0.2% cholesterol and 5% of the gum and were meal fed to ten rats for fourteen days.

b) Cholesterol biosynthesis study, as estimated by determining the activity of labelled digitonin-precipitable ^{14}C-sterols biosynthesised from labelled glucose. Gum arabic and pectin increased digitonin-precipitable ^{14}C-sterols in the carcass and gum arabic also increased

the activity in the intestine. The whole body (total) digitonin-precipitable ^{14}C-sterols were significantly greater in the pectin and gum arabic groups than in the controls. Gum arabic also increased the total activity of ^{14}C-long chain fatty acids by 22%. In rats fed a "cholesterol free" diet, both pectin and gum arabic resulted in a decrease in serum digitonin-precipitable ^{14}C-sterols and in liver long chain fatty acids. There was no difference in carcass or total digitonin-precipitable ^{14}C-sterols, but gum arabic feeding increased the carcass ^{14}C-long chain fatty acids. The diets and feeding periods were the same as in a) but using eight animals per group.

c) Turnover study, as estimated by tissue analysis two, four, eight and sixteen days after injection of labelled cholesterol. This followed fourteen days feeding on the experimental diets - eight rats were fed the same cholesterol supplemented diet as in a) and eight others received a cholesterol-free diet. In the animals fed the cholesterol supplemented diet, the activity of ^{14}C-cholesterol in the liver was significantly decreased by pectin at four, eight and sixteen days, but gum arabic and agar did not have an effect on the radioactivity of cholesterol in liver, carcass or whole body. In the experiment with the cholesterol-free diet, neither pectin nor gum arabic affected ^{14}C-cholesterol radioactivity in the liver or carcass at ten days, but pectin significantly decreased the activity in serum and liver at twenty days. Gum arabic had no such effect. Thus, compared with pectin, gum arabic is not as potent a hypocholesterolemic agent, does not have a strong effect on cholesterol absorption and does not increase cholesterol turnover. When rats were fed the cholesterol-free diet, the differences between pectin and gum arabic became less distinct.

Earlier, in 1957, Lin *et al.* (85) investigated the effect of dietary pectin, protopectin and gum arabic on cholesterol excretion in rats. A group of ten rats were each given a diet containing approximately 2.5% gum arabic (500 mg per rat per day) and 0.25% cholesterol (50mg per rat per day) for six days. The faeces collected during the gum arabic feeding were more bulky, presumed due to the presence of unaltered gum arabic. The addition of the gum to the diet brought about a slight increase in the excretion of total lipid, nonsaponifiable material and sterols that were analysed gravimetrically and colorimetrically, i.e. indicating a decrease in absorption of dietary cholesterol.

iii. Interference with drugs:

Various gums are used in the preparation of medicinal tablets as diluents, binders, disintegrants and lubricants. The view has been expressed e.g. by Asker *et al.* (86), that these materials may not be truly inert and may affect the stability and availability of drugs incorporated in the preparation, depending on the method of tablet manufacture.

Asker's paper investigates the influence of formulation and processing factors on the stability of tetracycline hydrochloride and chloramphenicol, dealing specifically with the effects of the tablet binders. The tablets were made by wet granulation techniques and by partial granulation using acacia, Luviskol K90 (a special grade of polyvinyl-pyrrolidone), polyethylene glycol 6000 and sodium carboxymethylcellulose as binders. The antibiotic activities were determined after four months storage in humid conditions.

It was found that, regardless of the method of preparation of the tablets, any of the binders studied elicited a decrease in the

anti-microbial activity of either tetracycline hydrochloride or chloramphenicol. The effect on tetracycline hydrochloride was more pronounced than that on chloramphenicol, due possibly to the greater number of functional groups in the tetracycline molecule capable of interaction and the susceptibility of tetracycline to oxidation and metal complexation. The decreases in activity generally followed a first order rate, being higher during the first month of storage. This change in rate is not unexpected as the drug is initially present in excess as compared to the adjuvant. Some loss of activity in the subsequent months would arise from normal drug degradation and interaction with degradation products.

The method of wet granulation was found to produce a higher loss of activity of either drug than that obtained by partial granulation. Again, this was not surprising since, in wet granulation, both drug and binder are in a more direct contact within the granules and are simultaneously subjected to the action of water and heat during processing.

Possible mechanisms of activity loss were discussed. It was shown that acacia contained the oxidising enzyme, peroxidase, as detected by a deep blue colour when treated with two per cent alcoholic solution of guaiacum resin. The decrease in the antimicrobial activity of tetracycline hydrochloride may be due to the oxidising action of this enzyme and to an interaction between the positively charged amino groups of the antibiotic and the negatively charged polysaccharide molecules of the acacia.

With partial granulation, acacia bound tetracycline hydrochloride showed an activity loss of 40% and chloramphenicol a loss of 23%. For wet granulation, the losses were 30% and 27%. Losses with the other binders were generally slightly higher.

II. 4. CONCLUSIONS

As much of the available literature as possible on the toxicity of the naturally occurring acidic polysaccharide, gum arabic, has been reviewed.

Although the gum finds extensive use as a food additive, toxicity feeding studies are very sparse. Only a few short-term studies involving a very limited number of animals have been reported; no toxic effects were found. These studies however did suggest almost complete digestibility of the gum. One case of an allergic reaction in man to orally ingested gum arabic was reported, but was confined to one sensitive individual. The gum was reported to be toxic, at high levels, to pregnant animals of one species.

A further interesting effect is known, namely that gum arabic, in common with some other polysaccharides known as "dietary fibre", exerts a hypocholesterolemic action as a result of binding of bile acids in the intestine. This may have a clinical application for cardiovascular disease and deserves further investigation. As an acidic polysaccharide, the gum was also expected to affect mineral availability and absorption in the intestine, though the exact effect has not been demonstrated.

No long-term studies on animals were found and no human studies of any kind have been reported on oral toxicity of the gum.

Clearly, much more research is required before the action and fate of this gum when ingested in food are thoroughly known and its safety as a foodstuff ingredient can be evaluated. The great need is for well planned and careful investigation of any toxicity,

interference, breakdown, absorption, metabolism or accumulation of the gum as a result of short-term feeding, periodic feeding and especially long-term continuous intake. The possibility of sudden toxic thresholds and the slow development of a gradual toxic build up require to be adequately investigated. The effect on the unborn, the effect of unique impurities, especially microbial contaminants, conversion to toxic metabolites and interaction with food and other additives must all be carefully studied, particularly in man.

It must be emphasised that restraint must be exercised when extrapolating animal data to man. Allowance must be made for life-time differences, differences in ability to assimilate and cope with different dose levels of foreign compounds as a result of body weight and size differences and of course, physiologic and metabolic differences. The significance of such experiments must be carefully assessed in the light of estimated consumer exposure data, consumption habits and "safety margins".

A large number of papers were published pre 1940 on the effects and merits of gum arabic solution, when injected intra-venously, for various clinical conditions. From a toxicological point of view, the gum was shown not to be inert, but to be deposited mainly in liver, spleen and kidney, leading to liver damage and function impairment and to harmful haematological effects.

Environmental contact with the gum has shown it to be an allergen in a proportion of individuals. This represents an industrial hazard for workers, for example in mills, food and confectionery factories and in other works using gum powder, solutions or sprays such as are used in the printing industry. This effect is not considered to affect a large enough proportion of the population to justify restricting the material, though epidemiological surveys might be desirable to prove this.

The main concern, rightly so, of organisations such as the U.S. Food and Drug Association, the U.N. Food and Agriculture Organisation and the World Health Organisation is the safety of the gum as a food additive for direct human consumption. Sound, adequate scientific findings on this are lacking and therefore long human experience, free from attributable toxic effects, has been used as a guide. At present, no limited Acceptable Daily Intake (ADI) has therefore been laid down, other than the quantities required in "good manufacturing practice". Thus the need is strongly presented for future careful and specialised metabolism and toxicity research on this compound, most particularly in man. That the gum is safe is still simply a non-scientific assumption, but to be considered in the light of the truth that there are inherent risks in anything that man eats in order to live, as gently hinted at by Paracelcus (87).

The Federation of American Societies for Experimental Biology has a Select Committee on Generally Recognised as Safe (GRAS) food substances and this committee concludes that, "There is no evidence in the available information on gum arabic that demonstrates a hazard to the public when it is used at levels that are now current and in the manner now practised. However, it is not possible to determine, without additional data, whether a significant increase in consumption would consitute a dietary hazard" (1, 17).

> "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy."
>
> PARACELCUS (1493 - 1541).

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CHAPTER III

EXPERIMENTAL METHODS

III. 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.1 single-pan balance, having an accuracy of ± 0.1mg.

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

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

Reductions in volume were carried out with a rotary evaporator at temperatures below 40°C.

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

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

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

Methoxyl contents were determined by a vapour phase infrared method (1,2); a calibration curve was based on known weights of methyl iodide. Infrared spectroscopy was carried out with a Perkin-Elmer 137 spectrophotometer.

Equivalent weight determinations on exhaustively electrodialysed polysaccharides were carried out by direct titration with standard

sodium hydroxide solution (ca. 0.01N).

Uronic acid contents were calculated from the equivalent weights as (1·7600/E.W.), i.e. values are expressed as uronic anhydride.

Quantitative estimations of sugars.

1. Sugars were separated from hydrolysates by chromatography in solvent (b) on Whatman 3MM papers. After elution from the paper in boiling water, sugars were estimated colourimetrically by the phenol-sulphuric acid method (3). The optical density was read on a Unicam SP 1300 spectrophotometer using filter 2. Calibration curves were obtained from known weights of sugars.

2. Sugars were determined by gas-liquid chromatography of their alditol acetates on a 3% ECNSS-M column at 190°C (4). The hydrolysate was reduced with sodium borohydride (ca. 10mg) 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 co-distilled several times with methanol to remove borate as volatile methyl borate. The dry residue was heated under reflux for four hours with a pyridine/acetic anhydride (1/1, v/v) mixture. The solution was then cooled and injected into the chromatograph.

III. 2. PHYSICAL METHODS

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

Viscosity determinations were carried out in M-sodium chloride solution in an Ubbelohde suspended-level dilution viscometer at 25.0 ± 0.1°C. Solutions were filtered carefully before additions were made

to the viscometer. Flow times were measured to within 0.1 second by means of a stop watch. The isoionic dilution technique was used; a solution of the gum (6ml, 1-2%) was placed in the viscometer and the flow time measured. Flow times were also obtained for successive dilutions with M-sodium chloride solutions (four additions of 2ml each). Since preliminary experiments had indicated that any loss of gum from M-sodium chloride solution on filtering was negligible, concentration values were estimated from the dry weight of gum dissolved in a known volume.

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

$$[\eta] = \lim_{c \to o} \frac{\eta \, sp}{c} = \lim_{c \to o} \frac{t-to}{cto}$$

where c is the concentration of gum (g/ml) and t_o and t are the flow times (seconds) for solvent and solution respectively. Extrapolation of the linear plot of $\frac{t-t_o}{ct_o}$ against c to t = 0 gives $[\eta]$.

Light scattering measurements, for molecular weight determinations, were carried out at 27 ± 0.5°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 as solvent, gum solutions were accurately prepared (0.1 - 0.3g in 50 ml). Dilutions of this solution were made; the molecular weight was calculated as an average of three of these solutions. The solutions were clarified and made dust-free by passage through filters of average pore size 0.80 μm and then through average pore size 0.22 μm (Millipore Ltd., Bedford, Mass., U.S.A.), using a

stainless steel filter holder attached to a 20 ml syringe. Concentrations of gum solutions were assumed to be unaltered by ultrafiltration (5).

For each concentration, the intensity of scattered light at various angles between 30° and 150° was recorded and corrected, and corrected scale readings I_{θ} for angle θ were calculated (6) from the equation:

$$I_{\theta} = \frac{(Isoln - Isol)\sin\theta}{1 + \cos^{2}\theta}$$

where I_{soln} and I_{sol} are the scale readings for polymer solution and solvent respectively. The reciprocal corrected scale reading $1/I_{\theta}$ is plotted against $\sin^{2}\theta/2$. Extrapolation of the linear portion of this graph to $\theta = 0$ gives a value for $[1/I_{\theta}]_{\theta=0}$. The downward curvature of these graphs at low angles is thought to be caused by dust particles suspended in solution (7).

Molecular weights are then calculated 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 (546 nm.)

c = concentration in g/ml

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 <u>Acacia</u> gums, the equation is simplified to:

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

III. 3. CHEMICAL METHODS

Small scale polysaccharide hydrolyses were carried out overnight with N-sulphuric acid on a boiling water bath, unless otherwise stated. Hydrolysates were neutralised with barium carbonate, filtered, deionised with Amberlite IR-120(H) resin and concentrated to a syrup 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 (2ml) and sodium hydroxide (2ml, 30% w/v) were added dropwise with stirring to the polysaccharide (100 - 500mg) in water (10ml) over a period of one hour. Acetone (5ml) was added to the reaction mixture and six further additions of dimethyl sulphate (12ml) and sodium hydroxide (17ml) 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 vigorously through the solution. After cooling, the reaction mixture was neutralised with 4N-sulphuric acid and made slightly acid (pH 4); 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°C, 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 six hours in the dark in a dry flask fitted with a water condenser and a calcium chloride tube. The mixture was cooled and filtered through sintered glass and the residue extracted six times with hot chloroform (ca. 50 ml). The combined filtrate and extracts were reduced in volume and any dissolved silver ions were removed by passing hydrogen sulphide through the solution and refiltering. After concentration to small volume, the syrup was poured into light petroleum (b.p. 60 - 80°C, ca. 400 ml) with stirring. After filtration and drying, the precipitated methylated polysaccharide was isolated as a white amorphous powder.

Small scale oligosaccharide methylations - the Kuhn method (11,12): The oligosaccharide (0.5 - 2.0 mg) was shaken with methyl iodide (0.2 ml), N,N - dimethylformamide (0.2 ml) and silver oxide (0.2 g) at room temperature in the dark for 18 hours. The mixture was filtered and the residue washed with chloroform. The combined filtrate and washings were concentrated to a syrup on a rotary evaporator.

Methanolyses were carried out under reflux for six hours with methanolic 5% hydrogen chloride. Solutions were cooled and were taken to dryness in a vacuum desiccator filled with calcium chloride and containing a few pellets of potassium hydroxide. The residue was taken up in chloroform and concentrated to a small volume.

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

a) Reduction of periodate. The amount of periodate reduced by a polysaccharide was estimated by back-titration of excess periodate.

Excess potassium iodide (as 10% solution) was added to a portion (1 ml) of the polysaccharide/periodate solution and the iodine liberated was titrated, after the addition of sodium bicarbonate (200 mg), with standard sodium arsenite solution (ca. 0.0125M) using "Thyodene" as indicator (13).

b) The formic acid released was estimated titrimetrically (14) with standard sodium hydroxide (ca. 0.1M) for portions (1 ml) of the solution. Methyl red was used as the indicator.

III. 4. CHROMATOGRAPHIC SEPARATIONS

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

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

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

(c) ethanol, phosphoric acid (0.1N), butan-1-ol (10:5:1) (15)

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

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

Before using solvent (c), 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°C for ca. 3 minutes.

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

R_f values of sugars refer to distances moved relative to that of the solvent front; R_{gal} values of sugars refer to distances moved relative to that of D-galactose and R_g values of O-methyl sugars refer to distances moved relative to that of 2,3,4,6-tetra-O-methyl-D-glucose.

Gas liquid partition chromatography (g.l.c.) of mixtures of O-methyl sugars was carried out with a Pye Series 104 chromatograph fitted with a flame ionisation detector and using nitrogen as the carrier gas, at a flow rate of 60 ml/min. The column (200 x 0.3 cm) was packed with 15% (w/w) polyethylene glycol adipate on Universal B (phase sep.) and used at 170°C.

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

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CHAPTER IV

AN ANALYTICAL STUDY OF GUM EXUDATES FROM THE GENUS *PROSOPIS*

IV. 1. INTRODUCTION

The genus Prosopis Linnaeus is an important gum-producing genus, belonging to the family Leguminosae, subfamily Mimosoideae. About forty-four species, distributed in semi-arid areas of South-west Asia, Africa and predominantly in America from Western North America to Patagonia (1) have been described. The genus includes the mesquites and screw-beans, plants which rank high among the trees and shrubs that give unique character to the desert. These species are important as range food plants having highly nutritious seeds and shoots, as bee plants and as timber, being much used for fencing, fuel and for carving (2). Some species, because of their tremendous power of invasion, have become nuisances to ranchers and are rigorously controlled.

Botanical classification of the genus has been in almost constant revision and dispute for over a century, particularly of those species from North America where much intergrading makes identification difficult. Bentham (1875) treated Prosopis as a polymorphic genus with several sections (3) and took active issue with Englemann and Gray (1845) (4), who considered that the U.S. species included two genera. Less than unanimous viewpoints have continued. In 1928, Britton and Rose (5) divided the North American Prosopis into three genera and this position was reiterated by Hutchinson in 1964 (6). On the other hand, Burkart has taken up and expanded the Bentham delimitation (7), a stance assumed by most U.S. authors (8). Recognised as an expert on the Leguminosae, Burkart, in his latest revision of 1976 (1), commented that karyological, palynological and chemotaxonomic researches did not support the radical subdivision into three or four genera, a subdivision based only on purely vegetative characters. Bentham's original classification of 1875 and

Burkart's revision of 1976 are illustrated in Tables IV.1 and IV.2, the latter being assumed in this present study.

Table IV.1

BENTHAM'S CLASSIFICATION OF THE GENUS *PROSOPIS*, 1875

Prosopis Linnaeus

Section 1	Adenopis	SW. Asia
Section 2	Anonychia	Africa
Section 3	Algarobia	N. and S. America
Section 4	Strombocarpa	N. and S. America

The classification of the North American mesquites (*Prosopis juliflora*, *P. glandulosa* and their varieties) is particularly complex since there are so many intergrades in many localities. Botanists have alternated between treating them as one polymorphic species, or segregating several of the variants as specific entities.

Bentham in 1875 (3) included nearly all the plants of *Prosopis* section Algarobia in one polymorphic species, *P. juliflora*. In 1928, Britton and Rose (5) recognised the entities *P. glandulosa* and *P. velutina* and Burkart (1940) recognised a number of species (7), but Benson in 1941 (2) recognised only one species in the United States, *P. juliflora* with three varieties - *glandulosa*, *velutina* and *torreyana*. Standley, 1926 (9) felt that *Prosopis juliflora* represented the N. American phase of the S. American *P. chilensis*, i.e. conspecific, but Benson (2), Johnston (10) and Burkart (1) reject this viewpoint.

Johnston in 1962 (10) reversed the trend of Benson, affirming Burkart's stance (7) that the N. American plants were distinct species. He recognised six species of mesquites and supported Burkart's theory of hybridisation (7) to explain indistinct morphic boundaries.

Table IV. 2

BURKART'S REVISION OF THE GENUS PROSOPIS, 1976

Prosopis Linnaeus emend. Burkart

Taxon	Distribution	Species
I. Section Prosopis (syn. sect. Adenopis)		
	SW. Asia, N. Africa	3 spp.
II. Section Anonychium		
	Tropical Africa	1 sp.
III. Section Strombocarpa (syn. Spirolobium)		
Ser. Strombocarpae		
	SW. United States and Mexico to Chile and Argentina	7 spp.
Ser. Cavenicarpae		
	S. America	2 spp.
IV. Section Monilicarpa		
	W. Argentina	1 sp.
V. Section Algarobia (syn. Neltuma, Algarobia)		
Ser. Sericanthae		
	Argentina, Paraguay	2 spp.
Ser. Ruscifoliae		
	Argentina, Paraguay	4 spp.
Ser. Denudantes		
	SW. Argentina	4 spp.
Ser. Humiles		
	Central Argentina, Paraguay	2 spp.
Ser. Pallidae		
	Argentina to Mexico	7 spp.
Ser. Chilenses		
	SW. United States and Mexico to Chile, Argentina and Uruguay	11 spp.

A total of forty-four species and twenty-seven varieties described (1), in Prosopis.

In 1972, Isely (8) followed Johnston (10) and under this interpretation, the traditional *Prosopis juliflora* does not occur in the United States. The principal U.S. mesquite is then *P. glandulosa*, its range being interrupted only by a zone of *P. velutina* in Arizona and local *P. laevigata* in Nueces County, Texas. Isely also follows Benson (2) and Johnston (10) in distinguishing an Eastern and Western segment of *P. glandulosa* - variety *glandulosa* and variety *torreyana* respectively.

Johnston, Benson and Isely all refer to the observed problem of mixing between *P. glandulosa* var. *torreyana* and *P. velutina* and Isely has designated intermediates approximately as follows:

P. velutina towards *torreyana* - 1. *velutina* leaflet spacing and size, but reduced pubescence; 2. *velutina* leaflet size and moderate pubescence, but *torreyana* spacing.

P.glandulosa var. *torreyana* towards *velutina* - *torreyana* leaflet spacing and size but mildly pubescent.

In his 1976 classification (1), Burkart establishes the pattern of Isely and Johnston and describes eleven species, sixteen varieties and one forma in the Series Chilenses, illustrated in Table IV.3.

The chromosomes of mesquite have been counted many times, but a haze of uncertainty remains. A critical correlation of taxonomic hypotheses and genome analyses remains to be accomplished (8). Thus the Section Algarobia species are a challenge for the new refined taxonomic techniques of cytogenetical, ecological, biometric and phytochemical analysis.

In the light of such inherent difficulties in studying the taxonomy of this genus, the chemical studies on *Prosopis* gum exudates presented in this chapter are of interest both to the botanist and the chemist. The gums studied are mainly from species of the N. American

Table IV.3

BURKART'S CLASSIFICATION OF THE MESQUITES, PROSOPIS SECT. ALGAROBIA SER. CHILENSES[a].

34. P. chilensis
    var. chilensis
    var. riojana
    var. catamarcana

35. P. juliflora
    var. juliflora
    var. inermis
    var. horrida

36. P. nigra
    var. nigra
    var. ragonesei
    var. longispina

37. P. caldenia

38. P. laevigata
    var. laevigata
    var. andicola

39. P. flexuosa
    forma subinermis

40. P. glandulosa
    var. glandulosa
    var. torreyana
    var. prostrata

41. P. alpataco

42. P. alba
    var. alba
    var. panta

43. P. velutina

44. P. pugionata

a Species numbering after Burkart (1).

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mesquites and the work is believed the first study of this genus to use botanically certified samples. Previous chemical studies on "mesquite gum" did not mention the botanical authenticity of the sample used (11 - 13).

A number of isolated chemical studies have been published on various *Prosopis* alkaloid and tannin extracts, but the most useful appear to be those of Gianinetto *et al.* (14), Bragg *et al.*(15) and Carman (16). Gianinetto *et al.* reported a considerable variety in the numbers of flavonoids from various S. American species within section Algarobia, while Bragg *et al.* reported a comprehensive study of flavonoids within the N. American *P. juliflora* complex. They noted that, in contrast to their considerable morphological variation, all members of the *P. juliflora* complex exhibited similar flavonoid patterns. Carman's findings are in agreement with those of Bragg *et al.*, for the N. American species and Gianinetto *et al.* for the other S. American species, in that the latter show different flavonoid contents. It is of interest to note that these results substantiate the integrity of those taxa as species - Burkart (17).

A detailed analytical study of the gum exudates from six *Prosopis* species, Section Algarobia, Series Chilenses, namely *P. juliflora*, *P. glandulosa*, *P. glandulosa* var. *glandulosa*, *P. glandulosa* var. *torreyana*, *P. alba*, *P. velutina* and Honey mesquite, plus a sample of commercial mesquite gum, is now presented.

IV. 2. ORIGIN OF GUM SPECIMENS

Commercial gum mesquite (Prosopis species) was kindly provided in 1964 by Mr. George Meer, Meer Corporation, New York.

Gum from Prosopis juliflora (Swartz) DC. was received from Dr. X. A. Dominguez, Chemistry Department, Technological Institute of Monterrey, Mexico, in January, 1970.

Gum from Prosopis glandulosa Torrey was collected on July 1st, 1974, at Puente la Poza, 12 miles south of Hermosillo, Sonora, Mexico. A voucher specimen (Chiang and Wendt 347) is deposited at the University of Texas Herbarium.

Gum from Prosopis glandulosa Torrey var. torreyana (Benson) Johnston (tree number 002) and from Prosopis alba Grisebach (tree number 013) was sent by Dr. Peter Felker, University of California, Riverside, California on August 15th, 1978.

Two samples of gum from Prosopis glandulosa Torrey var. glandulosa (honey mesquite) were collected by Dr. R.E. Meyer, U.S. Dept. of Agriculture Research Service, Texas Agricultural and Mechanical University, College Station, Texas, on July 27th, 1970 at College Station. The first sample (called Honey mesquite gum in this discussion) was gum recovered after the bark had been stripped off the tree and the second sample (referred to by its botanical name) was recovered in the normal way from a tree which had been sprayed with defoliant.

Gum from Prosopis velutina Wooton (velvet mesquite) was sent by Professor P.R. Morey, Texas Technological University on April 23rd, 1979.

IV. 3. PURIFICATION OF SAMPLES

The samples (4 - 15g), all very clean, some almost colourless, some dark amber, were dissolved in distilled water (approximately 10% solution) for two days. Dissolution in all cases was rapid and complete, yielding relatively non-viscous solutions. The solutions were filtered through muslin to remove small pieces of bark etc., then through Whatman No. 1 and No. 42 filter papers, and dialysed against running tap water for forty-eight hours. After refiltering, the polysaccharides were obtained as the freeze-dried products. The gum from Honey mesquite was dissolved by filling the collecting-bottle with distilled water (250 ml). The commercial mesquite gum was not purified as its botanical origin was probably uncertain.

IV. 4. RESULTS

Apart from the gums from the Honey mesquite, P. velutina and P. glandulosa var. glandulosa, samples of the crude gums were also analysed and the data obtained for these are shown in Table IV. 4. The results for the purified samples are shown in Table IV. 5.

Hydrolysis of the samples with N-sulphuric acid followed by chromatographic examination of the hydrolysates in solvents (a) and (b) showed the presence of galactose, arabinose and small amounts of rhamnose. The aldobiuronic acid, 6 - O - (β - D - glucopyranosyluronic acid) - D - galactose was detected in all of the samples [R_{gal} 0.22 in solvent (a), 0.19 in solvent (b)] and 4 - O - (4 - O - methyl - α - D - glucopyranosyluronic acid) - D - galactose [R_{gal} 0.57 in solvent (a), 0.47 in solvent (b)] was detected in all of the samples except P. juliflora. These two aldobiuronic acids have been commonly observed in Acacia gum exudates (18). In addition, a third

TABLE IV.4

ANALYTICAL DATA FOR CRUDE GUM POLYSACCHARIDES FROM

PROSOPIS SPECIES, SECTION ALGAROBIA, SERIES CHILENSES

	"mesquite gum"	*P. juliflora*	*P. glandulosa*	*P. glandulosa* var. *torreyana*	*P. alba*
Moisture, %	12.7	12.9	13.4	14.0	13.9
Ash, %[a]	3.3	3.3	2.5	2.8	2.8
Nitrogen, %[a]	0.74	0.17	0.67	0.38	0.89
Hence protein, % (N x 6.25)[a]	4.6	1.1	4.2	2.4	5.6
Methoxyl, %[b]	2.5	0.63	2.5	1.8	2.0
$[\alpha]_D$ in water, (degrees)[b]	+72	-40	+94	+98	+73
Intrinsic viscosity, $[\eta]$, ml/g [a]	13	15	9.1	11	10
Molecular weight, ($\bar{M}_w \times 10^5$) [a]	3.0	2.5	2.4	3.9	4.5
Equivalent weight[b]	1075	970	1134	984	809
Hence uronic anhydride, %[b,c]	16.4	18.1	15.5	17.9	21.8
Sugar composition after hydrolysis, %					
4-*O*-Methylglucuronic acid[d]	15.0	3.8	15.0	10.8	12.0
Glucuronic acid	1.4	14.3	0.5	7.1	9.8
Galactose	43	47	39	39	47
Arabinose	36	24	46	43	28
Rhamnose	5	12	trace	trace	3

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.

TABLE IV.5

ANALYTICAL DATA FOR PURIFIED GUM POLYSACCHARIDES FROM PROSOPIS SPECIES, SECTION ALGAROBIA, SERIES CHILENSES

	Honey mesquite	*P. glandulosa*	*P. glandulosa* var. *torreyana*	*P. glandulosa* var. *glandulosa*
Moisture, %	12.3	8.6	10.7	4.5
Ash, %[a]	2.5	2.5	2.6	2.2
Nitrogen, %[a]	0.33	0.58	0.37	0.54
Hence protein, % (N x 6.25)[a]	2.1	3.6	2.3	3.4
Methoxyl, %[b]	1.5	1.9	2.4	1.1
$[\alpha]_D$ in water, (degrees)[b]	+73	+61	+74	+68
Intrinsic viscosity, $[\eta]$, ml/g [a]	12	10	10	10
Molecular weight, (M_w x 10^5)[a]	6.8	2.5	4.5	1.8
Equivalent weight[b]	1101	1134	1107	1371
Hence uronic anhydride, %[b,c]	16.0	15.5	15.9	12.8
Sugar composition after hydrolysis, %				
4-*O*-Methylglucuronic acid[d]	9.0	11.4	14.4	6.7
Glucuronic acid	7.0	4.1	1.5	6.1
Galactose	43	41	41	41
Arabinose	41	40	38	42
Rhamnose	trace	3	5	4

Notes a - d as in Table IV.4.

TABLE IV.5 (Continued)

ANALYTICAL DATA FOR PURIFIED GUM POLYSACCHARIDES FROM

PROSOPIS SPECIES, SECTION ALGAROBIA, SERIES CHILENSES

	P. juliflora	*P. alba*	*P. velutina*
Moisture, %	10.9	8.5	1.9
Ash, %[a]	3.4	2.8	3.3
Nitrogen, %[a]	0.19	0.95	0.35
Hence protein, % (N x 6.25)[a]	1.2	5.9	2.2
Methoxyl, %[b]	n.d.	2.0	1.5
$[\alpha]_D$ in water, (degrees)[b]	-36	+70	+71
Intrinsic viscosity, $[\eta]$, ml/g [a]	14	11	4.3
Molecular weight, ($\bar{M}_w \times 10^5$)[a]	3.1	4.6	0.83
Equivalent weight[b]	1004	1093	1206
Hence uronic anhydride, %[b,c]	17.5	16.1	14.6
Sugar composition after hydrolysis, %			
4-*O*-Methylglucuronic acid[d]	n.d.	12.0	9.1
Glucuronic acid	n.d.	4.1	5.5
Galactose	50	40	43
Arabinose	21	40	38
Rhamnose	11	4	4

Notes a - d as in Table IV.4. n.d. not determined

aldobiuronic acid, 6 - O - (4 - O - methyl - β - D - glucopyranosyluronic acid) - D - galactose [R_{gal} 0.42 in solvent (a)] was detected in P. glandulosa var. glandulosa and in the commercial mesquite.

The presence of the acidic sugar, 4 - O - methyl glucuronic acid in a N - hydrolysate has been reported recently by Bell (19) and Gill (20). The presence of this sugar is unusual in a N - hydrolysate; 2 N - hydrolysis with sulphuric acid is normally required to break uronosyl linkages. Its presence in N - hydrolysates is further reported here, being detected in all of the samples except P. juliflora, R_{gal} 2.28 in solvent (a).

The data for the crude and purified materials are similar with a general slight drop in specific rotation and slight rise in molecular weight as low molecular weight materials are eliminated during purification.

IV. 5. DISCUSSION

The general physical and chemical properties of the Prosopis gum exudates studied are typical of the majority of plant gums investigated so far. In terms of sugar composition, they are less complex than gums from the genera Combretum, Terminalia, Anacardium (19) and Grevillea (21), which additionally contain the sugars mannose, xylose and galacturonic acid. The samples are in fact similar to many Acacia exudates, which is not altogether unexpected as both Prosopis and Acacia are classified botanically in the same subfamily, Mimosoideae, though in different tribes.

The samples studied are all from the same taxonomic grouping, i.e. Series Chilenses in Section Algarobia of Prosopis (1) and the analytical parameters are remarkably similar, readily supporting this grouping.

The purified gums are characterised by a high methoxyl content

(1.1 - 2.4%), a high positive specific rotation (+61° - +74°) and by intermediate values of viscosity ($[\eta] = 9.5 - 12.4$), molecular weight ($0.83 - 6.8 \times 10^5$), nitrogen (0.33 - 0.95%) and acidity (12.8 - 17.5% U.A.). The gums also contain a significant proportion of arabinose (ca. 40%) and low amounts of rhamnose (< 5%).

The gum from P. juliflora however, is immediately seen to be highly anomalous when compared with the other exudates. It has a lower methoxyl content (0.63%), an almost opposite specific rotation (-36°), noticeably decreased arabinose (21%) and nitrogen (0.19%) contents and a significant proportion of rhamnose (11%). The viscosity is also slightly increased, though the molecular weight and acidity are comparable. This may indicate a different structural arrangement on the periphery of the P. juliflora gum molecule to accommodate the rhamnose. Structural studies on rhamnose-containing Acacia gum exudates have shown the rhamnose generally to be attached to end-group, i.e. peripheral uronic acid residues (22), an arrangement considered necessary for good emulsifying properties (23).

As expected from a lower methoxyl content, no methyl-substituted aldobiuronic acids were detected in the small-scale hydrolysate of P. juliflora gum, whereas 4 - O - (4 - O - methyl - α - D - glucopyranosyluronic acid) - D - galactose was detected in all of the other exudates. 6 - O - (4 - O - methyl - β - D - glucopyranosyluronic acid) - D - galactose was detected in the commercial sample and in the gum from P. glandulosa var. glandulosa. The non-substituted aldobiuronic acid, 6 - O - (β - D - glucopyranosyluronic acid) - D - galactose was noticed in all of the samples.

P. juliflora is regarded as being the usual source of the commercially available mesquite gum, but the commercial sample analysed

in this study, however, is not comparable with P. juliflora gum. The commercial sample, with its low rhamnose content, higher arabinose content and positive specific rotation, is in fact almost identical to the other Series Chilenses gums studied.

The methoxyl contents of the gums reported in this study are high. The value for P. glandulosa var. torreyana gum and for the commercial sample (2.5%) just exceeds the value for Acacia giraffae gum (2.4%), which for a long time was regarded as the highest (24). A number of other Acacia gums, Series Phyllodineae, subseries Juliflorae have recently been shown (20) to have comparable and higher methoxyl contents, e.g. A. leptostachya (2.2%), A. kempeana (2.5%) and A. microneura (3.4%).

The range of nitrogen contents found here is well within the range commonly given by many gum exudates, e.g. 0.02 - 1.66% in the Acacia genus (25).

The intermediate values of molecular weight and acidity are similar to those found for species of Acacia in Series Vulgares, which includes A. senegal, the source of the commercially valuable gum arabic (26). The molecular weight of P. velutina gum (the velvet mesquite) is slightly less than the other gums in the group, as is its viscosity.

The gums are relatively non-viscous, with values almost too low to be of use commercially - good quality gum arabic has an intrinsic viscosity of about 17 - 20 ml/g.

The arabinose and rhamnose contents of ca. 40% and 4% respectively are noticeable constant features of the gums. Such low rhamnose contents are characteristic of Acacia species in Series Phyllodineae (27), while the practically invariant galactose:arabinose ratio of 1:1 (ca. 40% each) throughout has been observed in some Acacia, Series Botryocephalae species (28).

Reflecting the constant sugar compositions, the specific rotations shown by the group of gums are also remarkably constant, particularly those of the purified samples, which are all around +70°.

The honey mesquite tree is generally recognised as being P. glandulosa and has been mentioned as possibly being the most important gum producing plant in N. America - Burkart (7). P. glandulosa var. torreyana is known as Western honey mesquite, while var. prostrata is sometimes known as running mesquite. The honey mesquite and P. glandulosa samples studied here are analytically similar (even though the exudation stimuli were different), but this is equally true of the other gums studied, e.g. P. alba, P. velutina and the commercial sample. It is interesting to note that in Burkart's classification of the Series Chilenses (Table IV.3), P. velutina, P. alba and the P. glandulosa complex are all classified together, whereas the P. juliflora complex is farther away.

The results obtained for the commercial sample generally agree with those of previous workers (11-13), except that the galactose and arabinose contents were reported as ca. 20% and 50% respectively (13). Rhamnose was not detected until as recently as 1970 by Aspinall and Whitehead (11), who also report a comprehensive structural study on the gum (11). An interesting contrast between gum arabic and mesquite gum is in the opposite signs of their specific rotations, while the gum from P. juliflora shows almost the same specific rotation as gum arabic.

The obvious exception in this discussion must be the gum from P. juliflora with its negative specific rotation and significant rhamnose content of 11%. It would seem more likely that the commercial sample was collected from P. glandulosa rather than P. juliflora - differing viewpoints on mesquite taxonomy have been discussed, reflecting

the botanical difficulties over identification in the field (Section IV.1.). The collection and study of further authenticated samples of P. juliflora would be required to confirm this interesting anomaly.

Although interesting comparisons can be drawn with gum exudates from other genera and series, the analytical parameters of the Prosopis gums studied here, taken as a whole, do not fit clearly into any one previously observed pattern. Although only a part of one series within the Section Algarobia has been studied, it would seem that this particular group of gums possesses a unique analysis, further supporting the usefulness of chemical studies as an aid to taxonomy, perhaps even bringing to light unnoticed divisions overlooked by botanists. The study of gum exudates from other Series and Sections of Prosopis is of course essential before an adequate picture of this interesting genus can be built up.

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CHAPTER V

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

V. 1. INTRODUCTION

The genus Acacia (Family Leguminosae, sub-family Mimosoideae) is very large and still provides many complex botanical problems of nomenclature and classification. The number of species in the genus is not known with certainty; Black (1) estimated the number at 500, Brenan (2) and Ewart (3) proposed 750 - 800, while Hutchinson (4) suggested 900. Tindale (5) listed 616 species native to Australia alone and the known indigenous African and American species are probably sufficient to bring the total for the genus to at least 900.

The genus was divided into six Series and fifteen Sub-series by Bentham (6) and into Sections and Sub-sections by Taubert (7) and although various revisions have been necessary with the discovery of new species, Bentham's main divisions or Series are still used. Recently Vassal (8) proposed a revision of Bentham's Series, but his proposals have not been accepted universally.

Bentham's divisions, based on habit, inflorescence and geographical distribution, are: Series 1, Phyllodineae, containing 570 spp. (5), subdivided into eight Sub-series; Series 2, Botryocephalae, 32 spp. (5); Series 3, Pulchellae, 14 spp. (5); Series 4, Gummiferae, 60 spp. (6), with 3 Sub-series; Series 5, Vulgares, 75 spp. (6), with 4 sub-series; Series 6, Filicinae, 2 spp. (6).

Species from Series 1 are native to Australia, Hawaii and New Caledonia; species from Series 2 and 3 are native to Australia; species from Series 4 and 5 are found throughout tropical and semi-tropical parts of the world; and species from Series 6 are native to South America.

The number of Acacia gum exudates studied has increased from thirteen in 1963 to thirty in 1970 and to forty-seven by 1976 and since then over forty further species have been under investigation (9).

Comparisons of the analytical and structural data for species belonging to Bentham's Series 1, 4 and 5 have been made (10); in general, the chemical evidence substantiates Bentham's taxonomic divisions and the broad bases of difference shown by gums belonging to the different series in Acacia have been tabulated (9, 10). Gum exudates from Acacia species in Series 3 and 6 of Bentham's classification have not yet been studied.

Series 1, Phyllodineae, comprises at least 570 species and it will be a long time before a sufficiently large number of these species have been analysed for a statistically acceptable evaluation to be possible. From the species already studied, however, it would appear that the gums of this Series (11) are characterised by low molecular weight, low acidity, low positive or negative optical rotations, low rhamnose content and a high ratio of galactose to arabinose.

Series 2, Botryocephalae, comprises 32 species. The data available so far for twelve species of this Series indicate that it may contain two chemically distinct types. Species of one of these types (type A) differ greatly in composition from gums of the Phyllodineae, in that they have significant negative optical rotations and a low galactose to arabinose ratio. The gums from the so-called type B species show strong resemblances to those of the Phyllodineae.

Series 4, Gummiferae, contains 60 species and is a predominantly African group of Acacias. Gums of this series are characterised mainly by highly positive optical rotations and high molecular weight, with a tendency towards intermediate values of acidity and viscosity and low proportions of rhamnose; wide variations in nitrogen and methoxyl contents occur, however.

Series 5, Vulgares, comprising 75 species, includes the most important source of commercial gum arabic, A. senegal, which has

consequently been the subject of more chemical investigations into seasonal and geographical variation, and more structural analyses, than any other Acacia species. The main distinguishing features of gums from the species in Vulgares appear to be significant negative optical rotations, intermediate molecular weights (of the order of 0.5×10^6), 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.

Chemical analyses of ca. ninety different species have now been carried out (9, 10) with the conclusion that each Acacia species exudes a gum that is characteristic of that species regardless of where it is grown geographically, i.e. the chemical composition and physical properties of the exudate from each Acacia species differ, often very considerably, from that of other species.

The large number of species in the Series Phyllodineae was subdivided by Bentham into eight Sub-series, namely: Alatae, Continuae, Pungentes, Calamiformes, Brunioideae, Uninerves, Plurinerves and Juliflorae. Relatively few species of the Phyllodineae have been examined chemically, although there have been studies of the distribution of amino acids in some seeds (12, 13) and of the flavonoid content of some heartwoods (14-16). To date, the gum exudates from thirty-one species in the Series Phyllodineae have been studied (11, 17-20). Of these, one is in Bentham's Sub-series 4C (11), fourteen are in Sub-series 6F (11,18), three are in Sub-series 7F (11,18) twelve species are in Sub-series 8 (17,19,20) and one species was not conclusively allocated (18).

The characteristic analytical features of the Series Phyllodineae have been mentioned above and data (11) for five gums considered to be typical of the species in Sub-series 1 - 7 of this Series are given in

Table V. 1. The most recent data however, obtained by Leon de Pinto (18) for Phyllodineae species have considerably extended the analytical values thought to be representative of this Series. Results for five such gums are given in Table V. 2. Clearly, many more _Acacia_ species, from as many of the various Phyllodineae Sub-series as possible, will have to be studied before an acceptable picture of this interesting group of gums can be obtained.

Special attention has recently been given to the Sub-series _Juliflorae_ in Phyllodineae. Botanically, the _Juliflorae_ is considered to be one of the largest and most complex groups of Phyllodinous wattles occurring in both tropical and more temperate regions of Australia, Malaysia and the Pacific Islands (21). Tindale (15) has stated that the _Juliflorae_ are regarded as the most highly evolved of the _Acacia_ species, both morphologically and chemically.

In the first analytical study of _Juliflorae_ gum specimens, Anderson and Gill (19) reported that the five species studied gave gums which differed considerably from the majority of _Acacia_ gums studied so far (9). Their results, given in Table V. 3, suggested that gums from _Juliflorae_ species appear to be more proteinaceous, more acidic, more viscous, with higher methoxyl contents and higher molecular weights, but with lower proportions of rhamnose and arabinose.

This chapter presents an analytical study of five _Juliflorae_ gum exudates, bringing the total number of the _Juliflorae_ to be studied to date to seventeen. The gums studied are from _Acacia torulosa_ (two samples), _A. acuminata_, _A. resinomarginea_, _A. stipiligera_ and _A. tumida_.

TABLE V. 1

ANALYTICAL DATA FOR GUM EXUDATES OF SOME PHYLLODINEAE SPECIES OF *ACACIA*

	A. calamifolia	*A. difformis*	*A. falcata*	*A. mabellae*	*A. retinodes*
Moisture, %	12.9	10.2	9.8	11.5	11.4
Ash, %[a]	2.0	1.5	1.8	1.7	2.1
Nitrogen, %[a]	0.26	0.28	0.21	0.23	0.48
Hence protein, % (N x 6.25)[a]	1.6	1.8	1.3	1.4	3.0
Methoxyl, %[b]	0.87	0.64	0.49	0.41	0.41
$[\alpha]_D$ in water, (degrees)[b]	+4	-5	+9	+4	+1
Intrinsic viscosity, $[\eta]$, ml/g [a]	5.8	6.2	5.1	5.8	9.5
Molecular weight, ($\bar{M}_w \times 10^4$)[a]	24	4.7	7.9	12	73
Equivalent weight [b]	2430	3420	2290	2870	1770
Hence uronic anhydride, %[b, c]	7.0	5.0	8.0	6.0	10.0
<u>Sugar composition after hydrolysis, %</u>					
4-*O*-Methylglucuronic acid[d]	5.0	3.5	3.0	2.5	2.5
Glucuronic acid	2.0	1.5	5.0	3.5	7.5
Galactose	84	75	85	76	76
Arabinose	8	19	7	17	12
Rhamnose	1	1	trace	1	2

a Corrected for moisture content.
b Corrected for moisture and protein contents.
c If all acidity arises from uronic acids.
d If all methoxyl groups located in this acid.

TABLE V. 2

ANALYTICAL DATA FOR GUM EXUDATES OF FURTHER PHYLLODINEAE SPECIES OF ACACIA

	A. saliciformis	A. xanthina	A. rostellifera	A. dictyophleba	A. cyclops
Moisture, %	10.4	4.0	10.2	9.6	9.1
Ash, %[a]	3.1	3.9	3.0	2.4	3.6
Nitrogen, %[a]	1.2	0.67	0.18	7.6	0.08
Hence protein, % (N x 6.25)[a]	7.5	4.2	1.12	52	0.55
Methoxyl, %[b]	1.1	1.1	0.75	2.3	0.55
$[\alpha]_D$ in water, (degrees)[b]	-54	+20	+90	-32	+26
Intrinsic viscosity, $[\eta]$, ml/g [a]	39	11	10	16	7.4
Molecular weight, $(\bar{M}_w \times 10^4)$[a]	200	143	95	28	30
Equivalent weight[b]	1397	908	687	481	888
Hence uronic anhydride, %[b,c]	13.0	19.0	26.0	36.0	20.0
Sugar composition after hydrolysis, %					
4-O-Methylglucuronic acid[d]	6.6	6.6	5.0	13.7	3.3
Glucuronic acid	6.4	12.4	21.0	22.3	16.7
Galactose	55	52	58	53	68
Arabinose	26	29	12	11	5
Rhamnose	6	trace	4	trace	7

Notes a - d as in Table V.1

TABLE V.3

ANALYTICAL DATA FOR GUM EXUDATES OF SOME *JULIFLORAE* SPECIES OF *ACACIA*

	A. auriculiformis	*A. holosericea*	*A. mangium*	*A. leptostachya*	*A. pubifolia*
Moisture, %	13.3	9.0	16.2	16.1	13.4
Ash, %[a]	4.8	5.1	5.4	5.8	3.4
Nitrogen, %[a]	1.1	0.28	0.98	0.66	1.7
Hence protein, % (N x 6.25)[a]	7.1	1.8	6.1	4.1	10.4
Methoxyl, %[b]	1.7	0.47	1.5	2.2	1.2
$[\alpha]_D$ in water, (degrees)[b]	-19	+3	+36	+58	-58
Intrinsic viscosity, $[\eta]$, ml/g [a]	22	19	28	17	26
Molecular weight, ($\bar{M}_w \times 10^6$)[a]	1.9	3.8	3.2	1.4	2.4
Equivalent weight[b]	590	1010	545	475	680
Hence uronic anhydride, %[b,c]	29.7	17.3	32.2	37.0	25.9
Sugar composition after hydrolysis, %					
4-*O*-Methylglucuronic acid[d]	10.2	2.8	9.0	13.4	7.2
Glucuronic acid	19.5	14.5	23.2	23.6	18.7
Galactose	58	56	56	54	46
Arabinose	9	20	10	7	25
Rhamnose	3	6	2	2	3

Notes a - d as in Table V.1.

V. 2. ORIGIN OF GUM SAMPLES

Gum from Acacia torulosa Benth. ex F. Muell. was collected 42 km. WNW of Lakeland Downs, Laura, Queensland, by R. Coveny and P. Hind on September 9th, 1975 (Voucher No. NSW107876). A second sample of A. torulosa gum was collected by P.K. Latz (Latz 5790) near Elliot, Northern Territory, on May 28th, 1975.

Gum from the following species was collected in Western Australia by B.R. Maslin (voucher specimens lodged at W.A. Herbarium, Perth): A. acuminata Benth. (BRM 4268) 7 km. north of Mullewa; A. resinomarginea N.V. Fitzg. (BRM 4236) beside the Great Northern Highway, 8 km. from the turn off to Mount Gibson Station.

Gum from A. stipiligera F. Muell. was collected by P.K. Latz in Waribri District, Northern Territory, 300 km. north of Alice Springs, on May 26th, 1975 and gum from A. tumida F. Muell. was collected (Latz 5999), 32 km. south of Elliot, Northern Territory, on May 30th, 1975.

V. 3. PREPARATION OF GUM SAMPLES FOR ANALYSIS

Many species of the Juliflorae yield gum only very sparingly, even after pruning or tapping wounds have been made; consequently, the amounts of crude gum available for purification and analysis were small (1 - 11g).

Both gum samples from A. torulosa were dark brown in colour and admixed with pieces of bark, particularly the Queensland sample. Dissolution in cold distilled water (ca. 10% solution) for two days (four days for the Queensland sample) was incomplete and in both cases the remaining swollen gel was dispersed by the addition of sodium borohydride (22) over two days. The gel from the Queensland sample was particularly difficult to dissolve and complete dissolution was only effected by the further addition of traces of alkali (pH8). The solutions were filtered through muslin to remove pieces of bark etc.,

through Whatman No. 42 and No. 1 filter papers and dialysed against running tap water for two days (four days for the treated residues). The purified solutions were refiltered and freeze-dried.

The gums from A. stipiligera and A. tumida, also dark brown in colour, were likewise not completely soluble in cold distilled water (7% solution) and required to be similarly treated with borohydride prior to filtration, dialysis and freeze-drying. During dialysis, the solution of A. stipiligera gum reverted to a gel; this unique transformation has not been reported before. Further treatment with borohydride and traces of alkali was required to break this gel. Subsequent dialysis and freeze-drying was successful.

In contrast, the amber-coloured gums from A. acuminata and A. resinomarginea dissolved completely without any treatment with borohydride and the freeze-dried products were obtained after the normal filtering and dialysis.

V. 4. RESULTS

Analytical data for the six samples studied are shown in Table V. 4. The water-soluble and water-insoluble fractions of the gums from A. torulosa, A. stipiligera and A. tumida were not analysed separately.

The samples were hydrolysed with N-sulphuric acid and examination of the hydrolysates by paper chromatography in solvents (a) and (b) revealed the presence of large amounts of galactose and some arabinose as the major sugar components. Rhamnose was only detected in the gums from A. acuminata, A. resinomarginea and A. torulosa (Queensland sample), the latter two showing only traces.

The hydrolysates also contained at least two aldobiuronic acids [R_{gal} 0.18 and 0.55 in solvent (a); 0.19 and 0.43 in solvent (b)], which correspond to 6 - O - (β - D - glucopyranosyluronic acid) - D - galactose and 4 - O - (4 - O - methyl - α - D - glucopyranosyluronic acid)- D - galactose respectively. These acids occur commonly in

TABLE V. 4

ANALYTICAL DATA FOR PURIFIED GUM POLYSACCHARIDES FROM

ACACIA SPECIES, SERIES PHYLLODINEAE, SUB-SERIES *JULIFLORAE*

	A. torulosa (Q.)	*A. torulosa* (N.T.)	*A. resinomarginea*	*A. acuminata*	*A. stipilligera*	*A. tumida*
Moisture	9.7	13.0	8.3	11.3	5.7	10.8
Ash, %[a]	3.7	2.4	5.3	5.7	4.1	3.0
Nitrogen, %[a]	7.2	7.8	0.83	0.72	6.3	7.1
Hence protein, % (N x 6.25)[a]	45	49	5.2	4.5	40	44
Methoxyl, %[b]	1.6	3.8	3.4	0.69	4.8	5.5
$[\alpha]_D$ in water, (degrees)[b]	+41	n.d.	+5	-29	-19	-9
Intrinsic viscosity, $[\eta]$, ml/g [a]	69	39	5	21	21	30
Molecular weight, ($\bar{M}_w \times 10^5$)[a]	10	1.7	1.8	8.9	4.8	1.7
Equivalent weight[b]	957	558	631	656	613	481
Hence uronic anhydride, %[b, c]	18.5	31.8	28.0	27.1	29.0	37.0
Sugar composition after hydrolysis, %						
4-*O*-Methylglucuronic acid[d]	9.4	22.8	20.5	4.1	28.8	33.0
Glucuronic acid	9.1	9.0	7.5	23.0	0.02	4.0
Galactose	53	43	65	50	46	49
Arabinose	28	25	7	11	25	14
Rhamnose	trace	-	trace	12	-	-

Notes a - d as in Table V.1. n.d. not determined.

Acacia gums with significant methoxyl contents (23). In addition, 4 - O - methylglucuronic acid [R_{gal} 2.49 in solvent (a); 1.44 in solvent (c)] was detected chromatographically; the unusual occurrence of this sugar in an N-hydrolysate has already received comment (17, 24, this thesis Chapter IV).

V. 5. DISCUSSION

Although generalisations cannot be drawn for the characteristic properties of gums from the 151 known Juliflorae species in terms of the few species studied to date, the analytical parameters presented here are further evidence of the unique differences between these gums and other Acacia gums.

With the exception of A. acuminata and A. resinomarginea, the gums studied have a very high nitrogen content (%N = 6.3 - 7.8) and likewise, all but A. acuminata show a very high methoxyl content (3.8 - 5.5%). The intrinsic viscosities are in general also much higher than those of the majority of Acacia gums, particularly those of A. torulosa (Queensland), A. torulosa (Northern Territory) and A. tumida ($[\eta]$ = 69, 39 and 30 ml/g respectively), although the molecular weights are not quite as high as was first observed in gums from Juliflorae species (19).

Several of the gums studied show a remarkably high uronic acid content, e.g. A. tumida (37%) and A. torulosa, Northern Territory (32%), a characteristic already reported for Juliflorae species (17, 19). Only one gum showed appreciable amounts of rhamnose (A. acuminata, 12%) and in only two others was rhamnose detected at all (A. torulosa, Queensland and A. resinomarginea, both traces).

Some of the values reported are of great chemical interest as they represent the lower or upper values found so far for several of the key analytical parameters; these in turn indicate extremes in structure-type of the complex polysaccharide involved.

Thus in this study, A. tumida gum clearly gives the highest value of methoxyl content recorded so far (5.5%); A. torulosa (Queensland) is much the most viscous ($[\eta]$ = 69 ml/g) and the Northern Territory sample contains the highest nitrogen content (%N = 7.8) of the Acacia gums studied to date. Another Phyllodineae species, A. dictyophleba, was recently reported (18) to have 7.5%N.

The rhamnose content of ca. 0.5% in A. torulosa (Queensland) and A. resinomarginea is the lowest recorded and A. stipiligera and A. tumida are the first Acacia gums in which rhamnose was not detected at all. Rhamnose was not found in the Northern Territory sample of A. torulosa, although a trace was detected in the Queensland species. The combined arabinose and rhamnose contents of A. resinomarginea is also extremely low (7.5%) and this, along with the other Juliflorae gums (17, 20) of high acidity, A. kempeana and A. microneura (arabinose plus rhamnose contents of 4 and 6% respectively) represents one of the extreme structure-types, based on a glucurono-galactan, in Acacia. Only the gums from A. falcata and A. calamifolia (also Phyllodineae species) with arabinose plus rhamnose contents of 7 and 9% respectively (11) have similar low values, but the acidity of these two species is very much less and they are probably therefore best regarded as approximating to branched galactans.

The uronic acid content of A. tumida (37%) is practically the highest known in Acacia, just exceeded by A. kempeana (38.6%) and A. microneura (39.7%) and similar to A. leptostachya (37%), all Juliflorae species (17, 20) and to A. dictyophleba (36%), a Phyllodineae species (18).

The extreme values found in this study further extend the remarkable ranges in analytical parameters previously reported for

Juliflorae gums. Thus, the methoxyl content now ranges from 0.47% in A. holosericea (17, 19) to 5.5% in A. tumida; the nitrogen content from 0.14% in A. acradenia (17, 20) to 7.8% in A. torulosa (Northern Territory); the intrinsic viscosity from $[\eta] = 4.5$ ml/g for A. resinomarginea to $[\eta] = 69$ ml/g for A. torulosa (Queensland); the specific rotation from -58° for A. pubifolia (17, 19) to $+58^{\circ}$ for A. leptostachya (17, 19); the molecular weight from 0.17×10^{6} for A. tumida to 3.8×10^{6} for A. holosericea (17, 19); the uronic acid from 15.3% in A. acradenia (17, 20) to 39.7% in A. microneura (17, 20); the rhamnose content from zero in A. torulosa (Northern Territory), A. stipiligera and A. tumida to 12% in A. beauverdiana (17, 20) and A. acuminata; and the galactose to arabinose ratio from 58:3 in A. kempeana (17, 20) to 50:32 in A. acradenia (17, 20).

These are large variations, considerably more extensive than have been commonly found for gum specimens assigned to other sub-divisions of the genus and they are manifestations that the Juliflorae is a complex and heterogeneous botanical group. It is interesting to note that recent work on some Phyllodineae species (18) has also indicated a greater range in analytical parameters than found before.

The chemical data now available for some seventeen Juliflorae species are also of considerable taxonomic interest. In Table V.5, the species studied are grouped in terms of Bentham's classification of 1864 (25). For species not actually ascribed to a Sub-series by Bentham, the first author to do so is cited in brackets (20).

Apart from reasonable similarities between A. acradenia and A. pubifolia (Rigidulae), the species listed under Bentham's groups C, D and E cannot be regarded as homogeneous groups on the basis of the chemical data available. It is becoming increasingly clear that on botanical grounds, Bentham's groupings are highly artificial and cannot

TABLE V. 5

JULIFLORAE GUM EXUDATES ARRANGED IN BENTHAM'S DIVISIONS

Juliflorae	A. RIGIDULAE	:	A. acradenia
			A. pubifolia (26)
			A. stipiligera
	B. TETRAMERAE	:	
	C. STENOPHYLLAE	:	A. aneura
			A. coolgardiensis
			A. microneura
			A. resinomarginea (27)
	D. FALCATAE	:	A. acuminata
			A. auriculiformis
			A. beauverdiana (27)
			A. kempeana (28)
			A. leptostachya
			A. stereophylla
			A. torulosa
			A. tumida
	E. DIMIDIATAE	:	A. holosericea
			A. mangium

even be regarded as useful groups of convenience (20). A regrouping within the Juliflorae therefore has recently been proposed (20) based on gross morphological grounds, but with careful consideration of the chemical data reported here and previously (19). The construction of this informal grouping, shown in Table V. 6, is an example of the usefulness of chemical analysis in helping taxonomists realise their aim of adequately reappraising the classification of Acacia.

TABLE V. 6

A SUGGESTED INFORMAL RE-GROUPING OF JULIFLORAE GUM EXUDATES

Group 1.	A. mangium; A. auriculiformis
Group 2.	A. holosericea
Group 3.	A. acradenia; A. pubifolia; A. tumida
Group 4.	A. leptostachya; A. torulosa; A. stipiligera
Group 5.	A. beauverdiana; A. stereophylla
Group 6.	A. resinomarginea; A. microneura; A. kempeana
Group 7.	A. acuminata
Group 8.	A. aneura; A. coolgardiensis

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CHAPTER VI

AN ANALYTICAL STUDY OF *ACACIA* GUM EXUDATES OF THE SERIES GUMMIFERAE AND VULGARES

VI. 1. INTRODUCTION

A general introduction to the genus Acacia is given in Chapter V of this thesis.

Bentham's Series 4, Gummiferae, is a predominantly African group of Acacias and is comprised of 60 species (1) as follows: Sub-series 1, Semibracteatae, 13 species; Sub-series 2, Medibracteatae, 39 species; Sub-series 3, Basibracteatae, 8 species.

To date, gums from the following species of the Series Gummiferae have been studied in detail (analytically and structurally): Acacia arabica (2), A. drepanolobium (3), A. nilotica (4), A. nubica (5), A. seyal (6) and A. xanthophloea (7). Analytical data for five of these gums (2, 8) are given in Table VI. 1. Analytical studies have been published (or are in progress) for ca. seventeen other Gummiferae species (7 - 9).

Bentham's Series 5, Vulgares, contains 75 species in 4 Sub-series: Sub-series 1, Gerontogeae spiciflorae, 25 species; Sub-series 2, Americanae spiciflorae, 19 species; Sub-series 3, Americanae capitulatae, 26 species; Sub-series 4, Gerontogeae capitulatae, 5 species.

Sub-series 1 contains the most commercially important Acacia gum, that from A. senegal (syn. verek) and this has been the subject of more chemical investigations into e.g. its inter-nodule, seasonal and geographical variation and more detailed structural analyses than the gum from any other Acacia species (10 - 15). Only two other gums from the Series Vulgares have been examined in fine detail - Acacia campylacantha (16) and A. laeta var. hashab (17). Table VI. 2 shows the analytical data for these gums (8, 18, 19).

Relatively few other Vulgares gums have been examined (8, 9): A. catechu (18), A. mellifera (20 - 22) and A. sundra (18), the results obtained largely supporting the characteristics noted in Table VI. 2.

TABLE VI.1

ANALYTICAL DATA FOR SOME GUMMIFERAE SPECIES OF ACACIA GUMS

	Acacia drepanolobium	Acacia arabica	Acacia nilotica	Acacia nubica	Acacia seyal
Ash, %[a]	n.d.	2.52	2.48	1.54	2.87
Nitrogen, %[a]	0.07	1.11	0.02	0.20	0.14
Hence protein, % (N x 6.25)[a]	0.44	6.94	0.13	1.25	0.88
Methoxyl, %[b]	0.88	0.43	0.96	0.05	0.94
$[\alpha]_D$ in water, (degrees)[b]	+112	+78	+108	+98	+51
Intrinsic viscosity, $[\eta]$, ml/g [a]	10	18	10	10	12
Molecular weight, ($\bar{M}_w \times 10^6$)[a]	2.3	0.95	2.2	0.87	0.85
Equivalent weight[b]	1880	1980	1890	3030	1470
Hence uronic anhydride, %[b,c]	10.0	9.0	9.0	7.0	12.0
Sugar composition after hydrolysis, %					
4-O-Methylglucuronic acid[d]	6.0	2.5	6.0	0.5	5.5
Glucuronic acid	4.0	6.5	3.0	6.5	6.5
Galactose	32	38	44	33	38
Arabinose	57	52	46	59	46
Rhamnose	0.4	1	0.4	1	4

a Corrected for moisture content. n.d. Not determined
b Corrected for moisture and protein content.
c If all acidity arises from uronic acids.
d If all methoxyl groups located in this acid.

TABLE VI.2

ANALYTICAL DATA FOR SOME VULGARES SPECIES OF ACACIA GUMS

	Acacia campylacantha	Acacia laeta	Acacia senegal
Ash, %[a]	2.92	n.d.	3.93
Nitrogen, %[a]	0.37	0.65	0.29
Hence protein, %(N x 6.25)[a]	2.31	4.06	1.81
Methoxyl, %[b]	0.29	0.35	0.25
$[\alpha]_D$ in water, (degrees)[b]	-12	-42	-30
Intrinsic viscosity, $[\eta]$, ml/g [a]	16	21	13
Molecular weight, ($\bar{M}_w \times 10^5$)[a]	3.12	7.25	3.84
Equivalent weight[b]	1900	1250	1100
Hence uronic anhydride, %[b,c]	9.0	14.0	16.0
Sugar composition after hydrolysis, %			
4-O-Methylglucuronic acid[d]	2.0	3.5	1.5
Glucuronic acid	7.0	10.5	14.5
Galactose	54	44	44
Arabinose	29	29	27
Rhamnose	8	13	13

Notes a - d as in Table VI.1.

This chapter presents an analytical study of gum exudates from eight species not studied previously, namely A. hebeclada, A. kirkii (two samples), A. nebrownii and A. reficiens (all of the Series Gummiferae) and A. erubescens, A. fleckii, A. mellifera subspecies detinens and A. mellifera subspecies mellifera (all of the Series Vulgares).

VI. 2. ORIGIN OF GUM SPECIMENS

Gum exudates from the following species belonging to the Series Gummiferae were collected on 6th September, 1975 at Otjitambi, 1915 - CC (Okaukuejo), District Outjo, Namibia, by Mr. W. Giess (SWA Herbarium, Windhoek): A. hebeclada DC. syn. A. stolonifera Burch. (Bentham No. 309), A. reficiens Wawra (Bentham No. 312) and A. nebrownii Burtt Davy; gum from A. kirkii Oliver was collected by Mr. Giess on 4th September, 1975 at Otjovasandu (nr. 183), 1915 - DB (Okaukuejo), District Outjo.

The gum from A. kirkii was divided into two parts: one contained freshly exuded gum which was hard, transparent and glossy, while the other was gum from a previous season which had been wet and dried a number of times during the rains and was very crumbly and opaque, with the appearance of frosted glass.

Gum exudates from the following species belonging to the Series Vulgares were collected as follows: A. fleckii Schinz by Mr. H.D. von Alvensleben at Kumkauas (n.552), 1917 CA/CB (Tsumeb), District Grootfontein, Namibia, on 29th September, 1975; A. erubescens Welw. ex Oliver (Bentham No.370) and A. mellifera subspecies detinens (Burch.) Brenan by Mr. W. Giess at Otjitambi, 1915 - CC (Okaukuejo), District Outjo, Namibia, on 6th September, 1975; and A. mellifera (Vahl.) Benth. subspecies mellifera Brenan by Mr. A.G. Seif-el-Din, Gum Research Officer, Republic of the Sudan at Gardud Forest Reserve, Republic of the Sudan on 20th March, 1978.

VI. 3. RESULTS

The quantities of gum available for analysis were small; the amount available from A. erubescens did not allow determinations of ash nor molecular weight to be made. After removal of small pieces of bark etc., the samples were ground to small pieces and analysed (without purification, to conserve material) as clean, crude gum. Both the fresh and weathered samples of A. kirkii gum were analysed for comparison.

Analytical data for the Gummiferae species are given in Table VI.3 and those for the Vulgares species in Table VI.4.

The samples were hydrolysed with N-sulphuric acid and examination of the hydrolysates by paper chromatography in solvents (a) and (b) revealed the presence of galactose and arabinose as the major sugar components along with some rhamnose.

Prolonged elution time (forty hours) of the hydrolysate from A. erubescens gum in solvent (b) showed clearly the presence of an additional neutral sugar which corresponded chromatographically to glucose (R_{gal} 1.19, arabinose 1.39). Further chromatograms were run in solvent (b) and developed with glucose oxidase reagents, this test being specific for glusoce. A purple/green spot (which quickly became pink) was observed and corresponded to a similar spot for a glucose standard; subsequent development of these chromatograms with aniline oxalate clearly showed the glucose oxidase spot to be the sugar seen at R_{gal} 1.19. These tests confirmed the presence of glucose in A. erubescens gum. Duplicate, clean samples of the gum were similarly checked for glucose and the results were all positive, rendering it unlikely that the glucose came from wood or bark contaminants.

The presence of glucose in plant gums was first detected in the gum from Anacardium occidentale (23,) but has never been found in Acacia

TABLE VI.3

ANALYTICAL DATA FOR SOME GUMMIFERAE SPECIES OF ACACIA GUMS

	Acacia hebeclada	Acacia kirkii	Acacia kirkii	Acacia nebrownii	Acacia reficiens
		fresh	weathrd.		
Moisture,%	12.5	10.8	12.7	13.0	11.7
Ash, %[a]	n.d.	1.4	4.2	4.2	2.4
Nitrogen, %[a]	9.4	0.09	0.33	0.14	0.65
Hence protein, %(N x 6.25)[a]	59	0.56	2.1	0.88	4.1
Methoxyl, %[b]	2.5	0.93	1.1	0.50	1.7
$[\alpha]_D$ in water, (degrees)[b]	+28	+54	+30	+43	+89
Intrinsic viscosity, $[\eta]$, ml/g [a]	13	8	15	13	12
Molecular weight, ($\bar{M}_w$ x 10^5)[a]	n.d.	2.08	2.75	3.65	3.77
Equivalent weight[b]	521	1817	905	777	1117
Hence uronic anhydride, %[b,c]	33.8	9.7	19.5	22.6	15.8
Sugar composition after hydrolysis, %					
4-O-Methylglucuronic acid[d]	15.0	5.6	6.7	3.0	10.1
Glucuronic acid	18.8	4.1	12.8	19.6	5.7
Galactose	44	36	44	45	41
Arabinose	14	46	24	27	35
Rhamnose	8	8	13	7	8

Notes a - d as in Table VI.1.

TABLE VI.4

ANALYTICAL DATA FOR SOME VULGARES SPECIES OF *ACACIA* GUMS

	Acacia fleckii	*Acacia erubescens*	*Acacia mellifera* ssp. *detinens*	*Acacia mellifera* ssp. *mellifera*
Moisture, %	12.5	13.2	12.1	8.7
Ash, %[a]	4.0	3.9	3.6	2.9
Nitrogen, %[a]	0.58	1.1	1.3	1.5
Hence protein, % (N x 6.25)[a]	3.6	6.8	8.1	9.1
Methoxyl, %[b]	0.47	1.4	0.82	1.7
$[\alpha]_D$ in water, (degrees)[b]	-32	-31	-45	-56
Intrinsic viscosity, $[\eta]$, ml/g	13	8	21	24
Molecular weight, ($\bar{M}_w \times 10^5$)[a]	4.15	2.0	10.4	4.1
Equivalent weight[b]	918	874	822	843
Hence uronic anhydride, %[b,c]	19.2	20.1	21.4	20.9
Sugar composition after hydrolysis, %				
4-*O*-Methylglucuronic acid[d]	2.8	8.4	4.9	10.2
Glucuronic acid	16.4	11.7	16.5	10.7
Galactose	39	39	44	43
Glucose	3	12	-	-
Arabinose	25	17	25	27
Rhamnose	14	12	9	9

Notes a - d as in Table VI.1.

gums to date. A glucose oxidase check was therefore carried out on the hydrolysates of the other gums reported in this study and a further positive glucose reaction was found for **A. fleckii** gum. Alditol acetate derivatives of fresh hydrolysates of these two glucose-containing gums were prepared (see page 39) and subsequent g.l.c. examination revealed 12% of glucose in **A. erubescens** gum and 3% in **A. fleckii** gum.

The gum from **A. fleckii** also contains more rhamnose than any other Vulgares species yet studied - 14%, and in view of this and its small glucose content (3%), a series of hydrolysis experiments was carried out to determine very approximately the structural locations of these sugars.

1) Mild hydrolysis: the gum was hydrolysed for 8 hours with 0.1N - sulphuric acid and the neutral, deionised hydrolysate was dialysed in distilled water for 30 hours. The dialysate was concentrated to a syrup and examined by paper chromatography in solvents (a) and (b). The degraded product, "degraded gum A", was recovered by freeze-drying and its sugar composition determined.

2) Autohydrolysis: the gum was hydrolysed for 96 hours with 0.01N - sulphuric acid and the neutral, deionised hydrolysate dialysed as in 1). The dialysate and the recovered degraded product, "degraded gum B", were examined as in 1).

The results, which do not include any uronosyl-linked galactose are summarised in Table VI.5.

Two commonly occurring aldobiuronic acids (20) were detected in the hydrolysates of the gums studied: the major fraction corresponded chromatographically to 6-O-(β-D-glucopyranosyluronic acid)-D-galactose [R_{gal} 0.20 in solvent (a), 0.19 in solvent (b)] and the minor fraction to 4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose [R_{gal} 0.59 in solvent (a), 0.42 in solvent (b)]. The methyl substituted aldobiuronic acid was only present in trace amounts and was not detected in the hydrolysate

of A. nebrownii gum, which (along with A. fleckii) has a lower methoxyl content than most of the other samples.

The hydrolysates from A. mellifera subspecies mellifera and from the Gummiferae species all contained 4-O-methylglucuronic acid [R_{gal} 2.61 in solvent (a)]. The reporting of this unusual sugar in an N-hydrolysate is becoming more frequent (24, 25, this thesis Chapters IV and V). Also unusual in an N-hydrolysate is the presence of glucuronolactone; this was detected in all of the hydrolysates [R_{gal} 2.60 in solvent (b)], except those from A. kirkii and A. hebeclada. Hydrolysis with 2N-sulphuric acid is normally considered necessary to break uronosyl linkages.

TABLE VI.5

SUGAR RATIOS[a] AFTER HYDROLYSIS EXPERIMENTS ON Acacia fleckii GUM

Mild Hydrolysis		Autohydrolysis		N-hydrolysis
Dialysate[b]	Degraded Gum A[c]	Dialysate[b]	Degraded Gum B[c]	A. fleckii gum[c]
1:1:1	18:7.5:1 Contained glucose	1:2:1	2.6:1:1 Contained glucose	1.4:1.5:1 Contained glucose

a Galactose : arabinose : rhamnose (excluding uronosyl-linked galactose).

b Estimated visually from chromatograms.

c Determined colourimetrically from an N-hydrolysate.

VI. 4. DISCUSSION

Acacia gums from the Series Gummiferae (Table VI.1) have been generally characterised by high, positive optical rotations, high molecular weights, intermediate values of acidity and viscosity and low proportions of rhamnose with wide variations in nitrogen and methoxyl contents (8,9). The range of values found has recently been increased (7); high uronic anhydride contents (14-20%), increased rhamnose (4-10%) and lower arabinose contents (16-46%) and a high methoxyl content of 2.4% were noted. In particular, gum from A. ehrenbergiana was found to have a low, negative optical rotation (-6%), a low viscosity ($[\eta]$ = 8 ml/g) and molecular weight (1.6×10^5) and a rhamnose content of 10%, features which are more comparable with gums from the Series Vulgares.

The results for the Gummiferae gums reported in this study also show the extended values noted above, although the optical rotations are quite typical. The viscosity of A. kirkii (fresh sample, $[\eta]$ = 8 ml/g) and the molecular weights of the gums ($2.1 - 3.8 \times 10^5$) are all low when compared with Gummiferae species studied previously (Table VI.1).

Two of the gums show a high methoxyl content - A. hebeclada, 2.5% and A. reficiens, 1.7%. For a long time, A. giraffae gum (also Series Gummiferae) had the highest methoxyl content of 2.4% (21), that is, excluding the unusual Juliflorae gums recently analysed (Chapter V); the occurrence of such high values in Gummiferae may not now be uncommon.

The uronic acid contents found for A. hebeclada (33.8%) and A. nebrownii (22.6%) are considerably higher than those generally observed before (8,9). Galactose is generally the major sugar component and the values found are typical of the majority of Gummiferae

gums. The rhamnose contents, however, are higher than those reported at first, (Table VI.1) and A. hebeclada gum has the lowest arabinose content yet observed in a Gummiferae species, the value found (14%) being more typical of some Series Botryocephalae species (26).

Several noticeable differences are apparent in the results for the fresh and weathered samples of A. kirkii gum. The viscosity ($[\eta]$ = 15ml/g), uronic acid (19.5%) and rhamnose content (13%) of the weathered sample are all approximately twice the values for the fresh sample and the arabinose content (24%) has dropped by half, with an accompanying change in optical rotation. In each rainy period to which the gum is exposed some change in composition would not be unexpected as readily soluble low molecular weight materials would be dissolved and washed away. Conditions may even have been suitable for some autohydrolysis to occur, which may account for the loss of arabinose (presumed peripheral) and relative rise in rhamnose and uronic acid.

The most interesting feature of the Gummiferae analyses reported, however, concerns the remarkably high nitrogen content (9.4%) found for A. hebeclada gum. Until other very recent work, in which a nitrogen content of 7.2% was reported (Chapter V) for the gum from A. torulosa (Juliflorae), a nitrogen content of 1.66% (27) was the highest recorded for an Acacia gum, also for a Juliflorae species. Previous studies (28) have shown the nitrogen content of the gums from Acacia and other genera to be proteinaceous in origin; attempts to free Acacia gum polysaccharides from proteinaceous matter without causing extensive degradation to the gum molecules were not successful (28). It is important that the role played by the proteinaceous material in the production of the physico-chemical properties that are characteristic of the gum exudates should be clarified and that

the existence of any direct polysaccharide-protein covalent linkage should be investigated. The knowledge of the existence of Acacia gums containing 7-9% of nitrogen, indicative of a possible protein or polypeptide content of 40-55%, now makes the study of such materials a matter of urgency.

The main distinguishing features of gums from the Series Vulgares (Table VI.2) appear to be significant negative optical rotations, intermediate molecular weights (of the order of 0.5×10^6) and the presence of significant proportions of rhamnose.

The Vulgares species examined here clearly fit into this category; the optical rotations are all strongly negative, the molecular weights are generally around 0.4×10^6 and all the gums have significant rhamnose contents (9-14%).

The results, however, are not without interesting features. The methoxyl contents are higher than found previously (Table VI.2), that for A. mellifera sub-species mellifera (1.7%) being the highest reported for a Vulgares species and the nitrogen content for this species (1.45%) is also the highest found in a Vulgares species.

The acidity of the gums is generally high and A. mellifera sub-species detinens is the most acidic gum found in this Series to date (21.4% uronic anhydride). Although the gums have rhamnose contents typical of Vulgares species analysed previously, the high acidity means that the previously noted rhamnose to uronic anhydride ratio of unity does not apply here (9). The analysis of more Vulgares species would be required to confirm if this interesting variation is more widespread.

The data presented for the two sub-species of A. mellifera, from widely differing geographical locations, indicate that the

compositions of their gum exudates are extremely similar. The major difference recorded involves the molecular weights of the two specimens; this was also the major difference found in a recent study (29) of the variation between 15 different samples of A. karroo gum from widely different African locations. Acacia laeta has long been suspected to be a natural hybrid between A. senegal and A. mellifera (19): the data now available for A. mellifera gum allow comparisons to be made with that published previously for the gums from A. laeta (17) and A. senegal (11). The compositions of the three species are closely similar; it is interesting that, where differences occur, the values of the relevant parameters for A. laeta gum are intermediate between those for A. mellifera and those for A. senegal. This is further evidence that closely related Acacia species give gum exudates that are closely similar in composition (29-31).

A noteworthy result is the presence of a significant amount of glucose (12%) in A. erubescens gum and a small amount in A. fleckii gum (3%). This is the first time glucose has been detected in Acacia gums - its occurrence in plant gum exudates may not be as uncommon as was first supposed (23).

The hydrolysis experiments on A. fleckii gum show that during autohydrolysis, only about two-thirds of the arabinose and half of the rhamnose were eliminated. This contrasts with previous studies on Gummiferae and Vulgares species (2-6, 15-17), in which all the rhamnose and almost all the arabinose were lost during autohydrolysis. The reason for this difference must await a full structural investigation, but it may possibly indicate more arabinose and rhamnose bound within the core of the gum molecule than has previously been known. The results suggest that the small glucose component in A. fleckii gum is

probably located in the core of the molecule, rather than being peripheral.

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CHAPTER VII

AN ANALYTICAL STUDY OF A BULK SAMPLE OF GUM ARABIC AND ITS CHANGES DURING PROLONGED STORAGE

VII. 1. INTRODUCTION

A number of studies on inter-nodule variation in some Acacia gum exudates (1 - 6) have shown that significant variations in physical and chemical properties occur between one nodule and another taken from one tree and between nodules taken from different trees of one particular species. Seasonal and climatic change, soil conditions, age of the tree and nature of the injury stimulating gum flow are all expected to affect the quality and chemical composition of the gum produced, although the variations found have been less than might have been expected for such a complex natural product (7).

In addition to these natural variations, commercial samples of "gum arabic" can seldom be relied upon to contain gum from a single botanical species. Good quality Sudanese gum arabic is very largely the exudate from A. senegal (syn. verek) trees, but the presence of some gum from other Acacia species - particularly A. laeta, A. seyal and A. arabica - and from other genera (e.g. Albizia sericocephala) must be expected to occur spasmodically (7). Thus a large consignment of commercial gum arabic containing many gum collections presents considerable difficulty for meaningful chemical analysis.

A further interesting problem in gum chemistry has occasionally been noticed; dialysed and purified samples of several gums which had been stored in the freeze-dried state for several months have been found by molecular-sieve chromatography and ultracentrifugation studies to develop a minor component of high molecular weight (8,9), with corresponding increases in viscosity (4) and insolubility. In one Anacardium sample studied (10), the weight average molecular weight was found to have trebled in two months; samples of Acacia senegal gum have shown

viscosity increases of 50% in similar periods (4). The high molecular weight components were absent from freshly purified samples and were considered to be molecular aggregation artefacts, arising during freeze-drying or subsequent storage of the purified polysaccharide.

A large consignment of high quality powdered Sudanese gum arabic was received for use in a metabolism and toxicity study in laboratory animals and therefore, the chemical composition of the entire quantity required to be thoroughly characterised. This chapter presents the work carried out to obtain a meaningful analysis of the gum, taking into account the analytical difficulties discussed above. The opportunity was also taken to investigate any changes in analytical parameters during long storage of a number of samples of the gum ("time study"), following the reports of molecular aggregation mentioned above.

VII. 2. ORIGIN AND SAMPLING PROCEDURES OF THE GUM

The gum, kindly provided by Rowntree Mackintosh Ltd., York, was received as a coarse powder contained in two 50kg sacks. This quantity had been amassed over a period of several months from the small test samples passed weekly to the quality control laboratory prior to clearance of bulk quantities for production purposes.

In order to characterise the gum adequately, fifteen small samples were taken from the consignment for analysis, as follows:

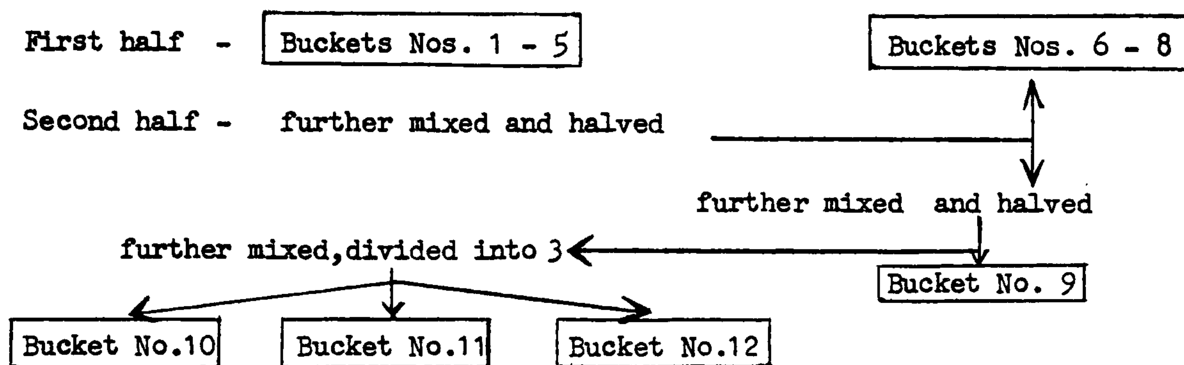
Samples A and B were taken from the first sack, purified and analysed by Dr. A. Stefani.

Samples C and D were taken from the second sack, purified and analysed by Dr. G. Leon de Pinto.

Sample E, taken from the first sack, and Sample F, from the second sack, were purified and analysed by the author, as were

all further samples.

The entire quantity of gum was then emptied on to a clean tarpaulin and thoroughly mixed. A number of heavy duty plastic pails with tightly fitting lids were then filled with the gum for convenient and separate storage. The following mixing and dividing technique was used after halving the mixed sacks:



Samples B1 to B5 were taken from the respective buckets. The gum in buckets 10 to 12 was considered to be the most thoroughly mixed and hence the most representative of the consignment and small samples were taken from these buckets and combined to become Sample G.

Four further samples were obtained in the same way as Sample G, labelled Samples H, J, K and L and treated as follows:

Sample H - kept as a reserve.

Sample J - ground in a pestle and mortar to a fine powder and mixed well.

Sample K - dissolved in distilled water (750 ml) for one day, filtered and freeze-dried without dialysis i.e. still considered as crude gum, but a homogeneous sample. Both these treatments were attempts to make the samples (considered to be representative) as homogeneous as possible.

Sample L - purified in the usual way (Section VII.3).

Samples G, J, K and B1 to B5 were then analysed in the crude gum form. Portions of Samples A - F were purified in the usual way and the freeze-dried products, along with Sample L, were also analysed.

VII. 3. PURIFICATION OF GUM SAMPLES

The gums to be purified (10g portions from Samples A - F and 75g Sample L) were dissolved in distilled water (10% solution) over two days, filtered through Whatman No.1 and No.42 filter papers and dialysed against running tap water for two days. The purified gums were recovered as the freeze-dried products.

VII. 4. RESULTS AND DISCUSSION

The analytical parameters for the crude samples, G, J, K and B1 to B5, are shown in Table VII.1 and those for the purified samples, A to F and L, are given in Table VII.2. An analytical comparison of the average results for the purified samples, the crude samples and purified A. senegal gum (11) is given in Table VII. 3.

The range of analytical parameters shown by the purified samples A to F is surprisingly small considering the usual difficulties involved in obtaining commercial gum originating from one botanical source. The crude samples, however, are probably of greater importance; the metabolism study involves this material since this is the form mostly used in foodstuffs and confectionery. The range of analytical parameters for samples B1 to B5 is also small, although differences are apparent from the purified material; the purified samples have a higher galactose content, lower arabinose and rhamnose contents and higher viscosities and molecular weights.

In contrast, crude sample G, which was chosen as being representative of all the crude material, has a sugar composition more similar to the purified samples than to samples B1 to B5. Although careful mixing was

TABLE VII.1

ANALYTICAL DATA FOR POWDERED GUM ARABIC SAMPLES

	G	J	K	B1	B2	B3	B4	B5
Moisture, %	13.0	13.8	7.0	13.1	13.6	13.4	13.2	13.2
Ash, %[a]	4.7	4.8	4.1	5.1	5.0	4.8	5.0	5.3
Nitrogen, %[a]	0.56	0.36	0.37	0.36	0.39	0.39	0.38	0.30
Hence protein, % (N x 6.25)[a]	3.5	2.3	2.3	2.3	2.4	2.4	2.4	1.9
Methoxyl, %[b]	0.50	0.49	0.55	0.44	0.56	0.56	0.59	0.54
$[\alpha]_D$ in water, (degrees)[b]	-30	-29	-27	-29	-27	-31	-30	-31
Intrinsic viscosity, $[\eta]$, ml/g[a]	20	17	27	21	19	18	18	17
Molecular weight, ($\bar{M}_w \times 10^5$)[a]	4.9	4.7	8.4	4.5	5.6	5.1	5.6	5.6
Equivalent weight[b]	880	880	903	871	917	822	846	989
Hence uronic anhydride, %[b,c]	20.0	20.0	19.5	20.2	19.2	21.4	20.8	17.8
Sugar composition after hydrolysis,%								
4-O-Methylglucuronic acid[d]	3.0	2.9	3.3	2.6	3.4	3.4	3.5	3.2
Glucuronic acid	17.0	17.1	16.2	17.6	15.8	18.0	17.3	14.6
Galactose	57	43	55	47	44	43	43	48
Arabinose	15	22	14	22	24	24	25	22
Rhamnose	8	15	12	11	13	12	10	12

a Corrected for moisture content. c If all acidity arises from uronic acids.
b Corrected for moisture and protein contents. d If all methoxyl groups located in this acid.

TABLE VII.2

ANALYTICAL DATA FOR PURIFIED GUM ARABIC SAMPLES

	A	B	C	D	E	F	Mean A-F	L
Moisture, %	7.5	4.1	8.8	8.8	6.1	6.5	7.0	11.7
Ash, %[a]	2.3	2.2	2.8	2.5	2.2	2.7	2.5	3.5
Nitrogen, %[a]	0.30	0.27	0.35	0.31	0.28	0.30	0.30	0.32
Hence protein,%(N x 6.25)[a]	1.9	1.7	2.2	1.9	1.8	1.9	1.9	2.0
Methoxyl, %[b]	0.71	0.74	0.74	0.51	0.69	0.69	0.68	0.68
$[\alpha]_D$ in water, (degrees)[b]	-29	-30	-24	-22	-28	-30	-27	-33
Intrinsic viscosity, $[\eta]$, ml/g[a]	28	28	28	28	27	27	28	24
Molecular weight, ($\bar{M}_w \times 10^5$)[a]	6.8	7.5	6.3	6.7	6.7	5.9	6.7	7.6
Equivalent weight[b]	923	1080	1030	1110	926	1020	1015	767
Hence uronic anhydride, %[b,c]	19.0	16.0	17.0	16.0	19.0	17.0	17.3	23.0
Sugar composition after hydrolysis,%								
4-O-Methylglucuronic acid[d]	4.3	4.4	4.4	3.1	4.1	4.1	4.1	4.1
Glucuronic acid	14.7	11.6	12.6	12.9	14.9	12.9	13.2	18.9
Galactose	54	56	54	56	57	57	56	47
Arabinose	19	18	20	18	14	18	18	18
Rhamnose	8	10	9	9	10	8	9	12

Notes a - d as for Table VII.1.

TABLE VII.3

ANALYTICAL COMPARISON OF GUM ARABIC SAMPLES AND

Acacia senegal GUM

	Mean A-F	G	B1	B4	*Acacia senegal*[e]
Moisture, %	7.0	13.0	13.1	13.2	
Ash, %[a]	2.5	4.7	5.1	5.0	3.9
Nitrogen, %[a]	0.30	0.56	0.36	0.38	0.29
Hence protein,%(N x 6.25)[a]	1.9	3.5	2.3	2.4	1.8
Methoxyl, %[b]	0.68	0.50	0.44	0.59	0.25
$[\alpha]_D$ in water, (degrees)[b]	-27	-30	-29	-30	-30
Intrinsic viscosity, $[\eta]$,ml/g[a]	28	20	21	18	13
Molecular weight, ($\bar{M}_w \times 10^5$)[a]	6.7	4.9	4.5	5.6	3.8
Equivalent weight[b]	1015	880	871	846	1100
Hence uronic anhydride, %[b,c]	17.3	20.0	20.2	20.8	16.0
Sugar composition after hydrolysis, %					
4-*O*-Methylglucuronic acid[d]	4.1	3.0	2.6	3.5	1.5
Glucuronic acid	13.2	17.0	17.6	17.3	14.5
Galactose	56	57	47	43	44
Arabinose	18	15	22	25	27
Rhamnose	9	8	11	10	13

Notes a - d as for Table VII.1.

e Anderson *et al.* (4); Anderson (11).

carried out, this difference only underlines the problems in adequately characterising samples of commercial gum. The chemical composition of each manageable portion of the consignment, i.e. of each bucket, is therefore a more representative result, rather than an average result, such as was found for sample G.

The material, as judged by samples B1 to B5, is very similar to A. senegal gum, particularly the sugar composition. The methoxyl content, viscosity and molecular weight of A. senegal gum are slightly lower than those of the commercial samples, but variations of these parameters are commonly found within species, as a recent study on on samples of A. karroo gum has shown (6).

VII. 5. TIME STUDY

Samples B1, B2 and B5 were chosen and placed in small paper trays which were then wrapped around with loosely closed muslin bags and stored on an open laboratory shelf for approximately two years. These conditions were an attempt to study the effects on the chemical composition of the gum of prolonged exposure to moisture and oxygen from the atmosphere. The samples were analysed at intervals of about six months. Only selected important parameters were determined namely methoxyl content, acidity, optical rotation, viscosity and molecular weight. The dates and results of these analyses are shown in Table VII. 4.

Although the results show a number of fluctuations, several trends are apparent. The methoxyl contents show a distinct decrease, dropping to half their initial values over the whole period and a noticeable change to more negative values is seen in the optical rotations, particularly during the earlier period of the investigation.

TABLE VII.4

SELECTED ANALYTICAL DATA FOR GUM ARABIC SAMPLES MEASURED OVER TWO YEARS

	Dates and results of analyses		
SAMPLE B1	3/1977	9/1978	4/1979
Methoxyl, %	0.44	0.25	0.23
$[\alpha]_D$ in water, (degrees)	-29	-34	-31
Intrinsic viscosity, $[\eta]$,ml/g	21	18	16
Molecular weight, ($\bar{M}_w$ x 10^5)	4.5	3.2	3.7
Uronic anhydride, %	20.2	20.7	21.1
SAMPLE B2	6/1977	6/1978	6/1979
Methoxyl, %	0.56	0.39	0.25
$[\alpha]_D$ in water, (degrees)	-27	-30	-28
Intrinsic viscosity, $[\eta]$,ml/g	19	16	17
Molecular weight, ($\bar{M}_w$ x 10^5)	5.6	4.4	4.9
Uronic anhydride, %	19.2	19.5	20.1
SAMPLE B5	2/1978	7/1978	7/1979
Methoxyl, %	0.54	0.56	0.28
$[\alpha]_D$ in water, (degrees)	-31	-35	-26
Intrinsic viscosity, $[\eta]$,ml/g	17	18	15
Molecular weight, ($\bar{M}_w$ x 10^5)	5.6	4.7	4.6
Uronic anhydride, %	17.8	18.8	19.1

In general, the viscosities and molecular weights are seen to drop slightly, although fluctuations may reduce any significance in this.

The most interesting trend in the results, however, is the apparent rise in acidity of the samples. In the past (12), it has been noticed that commercial gum arabic samples from the same source, but analysed after differing periods of transport and storage, have shown similar rises in acidity with time. The sugar composition of samples B2 and B5 was checked after the experimental period and a loss of arabinose was noted in both cases.

Clearly, the trends shown by this short investigation warrant further study, in particular the nature and extent of any oxidation of neutral sugar residues in the gum - the presence of the oxidising enzyme, peroxidase, has been shown in gum arabic (13), and in _Acacia nubica_ gum (14).

The results contrast with findings previously reported for freeze-dried samples of various gums (4, 8-10), in which a viscosity increase of 50% and a molecular weight increase of 300% were found. Such molecular aggregation clearly does not occur in crude gum. It may be that the purification process, i.e. dialysis and freeze-drying, removes certain salts or low molecular weight substances which inhibit molecular aggregation; or it may simply involve changes in physical state, water content, or overall electrical charge of the anionic polysaccharide molecules resulting in molecular attraction. This interesting phenomenon also warrants further investigation.

VII. 6. DATA ON TWO FURTHER BULK SAMPLES

On continuation of the metabolism and toxicity study mentioned

in part VII.1., two further bulk quantities of commercial gum arabic were received and, for completeness, the analyses of these consignments are reported here.

The first of these extra consignments was 50 kg powdered gum arabic collected in the same way as the original delivery and, after mixing, was divided into eleven buckets as before. A small sample was taken from each of buckets three, five, seven and nine and analysed without purification (labelled as samples BB3, BB5, BB7 and BB9).

The second extra consignment was 100 kg of gum arabic which had been spray-dried from an aqueous solution. This was to guarantee homogeneity of the whole quantity and so only one sample was chosen, at random, for analysis (labelled sample S).

The results of these analyses are given in Table VII. 5.

TABLE VII.5

ANALYTICAL DATA FOR POWDERED AND SPRAY-DRIED GUM ARABIC SAMPLES

	BB3	BB5	BB7	BB9	Mean BB3-BB9	S
Moisture, %	13.6	13.6	13.5	13.6	13.6	6.0
Ash, %[a]	4.1	4.2	4.1	3.9	4.1	3.0
Nitrogen, %[a]	0.39	0.29	0.34	0.24	0.32	0.31
Hence protein,%(N x 6.25)[a]	2.4	1.8	2.1	1.5	2.0	1.9
Methoxyl, %[b]	0.29	0.25	0.47	0.46	0.37	0.26
$[\alpha]_D$ in water (degrees)[b]	-35	-32	-30	-31	-32	-30
Intrinsic viscosity, $[\eta]$,ml/g [a]	16	16	14	20	17	17
Molecular weight, ($\overline{M}_w$ x 10^5)[a]	4.8	5.4	4.5	4.5	4.8	5.8
Equivalent weight[b]	896	889	829	831	861	1021
Hence uronic anhydride, %[b,c]	19.6	19.8	21.2	21.2	20.4	17.2
<u>Sugar composition after hydrolysis, %</u>						
4-<u>O</u>-Methylglucuronic acid[d]	1.7	1.5	2.8	2.8	2.2	1.6
Glucuronic acid	17.9	18.3	18.4	18.4	18.3	15.6
Galactose	47	42	46	43	44	46
Arabinose	16	22	19	25	21	24
Rhamnose	17	16	14	11	15	14

Notes a - d as in Table VII.1.

Samples BB3-BB9: powdered gum arabic.

Sample S: spray-dried gum arabic.

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CHAPTER VIII

A STUDY OF SOME OF THE STRUCTURAL FEATURES OF THE GUM EXUDATE FROM *Acacia deanei* SUBSPECIES *paucijuga*

VIII. 1. INTRODUCTION

Plant gum exudates are the most complex of the polysaccharide group of natural products and their full analytical and structural study presents the most formidable and time-consuming problems in carbohydrate chemistry. Although analytical data have been obtained (or are in progress) for almost ninety *Acacia* species (1), the number of full structural studies carried out on gums from this genus is much smaller. The majority of the species on which the more complete structural studies have been made belong to Bentham's Series 4, Gummiferae (2 - 6) and Series 5, Vulgares (7 - 9). The data available for some of these gums have been compared (10).

Only one gum from Series 1, Phyllodineae (Sub-series 8, *Juliflorae*) has been studied in detail (11); studies of four gums from Sub-series 6F, *Uninerves Racemosae* involved methylation analysis only (12).

No gums from Bentham's Series 2, Botryocephalae, have been analysed structurally, despite a suggestion, based on analyses of a number of these gums, that this Series may contain species that give gum exudates of two chemically distinct structure-types (13).

This chapter reports the first attempt to carry out a detailed examination of some of the structural features of a gum exudate from the Series Botryocephalae, that from *Acacia deanei* subspecies *paucijuga*.

VIII. 2. ORIGIN AND PURIFICATION OF SAMPLE

Gum nodules from *Acacia deanei* (R.T. Bak.) Welch, Coombs et McGlynn subspecies *paucijuga* (F. Muell ex N.A. Wakef.) Tindale were collected by Mr. E. Lassak (79 - 017) 22km W. of Gilgandra (along road to Warren), New South Wales, Australia on 23rd February, 1979.

The clean, fresh dark amber nodules (90g) were ground in a pestle and mortar; the nodules proved to be more resilient and less brittle than usual and did not break easily. Attempted dissolution in distilled water (ca. 5% solution) was incomplete after two days. A trace of alkali was added, but after a further two days, the pH decreased and a gel formed. (A similar result was observed during purification of Acacia stipiligera gum - see Chapter V). The gel was almost completely redissolved on the addition of alkali; the solution was filtered through muslin and No. 41 filter paper, dialysed against running tap water for two days and the purified polysaccharide was obtained as the freeze-dried product.

The gel residue was dissolved by treatment with sodium borohydride for two days; the polysaccharide was isolated by freeze-drying after dialysis for four days.

The low yield of pure gum (34g, 38%) necessitated the use of smaller quantities than is customary for the various stages of the following structural study.

VIII. 3. ANALYTICAL DATA

The analytical parameters for the gum are given in Table VIII.1, along with the data obtained for a previous sample from this species collected in Queensland, Australia in 1968 (13).

Hydrolysis of the gum with N-sulphuric acid followed by paper chromatography in solvents (a) and (b) showed the neutral sugars to be galactose, arabinose and some rhamnose.

The two samples are analytically similar, although the New South Wales sample has a higher molecular weight, viscosity, and rhamnose

TABLE VIII. 1

Acacia deanei subspecies paucijuga

ANALYTICAL PARAMETERS FOR THE WHOLE GUM EXUDATE AND ITS DEGRADED PRODUCTS*

	WG (NSW)	WG (Q)	DG	P1	P2	P3
Moisture, %	6.6	11.5	14.4	10.0	assumed	10.0
Ash, %[a]	2.8	2.4	n.d.	n.d.	n.d.	n.d.
Nitrogen, %[a]	0.76	1.3	2.0	2.2	3.9	Nil
Hence protein,% (N x 6.25)[a]	4.8	8.1	12.5	13.8	24.4	Nil
Methoxyl, %[b]	0.41	0.75	0.38	0.14	n.d.	n.d.
$[\alpha]_D$ in water, (degrees)[b]	-70	-66	+72	-23	-31	"
Intrinsic viscosity, $[\eta]$, ml/g[a]	20	13	4	13	10	"
Molecular weight, ($\bar{M}_w$ x 10^5)[a]	10.3	3.6	0.07	0.24	3.91	0.34
Equivalent weight[b]	1175	1350	1224	1910	1310	n.d.
Hence uronic anhydride, %[b,c]	15.0	13.0	14.4	9.2	13.4	"
Sugar composition after hydrolysis, %						
4-O-Methylglucuronic acid[d]	2.5	4.5	2.3	0.84	n.d.	"
Glucuronic acid	12.5	8.5	12.1	8.4	"	"
Galactose	30	38	68	65	79	"
Arabinose	43	43	9	18	8	"
Rhamnose	12	6	9	8	-	-
Smith-degradation data						
Periodate reduced, mmoles/g of polysaccharide[a]				5.73	5.17	3.70
Formic acid released, " " "				1.53	1.85	0.59
Yield of degraded product, %[a]				21	21	47

* WG (NSW) - Whole gum, New South Wales sample.
WG (Q) - Whole gum, Queensland sample.
DG - Degraded gum (0.01N acid hydrolysis).
P1 - 1st. Smith-degraded product.
P2 - 2nd. Smith-degraded product.
P3 - 3rd. Smith-degraded product.

Notes a-d as in Table VII.1 (Page 102), n.d. not determined.

content. A recent study of several different samples of Acacia karroo gum revealed large variations in molecular weight (14).

The gum, with its significant negative optical rotation, molecular weight and rhamnose content and intermediate value of acidity, clearly belongs to that division of the Botryocephalae, type A, as mentioned in the introduction to Chapter V (page 66).

VIII. 4. IDENTIFICATION OF NEUTRAL DISACCHARIDES

Gum from Acacia deanei subspecies paucijuga (2g) was hydrolysed with N-sulphuric acid (110ml) for eight hours in a boiling water bath. The neutralised hydrolysate was deionised, concentrated to a syrup and applied to a column (41.0 x 2.6cm) of Duolite A-4 resin in the formate form. Elution with distilled water (850ml) yielded the neutral disaccharides present in the hydrolysate. After concentration to a syrup, paper chromatography of the neutral fraction in solvents (a) and (b) showed the presence of galactose, arabinose and some rhamnose, together with five disaccharides, "A-E", as follows:-

Disaccharide A developed as a brown spot with R_{gal} 0.26 in solvent (a) and 0.23 in solvent (b). This was the major disaccharide present; it corresponded chromatographically to 6 - O -β - D - galactopyranosyl - D - galactose.

Disaccharide B was also a brown spot with R_{gal} 0.40 in solvent (a) and 0.50 in solvent (b); it corresponded chromatographically to 3 - O -β - D - galactopyranosyl - D - galactose.

Disaccharide C, a minor component, gave a pink spot at R_{gal} 0.51 in solvent (a) and was chromatographically identical to 3 - O -β - D - galactopyranosyl - L - arabinose.

Disaccharide D (a trace only) gave a brown spot at R_{gal} 0.61

in solvent (a) only. The R_{gal} value of this component and its non-elution in solvent (b) suggest that it is acidic and likely to be 4 - O - (4 - O - methyl -α- D - glucopyranosyluronic acid) - D - galactose. This aldobiuronic acid was detected in the acidic fraction of the whole gum hydrolysate - see Section VIII.5 below.

Disaccharide E gave a pink spot at R_{gal} 0.74 in solvent (a), 0.78 in solvent (b), and corresponded to 3 - O -β- L - arabinopyranosyl - L - arabinose.

These neutral disaccharides are commonly found in Acacia gums, although 3 - O -β - D - galactopyranosyl - L - arabinose has previously been detected in only the Series 5, Vulgares, gums from A. senegal (7), A. laeta (8) and A. campylacantha (9).

VIII. 5. IDENTIFICATION OF ALDOBIURONIC ACIDS

After recovery of the neutral disaccharides, elution of the Duolite A-4 column with 5% formic acid (500ml) yielded the acidic fraction of the hydrolysate. The eluate was concentrated to a syrup and formic acid was eliminated by repeated addition of water (2ml) followed by concentration. Paper chromatography of the syrup in solvent (a) indicated the presence of glucuronic acid (orange-brown spot, R_{gal} 0.91), 4 - O - methylglucuronic acid (pink spot, R_{gal} 1.27), glucuronolactone (pink spot, R_{gal} 2.00) and three aldobiuronic acids (brown spots) at R_{gal} 0.18 (the major component), 0.47 and 0.69 (minor components).

The first of these aldobiuronic acids corresponded chromatographically to 6 - O - (β - D - glucopyranosyluronic acid) - D - galactose, with the second one corresponding to 6 - O - (4 - O - methyl -β - D - glucopyranosyluronic acid) - D - galactose. These two aldobiuronic acids have often been observed in Acacia gums with

significant methoxyl contents and significant negative optical rotations (15).

The third aldobiuronic acid corresponded chromatographically to 4 - O - (4 - O - methyl - α - D - glucopyranosyluronic acid) - D - galactose. This is unusual; this acid has not previously been detected in Acacia gums having negative optical rotations, but has frequently been a component of gums with positive rotations (15). The total acid content of A. deanei subspecies paucijuga gum ($[\alpha]_D = -70^\circ$) is only 15% and the proportion of 4 - O - (4 - O - methyl - α - D - glucopyranosyluronic acid) - D - galactose with its α- linkage would not in itself be large enough to give the gum a positive optical rotation.

The detection of this α - linked aldobiuronic acid contrasts with the results previously obtained (15) for two Series Botryocephalae gums, A. dealbata ($[\alpha]_D = -25^\circ$) and A. mearnsii ($[\alpha]_D = -49^\circ$), which showed these gums to contain only the two β - linked aldobiuronic acids. The result published for A. dealbata gum, however, is not now regarded as being representative of that species (16).

A portion of the original gum (50 mg), hydrolysed with 2N-sulphuric acid, was examined by paper chromatography in solvent (c). Glucuronic acid and glucuronolactone were the only uronic acids detected, in agreement with the evidence obtained above from the aldobiuronic acid fractions.

VIII. 6. PARTIAL ACID HYDROLYSIS OF THE GUM

A portion of the gum (75 mg) was hydrolysed with 0.5N - sulphuric acid for one hour; paper chromatography of the hydrolysate in solvent (b) confirmed the presence of three of the disaccharides detected after longer hydrolysis (Section VIII.4), namely 6 - O - β - D - galactopyranosyl - D - galactose (major brown spot, R_{gal} 0.27),

3 - O - β - D - galactopyranosyl - D - galactose (major brown spot, R_{gal} 0.53) and 3 - O - β - L - arabinopyranosyl - L - arabinose (minor pink spot, R_{gal} 0.82).

VIII.7. METHYLATION OF THE GUM

The gum (312 mg) was methylated successively by the Haworth and Purdie methods to give a product (80 mg) with $[\alpha]_D$ = -123° (c 4.9 mg/ml in chloroform) and a methoxyl content of 33.2%. Methanolysis of a portion of the methylated product, followed by g.l.c. examination of the O-methyl glycosides, gave the results shown in Table VIII.2. The O-methyl glycosides were hydrolysed with N-sulphuric acid and the O-methyl sugars were examined by paper chromatography in solvents (d) and (e), as shown in Table VIII.2. This revealed the presence of 2 - O - methyl - D - galactose, R_g 0.33 in solvent (d), in addition to the O-methyl glycosides found by g.l.c. examination.

VIII. 8. PREPARATION AND ANALYSIS OF DEGRADED GUM

Purified gum (3.5g, dry weight) was hydrolysed with 0.01N-sulphuric acid (200ml) for ninety-six hours in a boiling water bath. The neutralised hydrolysate was deionised, concentrated and dialysed against distilled water (1l) for twenty-four hours. After further dialysis against running tap water for forty-eight hours, the degraded gum was recovered as the freeze-dried product (0.9g, yield 24%).

The distilled water dialysate was concentrated to a syrup and chromatographed in solvents (a) and (b). Large amounts of arabinose and some galactose and rhamnose were detected together with the four disaccharides previously identified in the whole gum (Section VIII.4), i.e. 6 - O - β - D - galactopyranosyl - D - galactose (R_{gal} 0.26 in solvent (a), 0.32 in solvent (b)), 3 - O - β - D -

TABLE VIII.2

O-METHYL SUGARS IDENTIFIED IN METHYLATED ACACIA DEANEI subspecies PAUCIJUGA GUM

Relative Retention Time (T) of Methyl Glycosides	R_g After Hydrolysis Solvent (d)	(e)	O-Methyl Sugar Identified	Relative Proportion (g.l.c.)
0.41			unknown sugar	20
0.49			2,3,4-tri-O-methyl-L-rhamnose	13
(0.60),0.68,(0.73)	0.92	0.92	2,3,5-tri-O-methyl-L-arabinose	5.5
0.83,(1.03)	0.86	0.75	2,3,4-tri-O-methyl-L-arabinose	4
1.17,(2.18)	0.86	0.75	3,5-di-O-methyl-L-arabinose	19.5
1.33, 2.36	0.86	0.75	2,5-di-O-methyl-L-arabinose	16.5
1.66	0.86	0.92	2,3,4,6-tetra-O-methyl-D-galactose	2.5
1.88	0.86	0.75	2,4- or 3,4-di-O-methyl-L-arabinose	2
(2.73)	-	-	2,3,4-tri-O-methyl-D-glucuronic acid*	5
(2.91)	0.75	0.48	2,3,6-tri-O-methyl-D-galactose	4
3.85,(4.42)	0.75	0.48	2,4,6-tri-O-methyl-D-galactose	2.5
(4.80),(5.20),(5.72),(6.06)	0.75		2,3,4-tri-O-methyl-D-galactose	3.5
7.15,7.91	-	-	2,3-di-O-methyl-D-glucuronic acid*	1
8.80,9.55	0.50		2,6-di-O-methyl-D-galactose	1
11.39	0.50		2,4-di-O-methyl-D-galactose	1
12.52	0.50	0.13	2,3-di-O-methyl-D-galactose	
	0.33		2-O-methyl-D-galactose	

Figures in parentheses indicate components which were not completely resolved.

*As methyl ester methyl glycoside.

galactopyranosyl - D - galactose (R_{gal} 0.30 in solvent (a), 0.51 in solvent (b)), 3 - O - β - D - galactopyranosyl - L - arabinose (R_{gal} 0.49 in solvent (a), 0.65 in solvent (b)) and 3 - O - β - L - arabinopyranosyl - L - arabinose (R_{gal} 0.78 in solvent (a), 0.81 in solvent (b)). Also detected was the aldobiuronic acid 4 - O - (4 - O - methyl - α - D - glucopyranosyluronic acid) - D - galactose, indicating a labile linkage of this acid to the remainder of the gum molecule.

The degraded gum (326 mg) was methylated by the Haworth and Purdie methods to give a product (57 mg) having $[\alpha]_D = -5^\circ$ (c 7.6 mg/ml in chloroform). A portion of this product was methanolysed and examined by g.l.c. The O-methyl glycosides identified are shown in Table VIII.3. Hydrolysis of the O-methyl glycosides with N-sulphuric acid followed by paper chromatography in solvents (d) and (e) (Table VIII.3) indicated the presence of 2 - O - methyl - D - galactose as well as the O-methyl glycosides identified by g.l.c.

VIII. 9. PREPARATION OF POLYSACCHARIDE 1 BY SMITH-DEGRADATION

Purified gum (24.3g, dry weight) was dissolved in water (620 ml) and 0.25M sodium metaperiodate solution (620 ml) was added. Oxidation was carried out in darkness at room temperature for ninety-six hours. Previous experience has shown that seventy-two to ninety-six hours oxidation time is ample for Smith-degradation of plant gums (6, 17). The extent of the oxidation was estimated by measuring the amount of periodate reduced, by titration, before and after the reaction, of 1 ml aliquots of the solution with 0.0126M sodium arsenite solution in the presence of sodium bicarbonate (200 mg) and potassium iodide solution (2 ml, 10%). The oxidation was also checked by measuring

TABLE VIII.3

O-METHYL SUGARS IDENTIFIED IN METHYLATED DEGRADED GUM

Relative Retention Time (T) of Methyl Glycoside	R_g After Hydrolysis Solvent (d)	(e)	O-Methyl Sugar Identified	Relative Proportion (g.l.c.)
0.32,0.38			unknown sugar	1.5
0.46			2,3,4-tri-O-methyl-L-rhamnose	0.5
(0.64),(0.68)			2,3,5-tri-O-methyl-L-arabinose	1
(0.82), 0.95	0.88	0.85	2,3,4-tri-O-methyl-L-arabinose	1
1.15,2.11	0.88	0.85	3,5-di-O-methyl-L-arabinose	34
1.31,(2.38)	0.88	0.85	2,5-di-O-methyl-L-arabinose	1
1.64	0.88	0.85	2,3,4,6-tetra-O-methyl-D-galactose	2.5
(1.91)			2,4- or 3,4-di-O-methyl-L-arabinose	trace
2.60	-	-	2,3,4-tri-O-methyl-D-glucuronic acid*	5
2.88,(3.20)	0.71	0.40	2,3,6-tri-O-methyl-D-galactose	12.5
4.80	0.71	0.40	2,3,4-tri-O-methyl-D-galactose	24.5
7.15	-	-	2,3-di-O-methyl-D-glucuronic acid*	1
9.60	0.48	0.24	2,6-di-O-methyl-D-galactose	1
11.50	0.48		2,4-di-O-methyl-D-galactose	4
12.50	0.48	0.13	2,3-di-O-methyl-D-galactose	9.5
	0.33		2-O-methyl-D-galactose	

Figures in parentheses indicate components which were not completely resolved.

* As methyl ester methyl glycoside.

the amount of formic acid released, by titration of 1ml aliquots of the solution with 0.0967M sodium hydroxide solution using methyl red as indicator.

After ninety-six hours, 5.73 mmoles periodate/g of polysaccharide had been reduced and 1.53 mmoles formic acid/g of polysaccharide had been released. The reaction was stopped by the addition of ethylene glycol (16ml) and the solution was dialysed against running tap water for forty-eight hours. Sodium borohydride (9.2g) was added and the mixture was kept at room temperature for thirty hours, then dialysed for a further forty-eight hours. The solution was made 1N with respect to sulphuric acid by the addition of 4N-acid and the polyalcohol was hydrolysed at room temperature for forty-eight hours. Following dialysis for a further forty-eight hours, polysaccharide 1 was isolated as the freeze-dried product (5.2g, yield 21%).

The analytical parameters for polysaccharide 1 are given in Table VIII.1. Polysaccharide 1 (256mg) was methylated to give a product (80mg) (Found: $[\alpha]_D = -4^{\circ}$ (c 7.0mg/ml in chloroform); OCH_3 32.1%). A portion of this was methanolysed and examined by g.l.c. The O-methyl glycosides found are shown in Table VIII.4. Hydrolysis of the mixture of O-methyl glycosides followed by paper chromatography in solvents (d) and (e) (Table VIII.4) indicated the presence of 2,3 - di - O - methyl - D - galactose and 2 - O - methyl - D - galactose in addition to the O - methyl glycosides identified by g.l.c.

VIII.10. PREPARATION OF POLYSACCHARIDES 2 AND 3 BY SEQUENTIAL SMITH-DEGRADATION

The following weights of polysaccharides, in a sequence of Smith-degradations, were oxidised with periodate, reduced with

TABLE VIII.4

O-METHYL SUGARS IDENTIFIED IN METHYLATED POLYSACCHARIDE 1

Relative Retention Time (T) of Methyl Glycoside	R_g After Hydrolysis Solvent (d)	(e)	O-Methyl Sugar Identified	Relative Proportion (g.l.c.)
0.35, 0.38			unknown sugar	9
0.48			2,3,4-tri-O-methyl-L-rhamnose	2
0.60,(0.65),0.75	0.98	1.00	2,3,5-tri-O-methyl-L-arabinose	4
0.88,(0.92),1.02	0.89	0.85	2,3,4-tri-O-methyl-L-arabinose	2
1.19, 2.23	0.89	0.85	3,5-di-O-methyl-L-arabinose	16
1.33, (1.85)	0.89	0.85	2,5-di-O-methyl-L-arabinose	23
1.71	0.89	0.85	2,3,4,6-tetra-O-methyl-D-galactose	4
2.79	-	-	2,3,4-tri-O-methyl-D-glucuronic acid*	33
(3.02)	0.73	0.43	2,3,6-tri-O-methyl-D-galactose	4
3.87	0.73	0.43	2,4,6-tri-O-methyl-D-galactose	trace
6.06	0.73	0.43	2,3,4-tri-O-methyl-D-galactose	1
10.62	0.49	0.28	2,6-di-O-methyl-D-galactose	1.5
	0.49	0.13	2,3-di-O-methyl-D-galactose	
	0.33	0.08	2-O-methyl-D-galactose	

Figures in parentheses indicate components which were not completely resolved.

* As methyl ester methyl glycoside.

borohydride, hydrolysed with sulphuric acid and the products recovered, all as described for polysaccharide 1. Polysaccharide 1 (4.23g) gave polysaccharide 2 (0.9g, yield 21%) with the reduction of 5.17 mmoles periodate/g of polysaccharide and the release of 1.85 mmoles formic acid/g of polysaccharide. Polysaccharide 2 (0.74g) gave polysaccharide 3 (0.35g, yield 47%) with the reduction of 3.70 mmoles periodate/g and the release of 0.59 mmoles formic acid/g. All weights are corrected for moisture.

Analytical data for polysaccharide 2 are given in Table VIII.1. A sample of polysaccharide 3 contained no nitrogen and its molecular weight was found, by light-scattering, to be 3.42×10^4. The g.l.c. trace of the O-methyl glycosides from methylated polysaccharide 3 (see below) indicated the presence of 11% of glucuronic acid in polysaccharide 3. The sugar composition of polysaccharide 3 (after hydrolysis) is therefore galactose (79%) and arabinose (10%), a composition very similar to polysaccharide 2 (Table VIII.1.). The ratio of non-uronosyl linked galactose to arabinose was 7.2 :1 in polysaccharide 3 and 8.3 :1 in polysaccharide 2.

Polysaccharide 2 (249 mg) was methylated to give a product (89 mg) (Found: $[\alpha]_D = -79^\circ$ (c 5.3 mg/ml in chloroform); OCH_3 34.9%). Polysaccharide 3 (232 mg) was methylated to give a product (131 mg) (Found: $[\alpha]_D = -12^\circ$ (c 0.52 mg/ml in chloroform); OCH_3 21.4%). Portions of each product were methanolysed and examined by g.l.c. The O-methyl glycosides found are shown in Tables VIII.5 and VIII.6. Hydrolysis of the O-methyl glycosides, followed by paper chromatography in solvents (d) and (e), showed the additional presence of 2 - O - methyl - D - galactose in both cases.

Methylation data for the whole gum, degraded gum and Smith-degraded polysaccharides 1 - 3 are summarised in Table VIII.7, and the relative proportions of O-methyl glycosides identified in each

TABLE VIII.5

O-METHYL SUGARS IDENTIFIED IN METHYLATED POLYSACCHARIDE 2

Relative Retention Time (T) of Methyl Glycoside	R_g After Hydrolysis Solvent (d)	(e)	O-Methyl Sugar Identified	Relative Proportion (g.l.c.)
0.38			unknown sugar	3
0.47			2,3,4-tri-O-methyl-L-rhamnose	1
(0.54),(0.59),(0.63),0.72	0.95		2,3,5-tri-O-methyl-L-arabinose	1
0.83, 0.96	0.87	0.87	2,3,4-tri-O-methyl-L-arabinose	0.5
1.11, 2.09	0.87	0.87	3,5-di-O-methyl-L-arabinose	27.5
1.27, 1.45, 2.32	0.87	0.87	2,5-di-O-methyl-L-arabinose	8
1.63	0.87	0.87	2,3,4,6-tetra-O-methyl-D-galactose	trace
1.82			2,4- or 3,4-di-O-methyl-L-arabinose	0.5
2.61	-	-	2,3,4-tri-O-methyl-D-glucuronic acid*	13
2.87, (3.41)	0.71	0.42	2,3,6-tri-O-methyl-D-galactose	26.5
4.74, 5.57	0.71	0.42	2,3,4-tri-O-methyl-D-galactose	7
6.82, 8.32	-	-	2,3-di-O-methyl-D-glucuronic acid*	2
9.08	0.50	0.27	2,6-di-O-methyl-D-galactose	4.5
11.13	0.50		2,4-di-O-methyl-D-galactose	1
12.13	0.50	0.13	2,3-di-O-methyl-D-galactose	2.5
	0.32	0.09	2-O-methyl-D-galactose	

Figures in parentheses indicate components which were not completely resolved.

*As methyl ester methyl glycoside.

TABLE VIII.6

O-METHYL SUGARS IDENTIFIED IN METHYLATED POLYSACCHARIDE 3

Relative Retention Time (T) of Methyl Glycoside	R_g After Hydrolysis Solvent (d)	(e)	O-Methyl Sugar Identified	Relative Proportion (g.l.c.)
0.39			unknown sugar	3.5
0.49			2,3,4-tri-O-methyl-L-rhamnose	1
(0.68), (0.72)			2,3,5-tri-O-methyl-L-arabinose	1
1.00	0.87	0.87	2,3,4-tri-O-methyl-L-arabinose	1
1.16, (2.19)	0.87	0.87	3,5-di-O-methyl-L-arabinose	22
1.35, (1.81)	0.87	0.87	2,5-di-O-methyl-L-arabinose	2
(1.68)	0.87	0.87	2,3,4,6-tetra-O-methyl-D-galactose	0.5
(1.89)			2,4- or 3,4-di-O-methyl-L-arabinose	1
(2.43), 2.69	-	-	2,3,4-tri-O-methyl-D-glucuronic acid*	11
2.97	0.76	0.42	2,3,6-tri-O-methyl-D-galactose	42
(3.80)	0.76	0.42	2,4,6-tri-O-methyl-D-galactose	2
4.99, (5.57), (6.06)	0.76	0.42	2,3,4-tri-O-methyl-D-galactose	2
7.26	-	-	2,3-di-O-methyl-D-glucuronic acid*	1
9.06, 9.85	0.52	0.28	2,6-di-O-methyl-D-galactose	8
13.0	0.52	0.13	2,3-di-O-methyl-D-galactose	1
	0.33	0.09	2-O-methyl-D-galactose	

Figures in parentheses indicate components which were not completely resolved.

* As methyl ester methyl glycoside.

product are compared in Table VIII.8.

TABLE VIII.7

Acacia deanei subspecies paucijuga

METHYLATION DATA FOR THE GUM AND ITS DEGRADATION PRODUCTS*

	WG	DG	P1	P2	P3
Weight polysaccharide taken, mg	312	326	256	249	232
Weight product, mg	80	57	80	89	131
Yield, %	26	17	31	36	56
Product OCH_3, %	33.2		32.1	34.9	21.4
Product $[\alpha]_D$, degrees	-123	-5	-4	-79	-12
($\underline{c}$, mg/ml in chloroform	4.9	7.6	7.0	5.3	0.52)

* WG - P3 as in Table VIII.1.

VIII. 11. DISCUSSION

Purified gum from Acacia deanei subspecies paucijuga was shown by complete hydrolysis with N-sulphuric acid to consist of D-galactose (30%), L-arabinose (43%), L-rhamnose (12%), D-glucuronic acid (12.5%) and its 4 - O - methyl derivative (2.5%).

Partial acid hydrolysis (0.5N) gave three disaccharides, namely 6 - O -β- D - galactopyranosyl - D - galactose (major component), 3 - O -β- D - galactopyranosyl - D - galactose and 3 - O -β- L - arabinopyranosyl - L - arabinose. In addition, 3 - O -β- D -

TABLE VIII.8

Acacia deanei subspecies paucijuga

RELATIVE PROPORTIONS OF O-METHYL SUGARS PRESENT IN

THE GUM AND ITS DEGRADATION PRODUCTS *

	WG	DG	P1	P2	P3
2,3,4-tri-O-methyl-D-glucuronic acid+	5	5	33	13	11
2,3-di-O-methyl-D-glucuronic acid +	1	1		2	1
2,3,4-tri-O-methyl-L-rhamnose	13	0.5	2	1	1
2,3,5-tri-O-methyl-L-arabinose	5.5	1	4	1	1
2,3,4-tri-O-methyl-L-arabinose	4	1	2	0.5	1
3,5-di-O-methyl-L-arabinose	19.5	34	16	27.5	22
2,5-di-O-methyl-L-arabinose	16.5	1	23	8	2
2,4- or 3,4-di-O-methyl-L-arabinose	2	trace		0.5	1
2,3,4,6-tetra-O-methyl-D-galactose	2.5	2.5	4	trace	0.5
2,3,6-tri-O-methyl-D-galactose	4	12.5	4	26.5	42
2,4,6-tri-O-methyl-D-galactose	2.5		trace		2
2,3,4-tri-O-methyl-D-galactose	3.5	24.5	1	7	2
2,6-di-O-methyl-D-galactose	1	1	1.5	4.5	8
2,4-di-O-methyl-D-galactose	1	4		1	
2,3-di-O-methyl-D-galactose	2.5	9.5	p.c.	2.5	1
2-O-methyl-D-galactose	p.c.	p.c.	p.c.	p.c.	p.c.

* WG - P3 as in Table VIII.1.　　+ As methyl ester methyl glycoside

p.c. detected qualitatively by paper chromatography.

galactopyranosyl - L - arabinose (minor component) was detected in the 1N hydrolysate. The presence of 3 - O - β - D - galactopyranosyl - L - arabinose has previously been a characteristic of Series 5 gums in *Acacia* (7 - 9).

Separation of a large-scale 1N-acid hydrolysate on a Duolite A-4 ion exchange resin column into neutral and acidic fractions revealed the presence of three aldobiuronic acids. The major aldobiuronic acid was identified as 6 - O - (β - D - glucopyranosyluronic acid) - D - galactose with the second one identified as its 4 - O - methyl derivative, 6 - O - (4 - O - methyl - β - D - glucopyranosyluronic acid) - D - galactose. The other aldobiuronic acid was identified as 4 - O - (4 - O - methyl - α - D - glucopyranosyluronic acid) - D - galactose, an acid which has previously only been a component of *Acacia* gums having a positive optical rotation (15). The negative optical rotation of this gum (-70°) suggests deep-seated structural differences from dextrorotatory gums i.e. *Acacia*, Series 4 (2 - 6).

The gum was methylated and on subsequent methanolysis and chromatographic examination, 2,3,4 - tri - O - methyl - L - rhamnose, 2,3,5 - and 2,3,4 - tri -, 3,5 -, 2,5 - and 3,4 - or 2,4 - di - O - methyl - L - arabinose, 2,3,4,6 - tetra -, 2,3,6 -, 2,4,6 - and 2,3,4 - tri -, 2,6 -, 2,4 - and 2,3 - di and 2 - O - methyl- D - galactose and 2,3,4 - tri - and 2,3 - di - O - methyl - D - glucuronic acid were identified.

Rhamnopyranose, arabinopyranose, arabinofuranose, galactopyranose and glucopyranosyluronic acid are therefore all present as non-reducing end-group residues, rhamnose and arabinose being the major ones. The three di - O - methylated galactose derivatives detected indicate the presence of 4,6 -, 3,6 - and 3,4 - di - O -

substituted galactose residues in the gum, with the 4,6 - di - O - substituted isomer the most frequent. This is indicative of a highly branched structure in the gum, as already suggested by the large amount of end-group residues and the high molecular weight with average viscosity of the gum. Also present in the gum are chains of β - (1→6) - and β - (1→3) - linked galactose residues as indicated by the identification of 2,3,4 - and 2,4,6 - tri - O - methyl - D - galactose and the disaccharides obtained above.

The identification also of 2,3,6 - tri - O - methyl - D - galactose infers the presence of 4 - O - substituted galactose residues, although (1→4) - linked galactobiose was not detected in the partial hydrolysate of the gum. This isomer may well have arisen from the (1→4) - linked aldobiuronic acid which was detected in the partial hydrolysate. The presence of small amounts of 2 - O - methyl - D - galactose is considered to be from incomplete methylation or from demethylation during hydrolysis. 2,6 - Di - O - methyl - D - galactose is often ascribed to undermethylation as well, but this is probably more important in the third Smith-degraded polysaccharide, which proved difficult to methylate (see later).

The major di - O - methyl - L - arabinoses identified were the 3,5 - (20 parts) and 2,5 - (16 parts) di - O - methyl derivatives, with a little (2 parts) of the 2,4 - or 3,4 - derivative also seen. This is evidence for (1→2) - and (1→3) - linked arabinofuranose chains in the molecule with a small amount of similarly linked arabinopyranose chains. 3 - O - β - L - Arabinopyranosyl - L - arabinose was the only arabinose disaccharide

detected in the whole gum partial hydrolysate. The presence of 2,3,4 - and 2,3,5 - tri - O - methyl L - arabinose indicates that the arabinose-containing chains are terminated in some cases by L-arabinopyranose and in other cases by L-arabinofuranose residues.

The identification of 2,3 - di - O - methyl - D - glucuronic acid shows that the gum contains 4 - O - substituted glucuronic acid residues. The substituent is probably rhamnose, a common feature in *Acacia* gum exudates (5).

Mild acid hydrolysis of the gum with 0.01N acid gave the degraded gum (yield 24%, Table VIII.1), which contained galactose (68%), arabinose (9%), rhamnose (9%), glucuronic acid (12%) and its 4 - O - methyl derivative (2%). That considerable degradation has taken place is indicated by the large decrease in molecular weight (from 10.3×10^5 to 7,041) and viscosity (from $[\eta]$ = 20 to 4 ml/g) and the unusual swing to a positive optical rotation. Such extensive degradation on mild acid hydrolysis is much greater than could result from simple removal of arabinose and rhamnose from the periphery of the gum molecule and there is no evidence for the presence of galactofuranose residues in the polysaccharide. It therefore seems probable that certain galactopyranosidic bonds in the gum are unusually sensitive to mild, acid hydrolysis, as has already been proposed for a number of *Acacia* gums (4,7,8,18,19).

The nitrogen content of the degraded gum (2.0%) is significantly higher than that in the whole gum (0.76%). This may have arisen from a resistant polypeptide in an interior part of the whole gum molecule. Evidence for this in *Acacia* exudates has recently been obtained by other workers (6, 11). Similar increases were observed in the Smith-degraded polysaccharides (see later).

The acidity of the degraded gum (14.5%) is practically the same as that of the whole gum, indicating uronic acid residues in the interior of the molecule; this, and the quantity of di - O - methyl - L - arabinose derivatives in the whole and degraded gums suggests the occurrence of many and unusually long arabinose-containing side chains linked to a densely branched interior in the polysaccharide.

The distilled water dialysate from the preparation of the degraded gum showed the following to be liberated during degradation of the gum: large amounts of arabinose with some galactose and rhamnose and 6 - O - β - D - galactopyranosyl - D - galactose (major disaccharide), 3 - O - β - D - galactopyranosyl - D - galactose, 3 - O - β - D - galactopyranosyl - L - arabinose (trace), 3 - O - β - L - arabinopyranosyl - L - arabinose and 4 - O - (4 - O - methyl - α - D - glucopyranosyluronic acid) - D - galactose. The presence of this acid indicates a labile linkage from the galactose moiety to the remainder of the gum molecule and similarly, the presence of arabinose disaccharides in this mild acid hydrolysate suggests these residues were originally present in the furanose form in the gum.

Methylation and methanolysis of the degraded gum gave 2,3,4 - tri - O - methyl - L - rhamnose, 2,3,5 - and 2,3,4 - tri -, 3,5 -, 2,5 - and traces of 2,4 - or 3,4 - di - O - methyl - L - arabinose, 2,3,4,6 - tetra -, 2,3,6 - and 2,3,4 - tri -, 2,6 -, 2,4 - and 2,3 - di - and 2 - O - methyl - D - galactose, 2,3,4 - tri - and 2,3 - di - O - methyl - D - glucuronic acid. The presence of 2 - O - methyl - D - galactose is ascribed to undermethylation. Thus, rhamnose, arabinopyranose, arabinofuranose, galactose and glucuronic acid are present as end-group residues; in comparison

with the whole gum, the proportion of end-group rhamnose and arabinose residues is considerably less, as expected. The total proportion of arabinose derivatives would appear to be higher than that measured colourimetrically; the arabinose-containing side chains are unusually long, with many (1→2) - linked arabinofuranose units as judged by the high proportion of 3,5 - di - O - methyl - L - arabinose. The di - O - substituted galactose residues are mainly 4,6 - and 3,6 - di - O - substituted revealing the highly branched nature of the gum structure, and the O - substituted galactose units are mainly the 6 - and 4 - linked isomers.

The original gum was subjected to three successive Smith-degradations, involving periodate oxidation, borohydride reduction and controlled acid hydrolysis. Polysaccharide 1 contained galactose (65%), arabinose (18%), rhamnose (8%) and uronic acid (9%). Non-reducing end-group galactose, arabinose, rhamnose, glucuronic acid as well as 6 - O - and 4 - O - substituted galactose and 4 - O - substituted glucuronic acid residues should be cleaved during the first Smith-degradation. Methylation evidence for the whole gum indicates that all the rhamnose and practically all the glucuronic acid residues are present as non-reducing end-groups and complete oxidation of these residues would be expected during the first treatment with periodate. Incomplete oxidation has been observed by previous workers (20), who considered the reason to be steric hindrance, although acetal linkages involving acid fragments are known to be difficult to hydrolyse with cold dilute acid (21). The possibility of uronic acid residues in a highly branched and therefore sterically hindered interior has been suggested for the

degraded gum; the yield of polysaccharide 1 (21%), however, indicates that considerable degradation of the gum molecule has occurred.

Methanolysis of the O - methyl derivative of polysaccharide 1 yielded 2,3,4 - tri - O - methyl - L - rhamnose, 2,3,5 - and 2,3,4 - tri -, 3,5 - and 2,5 - di - O - methyl - L - arabinose, 2,3,4,6 - tetra -, 2,3,6 -, 2,4,6 - and 2,3,4 - tri -, 2,6 - and 2,3 - di - and 2 - O - methyl - D - galactose and 2,3,4 - tri - O - methyl - D - glucuronic acid. The high proportion of di - O - methylated arabinose units suggests there are still long side-chains in polysaccharide 1 containing (1→3) and (1→2) - linked arabinofuranose units and terminated by both arabinofuranose and arabinopyranose residues. The main O - substituted galactose residues are (1→4) - linked with some (1→6) - and a trace of (1→3) - linked units, while the main di - O - substituted galactose units are 3,4 - and 4,6 - di - O - substituted.

A second Smith-degradation yielded polysaccharide 2 (21%), which contained galactose (79%), arabinose (8%) and uronic acid (13%). The O - methyl derivative of polysaccharide 2 contained, apart from 2,4,6 - tri - O - methyl - D - galactose, the same O - methyl glycosides as polysaccharide 1 plus a trace of 2,4 - or 3,4 - di - O - methyl - L - arabinose, 2,4 - di - O - methyl - D - galactose and 2,3 - di - O - methyl - D - glucuronic acid. The various proportions of these products indicate that polysaccharide 2 contains many (1→4) - linked and some (1→6) - linked galactose residues and that the branched galactose units are mainly 3,4 - di - O - substituted with some 4,6 - and 3,6 - di - O - substituted units as well. The relative proportion of (1→4) - linked galactose in polysaccharide 2 is apparently greater than that in polysaccharide 1.

According to the methylation evidence, polysaccharide 2 still contains a little end-group rhamnose together with end-group uronic acid, arabinofuranose, arabinopyranose and galactose residues. The arabinose-containing side-chains are mainly (1→2) - linked and still long. Polysaccharide 1 and the whole gum, in contrast, contain an approximately equal proportion of (1→2) - and (1→3) - linked arabinose side-chains.

A Smith-degradation of polysaccharide 2 gave polysaccharide 3 (47%), which still contained arabinose, uronic acid and some rhamnose. G.l.c. examination of the O - methyl derivative indicated the presence of 11% uronic acid (and 1% rhamnose) and this figure leads to a sugar composition of galactose (79%) and arabinose (10%), which is very similar to polysaccharide 2.

The O - methyl glycosides identified are practically the same as those found in polysaccharide 2, except that the proportion of 2,3,6 - tri - and 2,6 - di - O - methyl - D - galactose are considerably increased. The higher yield and lower méthoxyl content of methylated polysaccharide 3 indicate some under-methylation so these increased proportions and that of 2 - O - methyl - D - galactose are probably too great. 2,4 - Di - O - methyl - D - galactose was not detected in methylated polysaccharide 3 and this may suggest a greater preponderance of 3,4 - and 4,6 - di - O - substituted galactose units in the core of polysaccharide 3 than suggested in polysaccharides 1 and 2. For the O - substituted galactose units, a preponderance of (1→4) - linkages is suggested.

A trend of increasing nitrogen content is clearly seen as the gum is progressively degraded to polysaccharide 2; this may be due to a periodate-resistant polypeptide in the molecule (6, 11). Polysaccharide 3 however, was shown to contain no nitrogen.

It is clear from this structural analysis that three Smith-degradations were not sufficient to expose the "core" of the gum molecule and that no exclusive structure can be proposed for the original gum exudate. However, a number of structural inferences can be made. Polysaccharide 3 appears to contain a highly branched galactan framework composed of 3,4 - and 4,6 - di - O - substituted galactose units and containing 4 - O - substituted i.e. (1→4) - linked galactose chains with some (1→3) - and (1→6) - linked chains also present. There are many ways, of course, in which these residues could be joined together, although relatively high molecular weights and relatively low viscosities in Acacia gum exudates indicate compact, highly branched arrangements. Further Smith-degradations would be required to elucidate the "core" structure more clearly; sufficient starting material was not available for the present study.

The unusually long arabinose "side-chains" attached to this framework in polysaccharide 3 contain many (1→2) - linked arabinofuranose units, although a few (1→3) - linked arabinofuranose and (1→3) - and (1→2) - linked arabinopyranose units are also present. The chains are terminated by non-reducing arabinofuranose, arabinopyranose, some galactose and a significant quantity of glucuronic acid units. These acid units may possibly be attached to C-4 of galactose residues within the galactan framework. The evidence obtained does not exclude the possibility of galactose units along the arabinose chains, whether acid-substituted at C-4 or not, nor does it predict unequivocably at which position the arabinose chains are attached to the framework.

Polysaccharide 2 contains a significant quantity of periodate-vulnerable (1→4) - and (1→6) - linked galactose units and the

relatively high yield of polysaccharide 3 (47%) suggests some steric hindrance from the long side chains and highly branched molecular framework. Polysaccharide 2 also contains periodate-vulnerable 4,6 - di - O - substituted galactose residues, but in a lesser proportion to the periodate-resistant 3,4 - di - O - substituted isomer; this may also contribute to a greater yield of polysaccharide 3.

The structure of the gum from Acacia deanei subspecies paucijuga therefore appears to be a complex, highly branched arrangement of (1→6) -, (1→4) - and (1→3) - linked galactose units, attached to which are very long (1→2) - and (1→3) - linked arabinofuranose (and arabinopyranose) side chains terminated by non-reducing rhamnopyranose, arabinofuranose, arabinopyranose, galactopyranose and glucuronic acid residues. Other noticeable features of the laevorotatory gum are the presence of glucuronic acid linked α- (1→4) to galactose and the presence of 3 - O - β - D - galactopyranosyl - L - arabinose.

The presence of three aldobiuronic acids and long side-chains distinguish this Acacia gum structurally from those in Series 4, Gummiferae and Series 5, Vulgares. The former are dextrorotatory and generally contain four aldobiuronic acids and side-chains greater than eight units long (2 - 6); the latter are laevorotatory, and generally contain two aldobiuronic acids and short side-chains of under eight units long (7 - 9). Series 1, Phyllodineae gums are generally distinguished by being slightly dextro - or laevorotatory and contain, to date, only one aldobiuronic acid and very short side-chains not longer than two units, with the only disaccharides detected thus being galactobioses (12).

Further and more exhaustive analyses of more Series 2, Botryocephalae gums are required to confirm these findings, particularly the suggested division of this Series into two Sub-series.

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THE COMPOSITION OF EIGHT *ACACIA* GUM EXUDATES FROM THE SERIES *GUMMIFERAE* AND *VULGARES**

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Key Word Index—*Acacia*; Leguminosae; gum exudates; protein; glucose; chemotaxonomy; hybrid.

Abstract—An analytical study has been made of gum specimens from *Acacia hebeclada*, *A. kirkii*, *A. newbrownii* and *A. reficiens* (all of the series *Gummiferae*) and of *Acacia erubescens*, *A. fleckii*, *A. mellifera* ssp. *mellifera* and *A. mellifera* ssp. *detinens* (all of the series *Vulgares*). The data obtained give further support for the main chemotaxonomic differences between the *Gummiferae* and *Vulgares* species recorded previously. In addition, two of the species studied have exceptional features; the gum exudate from *A. hebeclada* contains 9.4% of nitrogen; that from *A. erubescens* contains 12% of glucose.

INTRODUCTION

The chemical compositions of the gum exudates from nearly 90 *Acacia* species are now known; the names of the species concerned have been listed recently [2] in series according to Bentham [3] under the subgenera of Vassal [4]. In continuation of the search in this laboratory for *Acacia* gums with unusual chemical features worthy of full structural investigation in the future, this report presents the analytical data obtained for eight *Acacia* species whose gum exudates had not been studied previously. In addition to revealing the existence of *Acacia* gums containing glucose and unusually high nitrogen contents, the analytical data obtained from this form of phytochemical survey are useful for chemotaxonomic purposes.

* Part 55 of the series "Studies of Uronic Acid Materials". For Part 54, see ref. [1].

RESULTS AND DISCUSSION

The analytical data obtained for the eight species studied are shown in Table 1. The four species belonging to the *Gummiferae* have highly positive specific optical rotations; the species belonging to the series *Vulgares* have strongly negative rotations. This substantiates a

Table 1. Analytical data for *Acacia* gums*

	Gummiferae				*Vulgares*			
	Acacia hebeclada	*Acacia reficiens*	*Acacia newbrownii*	*Acacia kirkii*	*Acacia fleckii*	*Acacia erubescens*	*Acacia mellifera* ssp. *detinens*	*Acacia mellifera* (Vahl) Benth. (Sudanese)
Moisture (%)	12.5	11.7	13.0	10.8	12.5	13.2	12.1	8.7
Ash (%)	n.d.	2.4	4.2	1.4	4.0	3.9	3.6	2.9
Nitrogen (%)	9.4	0.65	0.14	0.09	0.58	1.08	1.3	1.45
Hence protein (%) (N × 6.25)	59	4.1	0.88	0.56	3.6	6.8	8.1	9.1
Methoxyl (%)	2.5	1.7	0.50	0.93	0.47	1.4	0.82	1.7
Specific rotation, $[\alpha]_D$, degrees	+28	+89	+43	+54	−32	−31	−45	−56
Intrinsic viscosity, $[\eta]$, ml g^{-1}	13	12	13	8	13	8	21	23.5
Molecular weight, MW × 10^5	n.d.	3.77	3.65	2.08	4.15	2.0	10.4	4.1
Equivalent weight	521	1117	777	1817	918	874	822	843
Hence uronic anhydride (%)	33.8	15.8	22.6	9.7	19.2	20.1	21.4	20.9
% Sugar composition after hydrolysis:								
4-*O*-Methylglucuronic acid	15.0	10.1	3.0	5.6	2.8	8.4	4.9	10.2
Glucuronic acid	18.8	5.7	19.6	4.1	16.4	11.7	16.5	10.7
Galactose	44	41	45	36	39	39	44	43
Glucose	—	—	—	—	3	12	—	—
Arabinose	14	35	27	46	25	17	25	27
Rhamnose	8	8	7	8	14	12	9	9

* Collected in Namibia by Mr. Willy Giess, SWA Herbarium.

possible chemotaxonomic correlation that was noted previously [5].

The data presented for the two subspecies of *A. mellifera*, from widely differing geographical locations, indicate that the compositions of their gum exudates are extremely similar. The major difference recorded involves the MWs of the two specimens; this was also the major difference found in a recent study [1] of the variation between 15 different samples of *A. karroo* gum from widely different African locations. *Acacia laeta* has long been suspected to be a natural hybrid between *A. senegal* and *A. mellifera* [6]: the data now available for *A. mellifera* gum allow comparisons to be made with that published previously for the gums from *A. laeta* [7] and *A. senegal* [8]. The compositions of the three species are closely similar; it is interesting that, where differences occur, the values of the relevant parameters for *A. laeta* gum are intermediate between those for *A. mellifera* and those for *A. senegal*. This is further evidence that closely related *Acacia* ssp. give gum exudates that are closely similar in composition [1, 9, 10].

The gums from *A. fleckii* and *A. erubescens* have been found to contain glucose, as confirmed by the specific test involving glucose oxidase [11]. The presence of glucose in plant gums, first detected in the gum from *Anacardium occidentale* [11], may not be as uncommon as was at first supposed.

The most interesting feature of the analyses reported, however, concerns the remarkably high nitrogen content (9.4%) found for *A. hebeclada* gum. Until other very recent work, in which a nitrogen content of 7.2% was reported [12] for the gum from *A. torulosa* (*Juliflorae*), a nitrogen content of 1.66% [13] was the highest recorded for an *Acacia* gum. Previous studies [14] have shown the nitrogen content of the gums from *Acacia* and other genera to be proteinaceous in origin; attempts to free *Acacia* gum polysaccharides from proteinaceous matter without causing extensive degradation to the gum molecules were not successful [14]. It is important that the rôle played by the proteinaceous material in the production of the physico-chemical properties that are characteristic of the gum exudates should be clarified and that the existence of any direct polysaccharide–protein covalent linkage should be investigated. The relatively small protein content (< 10%) in the *Acacia* gums studied previously did not make such experiments particularly attractive, but knowledge of the existence of *Acacia* gums containing 7–9% of nitrogen, indicative of a possible protein or polypeptide content of 40–55%, now makes the study of such materials a matter of urgency.

EXPERIMENTAL

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Analytical methods. The standard analytical methods have been described [15]. The quantities of gum available for analysis were small; the amount available from *A. erubescens* did not allow determinations of ash nor MW to be made. The extraction of glucose, and its specific identification by means of glucose oxidase, has been described [11].

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Data for the chemical composition of the gum exudates from *Acacia acradenia, A. aneura, A. acuminata, A. beauverdiana, A. coolgardiensis, A. kempeana, A. microneura, A. resinomarginea, A. stereophylla* and *A. torulosa* are presented. Comparisons with the data reported previously for other species of the series *Juliflorae, viz. A. auriculiformis, A. holosericea, A. leptostachya, A. mangium* and *A. pubifolia* confirm that the gums from this series have interesting combinations of chemical properties that are unusual in *Acacia*. The data do not offer much chemotaxonomic support for the groupings of the *Juliflorae* suggested by Bentham (1864). On the basis of their gross morphological features, the species under consideration are re-grouped in a way that is also better supported chemically.

KEY WORDS:–Mimosoideae–*Acacia–Juliflorae*–gums–composition–chemotaxonomy.

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INTRODUCTION

The series *Juliflorae* Bentham, one of the largest and most complex groups of phyllodinous acacias, occurs in both tropical and temperate regions of Australia as well as in a number of Southeast Asian and Pacific Islands (see Pedley, 1975). Chemical studies of this interesting series are still sparse. The flavonoid content of the heartwood of fifteen species was studied by Tindale & Roux (1974, 1975); the distribution of amino acids in the seeds was determined for one species by

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possible chemotaxonomic correlation that was noted previously [5].

The data presented for the two subspecies of *A. mellifera*, from widely differing geographical locations, indicate that the compositions of their gum exudates are extremely similar. The major difference recorded involves the MWs of the two specimens; this was also the major difference found in a recent study [1] of the variation between 15 different samples of *A. karroo* gum from widely different African locations. *Acacia laeta* has long been suspected to be a natural hybrid between *A. senegal* and *A. mellifera* [6]: the data now available for *A. mellifera* gum allow comparisons to be made with that published previously for the gums from *A. laeta* [7] and *A. senegal* [8]. The compositions of the three species are closely similar; it is interesting that, where differences occur, the values of the relevant parameters for *A. laeta* gum are intermediate between those for *A. mellifera* and those for *A. senegal*. This is further evidence that closely related *Acacia* ssp. give gum exudates that are closely similar in composition [1, 9, 10].

The gums from *A. fleckii* and *A. erubescens* have been found to contain glucose, as confirmed by the specific test involving glucose oxidase [11]. The presence of glucose in plant gums, first detected in the gum from *Anacardium occidentale* [11], may not be as uncommon as was at first supposed.

The most interesting feature of the analyses reported, however, concerns the remarkably high nitrogen content (9.4%) found for *A. hebeclada* gum. Until other very recent work, in which a nitrogen content of 7.2% was reported [12] for the gum from *A. torulosa* (*Juliflorae*), a nitrogen content of 1.66% [13] was the highest recorded for an *Acacia* gum. Previous studies [14] have shown the nitrogen content of the gums from *Acacia* and other genera to be proteinaceous in origin; attempts to free *Acacia* gum polysaccharides from proteinaceous matter without causing extensive degradation to the gum molecules were not successful [14]. It is important that the rôle played by the proteinaceous material in the production of the physico-chemical properties that are characteristic of the gum exudates should be clarified and that the existence of any direct polysaccharide–protein covalent linkage should be investigated. The relatively small protein content ($< 10\%$) in the *Acacia* gums studied previously did not make such experiments particularly attractive, but knowledge of the existence of *Acacia* gums containing 7–9% of nitrogen, indicative of a possible protein or polypeptide content of 40–55%, now makes the study of such materials a matter of urgency.

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Data for the chemical composition of the gum exudates from *Acacia acradenia, A. aneura, A. acuminata, A. beauverdiana, A. coolgardiensis, A. kempeana, A. microneura, A. resinomarginea, A. stereophylla* and *A. torulosa* are presented. Comparisons with the data reported previously for other species of the series *Juliflorae, viz. A. auriculiformis, A. holosericea, A. leptostachya, A. mangium* and *A. pubifolia* confirm that the gums from this series have interesting combinations of chemical properties that are unusual in *Acacia*. The data do not offer much chemotaxonomic support for the groupings of the *Juliflorae* suggested by Bentham (1864). On the basis of their gross morphological features, the species under consideration are re-grouped in a way that is also better supported chemically.

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INTRODUCTION

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Seneviratne & Fowden (1968) and for a further six species by Evans, Qureshi & Bell (1977). An analysis of gums from *Acacia auriculiformis* A. Cunn. ex Bentham, *A. holosericea* A. Cunn. ex G. Don, *A. mangium* Willd., *A. leptostachya* Bentham and *A. pubifolia* L. Pedley by Anderson & Gill (1975) revealed a considerable difference in composition and properties from the majority of *Acacia* gums studied so far (Anderson, 1978). The gums from members of series *Juliflorae* appeared to be more proteinaceous, more acidic, more viscous, with higher methoxyl contents and higher molecular weights, but with lower proportions of rhamnose and arabinose.

This report presents the chemical composition of the gums from a further ten species: *Acacia acradenia* F. Mueller, *A. aneura* F. Mueller ex Bentham, *A. acuminata* Bentham, *A. beauverdiana* A. J. Ewart ex Sharman, *A. coolgardiensis* Maiden, *A. kempeana* F. Mueller, *A. microneura* Meissner, *A. resinomarginea* W. V. Fitzg., *A. stereophylla* Meissner, and *A. torulosa* Bentham ex F. Mueller. The analytical data for some of these species extend the interesting inter-species chemical differences indicated previously, and provide additional evidence that the botanical complexity of the series *Juliflorae* is reflected in the wide ranges of values shown for the various chemical parameters that characterize their gum exudates.

The data now available for fifteen species have been examined in terms of the groupings suggested for the series by Bentham (1864). There appears to be little correlation between the gum data and the systematic categories of Bentham. This appears to reinforce previous findings (Pedley, 1975) that Bentham's subgroups within the series *Juliflorae* are highly artificial and that a reappraisal of the classification is desirable. For the species of series *Juliflorae* for which chemical data are available, new (informal) groups based on gross morphological data (Maslin & Pedley, personal communication) are constructed. These informal groupings are supported much more strongly than the groups of Bentham by the chemical evidence available.

MATERIALS AND ANALYTICAL METHODS

Origin of gum specimens from Australia

Acacia acradenia: N.T., Napperby Station, 9. v. 1975, *Latz 5942*.
A. acuminata: W. A., 7 km N. of Mullewa, *Maslin 4268*.
A. aneura: N.T., 26 km W. of Alice Springs, 1975, *Maconochie 37064*.
A. beauverdiana: W.A., 13 km from Holt Rock towards Hyden, *Maslin 3936*.
A. coolgardiensis: W.A., 21.5 km N. of Holt Rock, near Modesty Downs Station, *Maslin 3938*.
A. kempeana: N.T., 16 km W. of Alice Springs, 1975, *Maconochie 37066*.
A. microneura: W.A., 15 km W. of Mukinbudin, *Maslin 3972*.
A. resinomarginea: W.A., Great Northern Highway, 8 km from turn-off to Mount Gibson Station, *Maslin 4236*.
A. stereophylla: W.A., 10 km N. of Southern Cross towards Bullfinch, *Maslin 3958*.
A. torulosa: Queensland, 42 km WNW. of Lakeland Downs, Laura, 9. ix. 1975, *Coveney & Hind NSW. 107876*.

Voucher specimens of the Maslin collection are lodged at Western Australian Herbarium, Perth.

Preparation of samples for analysis

Gum specimens from the members of series *Juliflorae* are difficult to obtain; many species yield gum very sparingly, even when pruning or tapping wounds are made. Consequently, the amounts of crude gum available for analysis were small (1–10 g for the specimens listed here). The gum from *A. torulosa* was dark brown in colour, admixed with pieces of bark; attempted dissolution in cold distilled water for four days gave only a swollen gel, which was dispersed by the addition of traces of dilute alkali and sodium borohydride (Anderson, Bell & King, 1972). This treatment was also necessary for other species of series *Juliflorae* (Anderson & Gill, 1975). The other gum specimens dissolved slowly (1–3 days) in volumes of cold distilled water that were calculated to give 5% gum solutions based on the weight of crude gum taken. After dialysis against tap water for 24 h, and then against distilled water for 2 x 24 h, the gum solutions were filtered through double layers of muslin, then successively through Whatman No. 41, No. 1 and No. 42 papers to obtain clear colourless or pale yellow-amber solutions. The gum was recovered by freeze-drying.

Analytical methods

The standard analytical methods used were described by Anderson, Bell & McNab (1972), with the exception that nitrogen contents were determined with a Perkin-Elmer Model 240 Autoanalyser in order to conserve the amounts of gum samples available for other analyses. For some specimens only 1–2 g of purified gum was available and the ash content was not determined.

RESULTS AND DISCUSSION

The analytical data for the ten *Acacia* species studied are given in Table 1. It is evident that there are extensive variations in the composition of these gum specimens. The parameters that indicate major structural differences in gum polysaccharide molecules are the ranges in values for the specific rotation, for the methoxyl content, for the rhamnose content, and for the ratio of arabinose to galactose. Previous studies of *Acacia* gum exudates, summarized recently by Anderson (1978), showed that gum specimens from *Acacia* species placed in Bentham's series *Gummiferae* have certain features in common. Similarly, the gums from Bentham's series *Vulgares* studied so far (Anderson, Farquhar & Pinto, 1978) show common chemical features and distinct differences from the *Gummiferae*. Previously, five gum specimens from the *Juliflorae* (Anderson & Gill, 1975) were found to have wide variations in chemical composition and properties, e.g., specific rotations ranging from −58° to +58°, methoxyl contents ranging from 0.47 to 2.24%, uronic acid contents ranging from 17 to 37%, intrinsic viscosities ranging from 16.7 to 25.6 mlg^{-1}, rhamnose contents ranging from 2 to 6%, and ratios of galactose to arabinose ranging from 46 : 25 to 54 : 7. The new data further extend these ranges. Thus the methoxyl content now ranges from 0.47% *(A. holosericea)* to 3.40% *(A. microneura)*; the nitrogen content from 0.14% *(A. acradenia)* to 7.2% *(A. torulosa)*; the intrinsic viscosity from 4.6 mlg^{-1} *(A. aneura)* to 69 mlg^{-1} *(A. torulosa)*; the uronic acid content from 15.3 *(A. acradenia)* to 39.7% *(A. microneura)*; the rhamnose content from a trace (0.5%, *A. torulosa, A. resinomarginea*) to 12% *(A. beauverdiana, A. acuminata)*; and the galactose to

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

	Acacia acradenia	*Acacia aneura*	*Acacia kempeana*	*Acacia stereophylla*	*Acacia microneura*	*Acacia coolgardiensis*	*Acacia beauverdiana*	*Acacia torulosa*	*Acacia acuminata*	*Acacia resinomarginea*
Moisture (%)	7.7	(10.0)	9.6	(10.0)	(10.0)	(10.0)	(10.0)	9.7	11.3	8.3
Ash (%)	4.1	n.d.	4.4	n.d.	n.d.	n.d.	n.d.	3.7	5.7	5.3
Nitrogen (%)	0.14	0.18	2.78	0.58	1.01	1.42	0.69	7.2	0.72	0.83
Hence protein (%) (N × 6.25)	0.84	1.12	17.4	3.6	6.3	8.9	4.3	45	4.5	5.2
Methoxyl (%)	0.89	1.50	2.50	0.73	3.40	0.82	0.68	1.60	0.69	3.4
Specific rotation, $[\alpha]_D$, degrees	−8.5	+5.8	−8.3	−12.5	+10.9	−2.2	−27.1	+41	−29	+4.6
Intrinsic viscosity, $[\eta]$, $ml g^{-1}$	13.7	4.6	14.2	12.2	8.1	12.3	10.1	69	21	4.5
Molecular weight, $\bar{M}_w \times 10^6$	0.97	n.d.	0.39	1.61	0.6	1.38	1.56	1.00	0.89	0.18
Equivalent weight	1150	765	456	761	443	501	624	957	656	631
Hence uronic anhydride (%)	15.3	23.0	38.6	23.1	39.7	35.2	28.2	19.0	27.1	28.0
% Sugar Composition after hydrolysis										
4-0-methylglucuronic acid	5.3	9.0	15.0	4.4	20.4	4.9	4.1	9.4	4.1	20.5
Glucuronic acid	10.0	14.0	23.6	18.7	19.3	30.2	24.1	9.6	23	7.5
Galactose	50	60	58	45	54	45	51	53	50	65
Arabinose	32	6	3	21	4	9	9	28	11	7
Rhamnose	3	11	1	10	2	10	12	trace	12	trace

rhamnose ratio ranges from 58 : 3 *(A. kempeana)* to 50 : 32 *(A. acradenia)*. These are large variations, considerably more extensive than have been found for gum specimens assigned to other subdivisions of the genus, and indicate that the series *Juliflorae* is a complex and heterogeneous botanical taxon.

Apart from the extent of the ranges in parameters shown by these species, some of the values found are of great chemical interest as they represent the lower or upper values found so far for several of the key analytical parameters; these, in turn, indicate extremes in structure-type of the complex polysaccharides involved. Thus, *A. holosericea* gives the *Acacia* gum of highest molecular weight (3.8×10^6); *A. microneura* (3.4%) and *A. resinomarginea* (3.4%) gums give the highest values of methoxyl content recorded so far; *A. torulosa* gum is much the most viscous (intrinsic viscosity = 69 mlg^{-1}), and most proteinaceous (%N = 7.2%) of the *Acacia* gums studied to date; the uronic acid contents of *A. kempeana* (38.6%), *A. microneura* (39.7%) and *A. leptostachya* (37.0%) gums are considerably higher than those found for other *Acacia* gums; and the arabinose contents of the gums from *A. kempeana* and *A. microneura* are the smallest recorded. Indeed, the structures of the gums from *A. kempeana, A. microneura* and *A. resinomarginea* (arabinose + rhamnose contents of 4, 6 and 7% respectively) represent one of the extreme chemical structure types, based on a glucurono-galactan, in *Acacia*. Only the gums from *A. falcata* Willd. and *A. calamifolia* Sweet ex Lindley (Anderson, Bell & McNab, 1972), with arabinose + rhamnose contents of 7 and 9% respectively, have similarly low values, but the acidity of these two species is very much less; they are probably best regarded at this stage as approximating to branched galactans.

The fifteen *Juliflorae* species of *Acacia* for which chemical data are now available are grouped below in terms of Bentham's classification (1864). For species not ascribed to a subseries by Bentham, the first author to do so is cited in brackets.

Juliflorae	A.	Rigidulae:	*A. acradenia*
			A. pubifolia (Pedley, personal communication)
	B.	Tetramerae:	No material available
	C.	Stenophyllae:	*A. aneura*
			A. coolgardiensis
			A. microneura
			A. resinomarginea (Gardner, 1931)
	D.	Falcatae:	*A. acuminata*
			A. auriculiformis
			A. beauverdiana (Gardner, 1931)
			A. kempeana (Maiden & Betche, 1916)
			A. leptostachya
			A. stereophylla
			A. torulosa
	E.	Dimidatae:	*A. holosericea*
			A. mangium

Apart from reasonable similarities between *A. acradenia* and *A. pubifolia (Rigidulae)*, the species listed under Bentham's groups C, D and E above cannot be regarded as homogeneous groups on the basis of the chemical data available. In consultation with Maslin and Pedley, the following informal grouping of the

species seems more natural from the morphological and chemical evidence now available:

Group 1. *A. mangium*; *A. auriculiformis*
2. *A. holosericea*
3. *A. acradenia*; *A. pubifolia*
4. *A. leptostachya*; *A. torulosa*
5. *A. beauverdiana*; *A. stereophylla*
6. *A. resinomarginea*; *A. microneura*; *A. kempeana*
7. *A. acuminata*
8. *A. aneura*; *A. coolgardiensis*

Clearly, the aggregation of these species-groups into a formal nomenclatural system must be done in the context of an overall reappraisal of the classification of *Acacia*. The suggestions tentatively offered here may be useful in stimulating and assisting taxonomists in their construction of such a system.

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We thank Rowntree–Mackintosh Ltd. (York) for financial support (to J.G.K.F. and M.C.L.G.) and the collectors mentioned in the text for providing gum specimens. We acknowledge the helpful, constructive advice of B. R. Maslin and L. Pedley, who suggested the new informal groupings listed here as a possible basis for reconciling the chemical data with the gross morphological characters of the species involved.

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