Phytochemistry, Vol. 24, No. 1, pp. 71–75, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

THE GUM EXUDATES FROM SOME CLOSELY RELATED *ACACIA* SPECIES OF THE SUBSERIES *UNINERVES RACEMOSAE* (SECTION *PHYLLODINEAE*)*

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(*Received* 31 *May* 1984)

Key Word Index—*Acacia*; *Phyllodineae*; Leguminosae; gum exudates; chemotaxonomy; amino acids.

Abstract—Australian gum specimens from *Acacia aestivalis*, *A. chrysella*, *A. jennerae* and *A. microbotrya* (five specimens differing slightly in some morphological characters) have been studied. These species, placed within Bentham's Series 1, subseries 6F (*Uninerves racemosae*) are closely related, forming part of the recognized *A. microbotrya* group. The five specimens from *A. microbotrya* show minor variations, similar in extent to those established previously for gums from other species. The gums from *A. chrysella* and *A. jennerae* are similar to those from *A. microbotrya* in chemical composition. The gum from *A. aestivalis* differs from those from *A. microbotrya*, *A. chrysella* and *A. jennerae* in two main respects: it is more acidic and has a much higher methoxyl content. Thus significant differences in gum composition can be shown by some species that differ only slightly in morphological characters. Data for the amino acid compositions of the proteinaceous components of the gums from *A. aestivalis*, *A. jennerae* and *A. microbotrya* differ considerably from those for the gums from other species belonging to the *Uninerves racemosae*, e.g. *A. saliciformis* and *A. xanthina*, which are much more viscous and have higher proteinaceous contents containing much higher proportions of the amino acids commonly involved in linkages with sugars. Of the closely related species studied, *A. aestivalis* is closer to *A. microbotrya* than *A. jennerae* in terms of the amino acid compositions of their gums, a reversal in the relative affinities shown by their polysaccharide parameters. Thus amino acid compositions are of interest chemotaxonomically and also in terms of the tertiary structures of *Acacia* gum exudates.

INTRODUCTION

The section *Phyllodineae* is by far the largest and most variable in *Acacia* [2]. Analytical data for the gum exudates from *ca* 20 phyllodine species (excluding the *Juliflorae*) belonging mostly to Bentham's [3] subseries 6F (*Uninerves racemosae*) and 7F (*Plurinerves nervosae*) are available [4]. The data for the gums from some of the species studied recently [4] extended considerably the ranges of values established previously for the first few species of these subseries studied.

In order to obtain additional data to test further the validity of earlier suggestions [2] that closely related species yield closely similar gum exudates, the opportunity has been taken to study gum specimens from five slightly differing specimens of *A. microbotrya*, and also to compare these analytically with the gums from three closely related species, viz. *A. chrysella*, *A. jennerae* and *A. aestivalis*, which are considered (Maslin, B. R., personal communication) to belong to a well-defined *A. microbotrya* group within the *Uninerves racemosae*. Although some data have been published for *A. microbotrya* gum [5], the full range of analytical parameters was not determined.

As knowledge of the proteinaceous components of gum exudates is one of the factors essential for a more complete understanding of their properties, biosynthesis and tertiary structures [4], the amino acid compositions of the gums from *A. aestivalis*, *A. jennerae* and *A. microbotrya* have been determined.

RESULTS AND DISCUSSION

The analytical data obtained are shown in Tables 1 and 2. The samples of gum from the five slightly different specimens of *A. microbotrya* have similar analytical parameters, yet they are not identical, in support of their collector's ability to discern slight morphological differences. It has long been established [6] that *Acacia* gum exudates are characteristic of a particular species, but that seasonal, geographical, genetic and biosynthetic factors cause slight variations in the composition of the gum nodules produced by different trees of any one species [7]. For *A. microbotrya*, the differences shown by five specimens (Table 1) are similar in extent to those established for other species, e.g. *A. senegal* [8, 9], *A. dealbata* [10], *A. sieberana* [10], *A. nilotica* [11] and *A. karroo* [12]. For *A. microbotrya* gum, the nitrogen content ranges from 0.06 to 0.16%; the methoxyl content from 0.43 to 0.72%; the specific rotation from +4 to −7°; the weight-average molecular weight from 42000 to 460000; the uronic anhydride content from 6 to 8.5%; the rhamnose content from a trace to 3%; and the ratio of galactose to arabinose ranges from 74:17 to 69:21. Such variations, which exceed the limits of experimental error, are not surprising for molecular structures as large and complex as typical gum molecules. Throughout polysaccharide chemistry, one of the major advances in recent years has involved the detection of irregular structural features, e.g. the presence of 'kinking' and other random sugar residues in otherwise

*Part 70 in the series "Studies of Uronic Acid Materials". For Part 69 see ref. [1].

Table 1. Analytical data for gum polysaccharides from *Acacia* species of the section *Phyllodineae*

	A. microbotrya specimen							
	A	B	C	D	E	*A. chrysella*	*A. jennerae*	*A. aestivalis*
Loss on drying, 105°, %	9.8	10.7	(10.0)	(10.0)	(10.0)	(10.0)	9.9	10.7
Total ash, 550°, %*	0.6	0.9	1.05	1.37	1.25	0.98	2.6	3.1
Nitrogen, %*	0.09	0.06	0.16	0.16	0.14	0.11	0.11	0.20
Hence protein (N × 6.25), %*	0.55	0.35	0.98	0.98	0.84	0.69	0.69	1.26
Methoxyl, %†	0.70	0.72	0.63	0.61	0.43	0.39	0.62	2 2
Sp. rotation, $[\alpha]_D$, °†	+4	+4	−5	−3	−7	+1	−8	−18
Intrinsic viscosity, ml/g†	5.4	6.7	6.6	5.9	6.6	4.7	6.5	6.5
MW × 10^5†	0.42	0.71	1.3	1.6	4.6	1 6	0.57	0.54
Neutralization equivalent (electrodialysis)	2980	2730	2110	2190	2310	2720	2450	1310
Hence uronic anhydride, %†‡	6	6.5	8.5	8	7.5	6.5	7	13.5
Sugar composition after hydrolysis, %								
4-*O*-Methylglucuronic acid§	4	4	3.5	3.5	2.5	2.5	2.5	13
Glucuronic acid	2	2.5	5	4.5	5	4	3.5	0.5
Galactose	74	74	72	69	71	72	69	66
Arabinose	17	17	19	21	21	16	22	20
Rhamnose	2	2	tr	3	tr	5	2	1

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

Table 2. The amino acid composition (residues per 1000 residues) of the proteinaceous components of *Acacia* gums from the subsection *Uninerves Racemosae*

	A. aestivalis	*A. microbotrya*	*A. jennerae*	*A. saliciformis**	*A. xanthina**
% Nitrogen	0.20	0.06	0.11	1.2	0.67
Alanine	78	84	100	38	66
Arginine	11	17	22	3	0
Aspartic acid	124	128	90	83	96
Cystine	1	2	9	1	0
Glutamic acid	51	52	34	17	45
Glycine	74	61	62	19	50
Histidine	42	38	36	42	51
Hydroxyproline	55	99	135	287	174
Isoleucine	42	33	31	14	24
Leucine	71	62	58	41	58
Lysine	57	44	29	9	32
Methionine	6	7	5	—	—
Phenylalanine	42	30	15	11	26
Proline	75	80	121	72	79
Serine	93	102	87	171	142
Threonine	75	72	72	85	61
Tyrosine	24	19	19	30	28
Valine	78	72	79	78	70

*Data from ref. [4].

regular sequences, leading to the postulation of much clearer models and more satisfactory explanations of polymer interactions and physico-chemical behaviour in solution.

The gums from *A. chrysella* and *A. jennerae* are closely similar in composition to those from *A. microbotrya*; the minor differences are the slightly lower arabinose and slightly higher rhamnose contents of *A. chrysella* gum, and the slightly higher arabinose content of *A. jennerae* gum. In contrast, the gum from *A. aestivalis* has several distinguishing features, particularly its higher methoxyl content, more negative specific rotation, lower neutralization equivalent, higher uronic anhydride content, and slightly lower galactose content.

Comparisons with the data for other phyllodine species [4, 13] indicate that the gums from *A. microbotrya*, *A. chrysella* and *A. jennerae* show similarities to those from *A. difformis* and *A. mabellae*, with *A. pycnantha* not far removed. The gum from *A. retinodes* is also close, but distinctly more acidic. The gums from *A. calamifolia* and *A. falcata* have distinct similarities. All these gums have characteristically small positive or small negative specific rotations, moderate nitrogen and methoxyl contents, low intrinsic viscosities, uronic anhydride and rhamnose contents, and high ratios of galactose to arabinose. All the other phyllodine species studied so far show the wide variability recognized at an early stage [2] and extended even further recently [4, 14]. The high methoxyl content of *A. aestivalis* gum is comparable with the values shown [4] by the gums from *A. murrayana* and *A. georginae* which are, however, much more acidic; in addition, *A. murrayana* gum is much more proteinaceous. There are several points of similarity between the gums from *A. aestivalis* and *A. rubida*, but *A. rubida* gum is much less acidic and has a much lower methoxyl content.

Thus there are renewed indications that the gum exudates from trees of a particular species show small but distinct variations in their compositions and properties, in keeping with both the complexity of the genus itself and the complexity of typical gum molecules. For some species, only marginal differences in external morphological characters are detectable by experienced fieldsmen; for other species the degree of differentiation is more extensive, leading to the recognition either of complexes or of distinct subspecies. The extent of the differences in gum composition shown by some subspecies has been studied [15, 16].

For species recognized as being closely related in terms of their morphological characters, it is clear that some may yield closely similar gums (e.g. *A. microbotrya*, *A. chrysella*, *A. jennerae*) whilst the gums from other apparently closely related species (e.g. *A. microbotrya*, *A. aestivalis*) differ considerably (Table 1). The constant reshuffling of genes and other inherited factors, particularly for Australian species, which lead to minor differences in some external characters, may, however, lead to more pronounced changes in secondary metabolic products so that some species long regarded as being closely related [e.g. *A. pycnantha* and *A. saligna* (syn. *A. cyanophylla*)] yield gum exudates that differ extensively in composition. When surprise concerning the extent of the differences shown by these two species was first expressed [17], data were available for very few *Acacia* exudates, comparisons in terms of subdivisions of the genus had not been undertaken, and the breadth of analytical parameters now known [4, 14] to be a feature of the gums from phyllodine species had not been established. Even yet it is clearly premature to draw conclusions from the data available for gum exudates as, even in the subsections most extensively studied to date (*Uninerves racemosae* and *Plurinerves nervosae*), only *ca* 25 out of an estimated total of some 300 species have been studied. Nevertheless, the

differences shown by closely related pairs of species, e.g. *A. saligna* and *A. pycnantha*, could scarcely have failed to attract reasonable, speculative comment which has served to stimulate interest and research activity. The recent additional data have shown that *A. pycnantha* remains central within a wide range of phyllodine species and that *A. saligna* gum is no longer as unique in composition as it was when first studied. The reason for the wide differences between *A. pycnantha* and *A. saligna* gums remains to be established, but now that other closely related pairs of species have shown considerable divergence in gum compositions, the need to suggest the reallocation of *A. saligna* to a different subsection of the genus is no longer justifiable. Rather than consider the data for gum exudates in isolation, it may be prudent chemotaxonomically to consider collectively the evidence, where available, for other secondary metabolic products, e.g. for flavonoids [18], amino acids [19]. Data for the amino acid compositions of the proteinaceous components of gum exudates may prove to be useful chemotaxonomic markers.

It has long been established [7] that one of the variable analytical parameters for gum exudates is their nitrogenous content; that the nitrogenous component is associated with high molecular weight fractions [20]; that the nitrogenous component is almost completely associated (from nitrogen recovery data) with proteinaceous material [21] whose separation from the polysaccharide components is not readily achievable for some, but not all, *Acacia* gums [7, 11]; and that the properties of *Acacia* gum solutions are best understood in terms of the involvement of their nitrogenous components [22]. The importance of their proteinaceous components remains central to a more complete understanding of the properties, tertiary structure, and biosynthesis of complex gum molecules [4].

The amino acid compositions for the gums from *A. aestivalis*, *A. jennerae* and *A. microbotrya* are now compared (Table 2) with those available for other species within the *Uninerves racemosae*, i.e. *A. saliciformis* and *A. xanthina* [4]. In addition to the considerable differences in nitrogen content shown by these gums (0.06–1.2%), the virtually complete recovery of the nitrogen content as amino acids after drastic hydrolysis of the proteinaceous matter has revealed that these nitrogen values reflect protein contents, respectively, ranging from *ca* 0.4 to *ca* 8% for the species under consideration. There are also interesting differences in their amino acid compositions, with the five gums (Table 2) showing reasonably constant values for some amino acids (e.g. cystine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tyrosine and valine) but widely different proportions of others. The closest correspondences are between *A. saliciformis* and *A. xanthina* (major components: hydroxyproline and serine, both in high proportions) and between *A. aestivalis* and *A. microbotrya* (major components: aspartic acid and serine, with hydroxyproline more minor, particularly for *A. aestivalis*). The amino acid composition for *A. jennerae* appears to be intermediate between these pairs, with hydroxyproline, proline and alanine as its major components. Before these differences can be rationalized, a knowledge of the amino acid compositions for many more gums is required. The proteinaceous content of the complex gum molecules may comprise a mixture of proteins, whose relative proportions vary for different *Acacia* species.

EXPERIMENTAL

Origin of gum specimens. Gum specimens from *A. microbotrya* Benth., *A. chrysella* Maiden et Blakely, *A. jennerae* Maiden and *A. aestivalis* E. Pritzel were collected in Western Australia by Mr. B. R. Maslin under the following numbers: *A. microbotrya* sample A (BRM 3823); sample B (BRM 3969); sample C (BRM 4098); sample D (BRM 4101); sample E (BRM 4124). Of these, sample A was regarded at the time of collection as being typical *A. microbotrya*, from which sample B differed very slightly; sample C was regarded as slightly atypical; sample D was secured from a number of trees and regarded as a local variant; sample E was from a tree that yielded gum unusually copiously and regarded as a northern form of *A. microbotrya*. *A. chrysella* was sent as BRM 4153; *A. jennerae* as BRM 3961 and *A. aestivalis* as BRM 4092.

Preparation of gums for analysis. The gums (1–3 g, as available) were dissolved in distilled H_2O (100 ml), with occasional stirring, for 48 hr. The solns were filtered (muslin, then Whatman No. 1 and No. 42 papers), dialysed (for 2 days *vs.* tap water; for 1 day vs. distilled water) then recovered by freeze-drying. All the gums gave pale brown solns of very low viscosity, and are unlikely to be of any commercial interest.

Analytical methods. The standard analytical methods used for the polysaccharide components have been described [13]. The amino acid analyses were carried out with an automated analyser (Rank-Hilger Chromaspek) after hydrolysis of the gum samples in boiling 6 M HCl for 20 hr in N_2.

Acknowledgements—We thank Rowntree-Mackintosh p.l.c. (York) for financial support (to M.C.L.G. and F. J. McD.) and Mr. Bruce R. Maslin (Western Australian Herbarium, Perth) for the collection of gum specimens and helpful comments on botanical relationships within the *Phyllodineae*.

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