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THE PROTEINACEOUS COMPONENTS OF THE GUM EXUDATES FROM SOME PHYLLODINOUS *ACACIA* SPECIES*

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

Abstract—*Acacia* gum exudates are proteinaceous polysaccharides; their protein content ranges from *ca* 0.2 to 45%. The data presented show that the amino acid compositions of the gums from 12 phyllodinous species (10 from Bentham's sub-series *Uninerves racemosae*, two from sub-series *Juliflorae*) also vary considerably, particularly in respect of their hydroxyproline content (55 residues per 1000 residues in *A. aestivalis* gum, 287 residues per 1000 in *A. saliciformis* gum). The proportions of some other amino acids, e.g. alanine, aspartic acid, proline and serine also vary considerably, but the proportions of others, e.g. cystine, methionine, histidine, threonine, tyrosine and valine, are remarkably constant. The amino acid composition of gums with a very low protein content (e.g. *A. victoriae* and *A. mycrobotrya*) is similar to that for a highly proteinaceous gum (*A. tumida*). There are, however, considerable differences between the amino acid compositions of the gums from *A. saligna* and *A. pycnantha* (South African and Western Australian specimens). This strengthens previous chemotaxonomic evidence, based on the polysaccharide parameters of their gums, that these two species are not as close taxonomically as was originally believed from morphological considerations.

INTRODUCTION

Following early observations that *Acacia seyal* gum contained a nitrogenous component [2] and that nitrogen was associated with high but not with low M_r fractions of *A. senegal* gum [3], it was reported that all plant gum exudates studied previously contained varying amounts of nitrogen ranging from traces (0.04%) to 5.6% and this nitrogenous content was shown [4] to be proteinaceous. Moreover, the amino acid compositions differed extensively [4] for different plant genera, e.g. *Acacia*, *Araucaria*, *Azadirachta* and *Lannea*. These differences have been confirmed in recent studies [5] of the gums from *Brachystegia* sp. (major components lysine and proline) and from *Julbernardia globiflora* (major components alanine and glutamic acid).

Further studies of *Acacia* gums have shown [6] that several (e.g. *A. torulosa*, *A. tumida*, *A. difficilis*, *A. eriopoda*, *A. stipuligera*) but not all (e.g. *A. dimidiata*, %N = 0.26) of the section *Juliflorae* are highly nitrogenous, corresponding to protein contents of 40–50%. This confirmed and extended previous polysaccharide evidence [7] that the *Juliflorae* comprises a number of species with widely differing chemical properties; it was suggested [7] that such species might be re-grouped more meaningfully taxonomically than they are at present. Botanically, the *Juliflorae* is the most complex group of phyllodinous *Acacias* [8].

Studies were, therefore, required to extend present knowledge [9] of the variations in amino acid composition shown by species assigned to Bentham's [10] sub-series *Uninerves racemosae* and to discover if the amino acid compositions of highly nitrogenous species differed from those with very low nitrogenous contents. In addition, studies of *A. pycnantha* gum (South African and Western Australian specimens) and of *A. saligna* gum (Northern Territory and Western Australian specimens) have been made to discover if their amino acid compositions differ significantly in view of the continued chemotaxonomic interest in these species.

RESULTS

The analytical data obtained for gum specimens from *A. pruinocarpa*, *A. pycnantha* (two specimens), *A. victoriae*, *A. tumida*, *A. ligulata*, *A. difficilis* and *A. saligna* (two specimens) are compared (Table 1) with the values reported [9] for *A. aestivalis*, *A. microbotrya*, *A. jennerae*, *A. xanthina*, and *A. saliciformis*. Of these, *A. tumida* and *A. difficilis* are placed within the *Juliflorae*, all others within Bentham's [10] sub-series *Uninerves racemosae*. To provide some basis, albeit arbitrary, for systematic comparisons, the data for these 12 species are arranged in terms of their increasing content of hydroxyproline, the amino acid showing the greatest range of values.

DISCUSSION

There are over 700 phyllodinous Australian *Acacias* (infrageneric terminology in *Acacia* is confusing and complex [Maslin, B. R., personal communication] with modern authorities (e.g. Pedley, Maslin) regarding section Phyllodineae as equivalent to Bentham's [10] series *Uninerves*); therefore, the number of species for which analytical data are available is small. Nevertheless, the

*Part 73 in the series "Studies of Uronic Acid Materials". For Part 72 see ref. [1].

Table 1. The amino acid composition (residues per 1000 residues) of the proteinaceous components of some phyllodinous *Acacia* gums

				A. pycnantha								*A. saligna*		
	A. aestivalis	*A. microbotrya*	*A. pruinocarpa*	South Africa	Western Australia	*A. jennerae*	*A. tumida*	*A. victoriae*	*A.xanthina*	*A. ligulata*	*A. difficilis*	Northern territory	Western Australia	*A. saliciformis*
% Nitrogen	0.20*	0.06*	0.65	0.25	0.28	0.11*	6.5	0.03	0.67*	0.25	7.2	0.18	0.12	1.2*
Alanine	78	84	67	88	72	100	55	81	66	69	42	61	55	38
Arginine	11	17	1	6	15	22	23	13	0	19	20	3	0	3
Aspartic acid	124	128	118	147	105	90	100	107	96	97	83	93	68	83
Cystine	1	2	6	13	9	9	5	0	0	0	5	2	2	1
Glutamic acid	51	52	64	40	43	34	50	53	45	44	43	30	41	17
Glycine	74	61	60	63	58	62	62	59	50	44	55	37	64	19
Histidine	42	38	35	27	29	36	32	41	51	37	26	28	29	42
Hydroxyproline	55	99	96	134	166	135	135	138	174	190	197	257	247	287
Isoleucine	42	33	21	31	25	31	45	23	24	20	45	21	19	14
Leucine	71	62	71	91	75	58	66	47	58	56	61	44	44	41
Lysine	57	44	42	31	38	29	46	41	32	25	36	23	17	9
Methionine	6	7	2	0	2	5	2	0	0	1	2	2	0	0
Phenylalanine	42	30	39	13	13	15	35	24	26	29	29	13	12	11
Proline	75	80	125	52	59	121	77	75	79	77	67	68	62	72
Serine	93	102	102	105	119	87	98	135	142	132	107	150	162	171
Threonine	75	72	51	50	54	72	64	72	61	57	64	62	60	85
Tyrosine	24	19	24	19	36	19	30	19	28	28	28	36	36	30
Valine	78	72	80	88	82	79	75	63	70	69	91	71	74	78

*Data from ref. [9].

species studied show a wide range of values for hydroxyproline: there are indications that the serine content tends to increase and the proportions of aspartic acid, glutamic acid, leucine and lysine to decrease as the hydroxyproline content increases. Data for nearly 200 gum specimens from a wide range of genera, acquired [Anderson, D. M. W. and McNab, C. G. A., unpublished results] during the past 3 years, are currently being evaluated prior to publication.

Although nitrogen contents of less than 0.1 % have been reported [4, 9, 11] e.g. for *A. leucoclada* (0.04 %), *A. microbotrya* (0.06 %) and *A. cyclops* (0.08 %), the nitrogen content of *A. victoriae* is even lower (0.02–0.03 %). Subsequently, it was shown that this, nevertheless, does represent a proteinaceous component with an amino acid composition similar to that of species with reasonably close affinities (*A. xanthina* and *A. ligulata*).

Table 1 also shows that highly nitrogenous [6] gums (*A. tumida* and *A. difficilis*) have amino acid compositions that correspond closely with those for species having low nitrogen contents and, moreover, with species currently assigned to different taxonomic sub-series. Thus, *A. tumida* (6.5 % N) corresponds well in its amino acid composition with *A. victoriae* (0.03 %) and *A. jennerae* (0.11 % N). Also, *A. difficilis* (7.2 % N, *Juliflorae*) corresponds well with *A. ligulata* (0.25 % N, *Uninerves racemosae*). Taxonomically this is an interesting, provisional, observation; morphological affinities within *Uninerves racemosae* have been confirmed for some species yet affinities, in at least this chemotaxonomic parameter, are also suggested between species in separate sub-series.

Table 1 shows that there is a good correspondence between the data for the gum specimens from *A. saligna* collected in Northern Territory and in Western Australia; the correspondence between the South African and Western Australian specimens of the gum from *A. pycnantha* is not so good. In view of the geographical differences involved, the adaptations in an introduced species and the very complex nature of these natural products, it is possibly surprising that the correspondence is as close as has been found. Good general agreement for many polysaccharide parameters with, however, marked geographical and seasonal variations, has been recorded for the gums from several *Acacia* sp. [12, 13]. Nevertheless, the differences in the amino acid profiles for *A. pycnantha* and *A. saligna* gums are of chemotaxonomic interest. Because the polysaccharide parameters of these two species differ so widely [4, 14], it has been suggested [14] that *A. saligna* should be re-classified within the *Juliflorae*. In terms of their amino acid compositions (Table 1) *A. pycnantha* and *A. saligna* each have closer affinities with other species (e.g. *A. saligna* with *A. saliciformis*) than they have with each other. This, therefore, confirms the previous polysaccharide evidence, summarized recently [9]. Although *A. saligna* and *A. pycnantha* have long been regarded as closely related morphologically, they do have very different numbers of flowers in each capitulum [4] and, on the basis of pollen data, are not very closely related, the pollen characters suggesting [Guinet, P., personal communication] that *A. saligna* has closer affinities with *A. dentifera*. Unfortunately, the gum from *A. dentifera* has not been available for chemical investigations. According to a leading expert in the taxonomy of Australian *Acacias*, *A. saligna* cannot be conceived as belonging to any group other than the *Uninerves racemosae* [Maslin, B. R., personal communication] although *A. saligna* and *A. pycnantha*, in the light of the chemotaxonomic evidence, are no longer regarded as being as close as was formerly believed; differences in certain minor morphological characters may have been underestimated in importance in the past [Maslin, B. R., personal communication]. It has also been concluded [9] that the chemical parameters for *A. saligna* gum can no longer be regarded as being so unusual for a member of the *Uninerves racemosae* as appeared to be the case earlier [4]. Nevertheless, *A. saligna*, on the basis of both its polysaccharide and its amino acid parameters, does have close chemical similarities with some *Juliflorae* species; equally, *A. dimidiata* [7] has chemical properties that are more typical of the *Uninerves racemosae* than of the *Juliflorae*. Such conflicts between the chemically and morphologically-based affinities may yet stimulate taxonomic re-considerations of the relative affinities of at least some species.

Table 1 shows that there are variations in the amino acid compositions of the gums from *Acacia* sp. assigned to one sub-series; the differences shown for *A. aestivalis* and *A. saliciformis* are extensive, particularly for those amino acids well-known [15] to participate frequently in sugar–amino acid linkages in glycoproteins and proteoglycans, as was recognized [4] when amino acid data for gum exudates were first published. Although the presence of proteinaceous components has continued to be reported for the gums from all the genera studied within this Chemistry Department, since that data, e.g. for *Combretum* [16], *Prosopis* [17], *Anacardium* [18], *Grevillea* [19] spp., etc., little interest in this aspect of gum chemistry has been shown by other groups of workers until recently, when a nitrogen value of 0.2 % was recorded [20] for one specimen of *A. karroo* gum, in agreement with the range of values recorded [12] for 15 different specimens of *A. karroo*, which is recognized as being highly variable. The value of 44 % arabinose reported [20] for *A. karroo* gum is not untoward for a species for which a range of 20–40 % has been recorded [12]. Even more recently there has, however, been confirmation [21] that the gum from *A. senegal* is a proteinaceous polysaccharide [3, 13]. Evidence of further strengthening of interest in protein–polysaccharide interaction arises from the recognition that arabinogalactans are often attached to greater or lesser amounts of protein in plant exudates [22], that a hemicellulosic arabinoxylan–protein complex gave a high M_r fraction having a peptide moiety rich in hydroxyproline and serine [23], and that glycogen of high M_r contains nitrogen and differs structurally from its low M_r counterpart [24]. The fact that *A. senegal* gum can be fractionated into nitrogen-enriched fractions of high M_r and nitrogen-depleted fractions of low M_r has long been recognized [3].

The data reported indicate that the protein–polysaccharide interactions and/or linkages are likely to be complex and that a better understanding is essential for more fundamental considerations of the true structure of natural gum exudates; recent re-evaluations of the complex highly branched structures proposed [25, 26] for *Acacia* gums have considered solely the polysaccharide component [27, 28]. The highly proteinaceous *Acacia* gums (Table 1) will serve as excellent model compounds, but with the knowledge that the proteinaceous components vary not only in the percentage to which they are present but also in terms of their amino acid compositions, it appears likely that the proteinaceous com-

ponent is of structural importance even in those species with very low nitrogen contents.

EXPERIMENTAL

Origin of gum specimens. The origins of the specimens from *A. aestivalis*, *A. microbotrya*, *A. jennerae*, *A. xanthina* and *A. saliciformis* have been given [9], as have those for *A. tumida* and *A. difficilis* [6]. The following specimens were kindly provided by Mr. B. R. Maslin: *A. victoriae* Benth. (BRM 4245); *A. saligna* (Labill.) H. Wendl. (BRM 3816); *A. pycnantha* Benth. (BRM 3982); *A. pruinocarpa* Tindale. The samples from *A. saligna* (Northern Territory) and *A. ligulata* Cunn. were sent by (the late) Mr. J. R. Maconochie, Alice Springs, N.T.

Analytical methods. The amino acid analyses were carried out with an automated analyser (Rank–Hilger Chromaspek) after hydrolysis of the samples in boiling 6 M HCl for 20 hr under N_2.

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