Phytochemistry, Vol. 23, No 9, pp. 1923–1926, 1984
Printed in Great Britain

0031-9422/84 $3 00 + 0 00

SOME *ACACIA* GUM EXUDATES OF THE SECTION *PHYLLODINEAE**

D. M. W. ANDERSON, M. C. L. GILL, C. G. A. MCNAB and G. DE PINTO

Chemistry Department, The University, Edinburgh EH9 3JJ, U.K.

(*Received* 22 *February* 1984)

Key Word Index—*Acacia*; Mimosaceae; gum exudates; chemotaxonomy, amino acids.

Abstract—Australian gum specimens from *Acacia salicíformis*, *A. xanthina*, *A. rostellifera*, *A. murrayana* (two specimens differing in the mode of initiation of gum exudation), *A. georginae*, *A. cyclops*, *A. implexa*, and an un-named species (Maslin 'P31') have been analysed. The first four of these are placed within Bentham's Series 1, subseries 6F, *A. georginae* within subseries 7E, and the remainder within subseries 7F. These data extend considerably the ranges of the analytical parameters reported previously for phyllodine species. The molecular weights of the gums from *A. cyclops* and *A. implexa* are much higher than those reported earlier for South African specimens; this may affect some taxonomic deductions based on their examination. The gum composition of *A. saligna* can no longer be regarded as atypical of a phyllodinous species; a suggestion that *A. saligna* should be transferred to the section *Juliflorae* may require reconsideration. The major difference between the specimens of gum from *A. murrayana* lies in their nitrogenous content. Data are reported for the amino acid compositions of the gums from *A. salicíformis* and *A. xanthina*.

INTRODUCTION

Since the suggestion [2] that analytical differences in the composition and/or structure of the gum exudates from different *Acacia* species might be useful taxonomically, the number of species studied has increased steadily, including recent contributions for species in Bentham's [3] sections *Gummiferae* [4, 5], *Vulgares* [4], *Juliflorae* [6, 7] and *Botryocephalae* [8]. Following the publication of analytical [9] and structural [10] data for species within the section *Phyllodineae*, studies begun in 1976 [11] were suspended within the period 1977–1983 so that priority could be given urgently to essential toxicological studies [e.g. 12, 13] of gum arabic (*Acacia senegal* (L.) Willd.) and gum karaya (*Sterculia*). Progress was, however, maintained for South African specimens of the gums from *A. saligna* [14, 15], *A. cyclops* and *A. implexa* [15, 16]; data for Australian gum specimens from *A. cyclops* and *A. implexa* are now reported.

Studies of exudates arising from unusual forms of stimuli may yield useful information concerning the mechanism of gum formation and biochemical gum precursors [17, 18]. Gum specimens from *A. murrayana* arising from (a) natural exudation and (b) chemical defoliation have therefore been studied.

At present, insufficient is known of the nitrogenous components of gum exudates in view of the correlation [19] between nitrogen content, intrinsic viscosity, and molecular weight; the unpredictable behaviour of the nitrogenous component [17]; polysaccharide/protein interactions [20, 21]; and the possibility [22] that the proteinaceous components may involve relictual fragments from enzymatic systems concerned in the process of gum biosynthesis. The amino acid [23] compositions of the gums from *A. saliciformis* and *A. xanthina* are therefore reported.

RESULTS AND DISCUSSION

The analytical data obtained are shown in Table 1. Data for about 20 phyllodine species, mostly belonging to Bentham's [3] subseries 6F (*Uninerves racemosae*) and 7F (*Plurinerves nervosae**) are now available. The section *Juliflorae* is complex and has been treated separately [6, 7]. From the data in Table 1 it is apparent that the gums of subseries 6F and 7F are even more variable in composition than was apparent previously, e.g. nitrogen (0.08–4.1%); methoxyl (0.4–2.9%); specific rotation (−54° to +90°); intrinsic viscosity (4–39 ml/g); rhamnose (trace–23%); and ratio of galactose/arabinose (from 2/1 for *A. salicíformis* and *A. xanthina* to 25/1 for *A. implexa* gum).

Following the suggestion that differences in the composition and structure of *Acacia* exudates might be useful taxonomically [2], the most frequently quoted differences have involved *A. saligna* and *A. pycnantha* gums [11, 14]. Perhaps fortuitously, it appeared from the data for the first few phyllodine species that happened to be studied [9] that the *Phyllodineae* were characterized by a low rhamnose content, low acidity, low intrinsic viscosity, and a high galactose/arabinose ratio. On this basis *A. rubida* gum [9] appeared to be slightly divergent and *A. saligna* gum [24] appeared [11] to be highly anomalous. In view of their accepted morphological resemblance, the undoubted chemical differences [9, 24] between the gums from *A. pycnantha* and *A. saligna* led to studies [14–16] in which a similarity between the gums from *A. saligna*, *A. implexa*, *A. cyclops* (all section *Phyllodineae*) and that from *A. longifolia* [25] (section *Juliflorae*) was observed. Recently, the gum from *A. maidenii* has been shown [7] to be closely similar to that from *A. longifolia* [25], perhaps not surprisingly as both these species are tetramerous

*Part 68 in the series "Studies of Uronic Acid Materials" For part 67, see ref [1].

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

	A. saliciformis	*A. xanthina*	*A. rostellifera*	*A. murrayana* (NE)	*A. murrayana* (D)	*A. georginae*	*A. cyclops*	New *Acacia* 'P31'	*A. implexa*
Loss on drying, 105°C, %	10 4	4.0	10.2	15.0	(10 0)	8.1	9.1	6.7	9.2
Total ash, 550°C, %*	3.1	4.0	3.0	5.2	n.d.	3.3	3.6	5.8	5 7
Nitrogen, %*	1.2	0.67	0.18	2.7	4.1	0.44	0.08	0.23	0.09
Hence protein (N × 6.25), %*	7.5	4.2	1.1	16.9	25 5	2.8	0.55	1.47	0.6
Methoxyl, %†	1.1	1.1	0.75	2.2	2.9	2.4	0.55	0.38	0 4
Sp. rotation, $[\alpha]_D$, degrees†	−54	+20	+90	−9	−24	−8	−21	−32	−31
Intrinsic viscosity, ml/g†	39	11	10	17	14	4	7	6	11
Molecular weight, $\bar{M}_w \times 10^5$†	20	14	9.5	7.4	5 1	0.95	3.0	5 1	1 2
Neut equiv. (electrodialysis)†	1400	910	690	540	520	610	890	560	680
Hence uronic anhydride, %†‡	13	19	26	33	34	29	20	33	26
Sugar composition after hydrolysis, %									
4-*O*-Methylglucuronic acid§	6.5	6.5	5	13	17	14 5	3.5	2.5	2 5
Glucuronic acid	6.5	12.5	21	20	17	14.5	16.5	30.5	23.5
Galactose	55	52	58	59	58	61	68	46	51
Arabinose	26	29	12	6	8	6	5	5	2
Rhamnose	6	tr	4	2	tr	4	7	16	23

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

members of the section *Juliflorae*. The new *Acacia* 'P31' (section *Phyllodineae*) also gives (Table 1) a gum having the particular combination of analytical parameters (significantly negative rotation, high uronic acid and rhamnose contents, low arabinose content) first recognised in *A. saligna* gum [24], which is, therefore, no longer seen as an anomalous [11] member of the *Phyllodineae*.

The chemical composition and structures of *A. saligna* and *A. longifolia* gums appear to be closely similar [14] and it was therefore suggested [14] that *A. saligna* should be regarded as being close to *A. longifolia*. The work of Pettigrew and Watson [26] was cited [14] in support of this suggestion, but it is only within their Fig. 2 that *A. saligna* and *A. longifolia* occur in the same group—they are clearly separated in Pettigrew and Watson's Figs 1.1 and 1.2 [26]. Correspondence with several leading international authorities on the taxonomy of *Acacia* species has indicated [cf. 9, 27] that *A. saligna* and *A. pycnantha* are clearly referable to the section *Phyllodineae*, but may not be quite so closely related as was originally held. The inclusion of *A. saligna* within section *Juliflorae* appears to be difficult to envisage taxonomically.

Nevertheless there exists the intriguing chemotaxonomic situation that *Acacia* species of close affinity (e.g. *A. difficilis*, *A. maidenii*) have closely similar gum compositions which are, however, also similar to those for species (*A saligna*; 'P31') that are not closely related. Vice versa, there have long been clear indications that species with close affinities (e.g. *A. saligna*, *A. pycnantha*) have widely differing gum compositions, and further examples of this can now be added; *A. xanthina* is close to *A. rostellifera* [B. R. Maslin, personal communication] and *A. saliciformis* is close to *A. mabellae* [M. D. Tindale, personal communication] yet the data available (Table 1 and ref. [9]) reveal considerable differences in several of their analytical parameters. Although the structure of gum polysaccharides must be regarded as a highly important factor in the taxonomy of the genus *Acacia* [14], the non-correspondence between gum composition and morphological affinity, already established for several species, suggests that gum chemistry may not be as useful taxonomically as was previously hoped [11, 14]. Suggestions that *A. cyclops* gum is similar to *A. podalyriifolia* gum [16], and that the gums from *A. implexa* [16] and *A. mabellae* [9] are similar, appear to be difficult to sustain in terms of their widely differing specific rotations, acidities, and proportions of neutral sugars.

Comparisons of the partial analytical data for South African [16] gum specimens from *A. cyclops* and *A. implexa* with the full data for Australian specimens (Table 1) reveals that there is excellent agreement for most parameters, but that the Australian specimens have much higher MWs than the South African specimens. There are chemical similarities between the gums from *A. saligna*, *A. implexa*, *A. cyclops* and *A. longifolia* [16] but it was suggested that *A. implexa* gum is simpler than *A. saligna* and *A. longifolia* gums and that *A. cyclops* gum is even more simple than *implexa* gum on the basis of their low MWs [15]. It may be possible that the South African gum samples available were not completely typical; the data reported for *A. cyclops* were obtained [16] for only 232 mg of gum. Previous comparisons between

Australian and South African gum specimens, e.g. from *A. pycnantha*, *A. saligna* and *A. dealbata*, have shown good agreements [cf. 9, 28].

When it proved difficult to obtain reasonable amounts of gum from *A. murrayana* and other *Acacia* in Northern Territory, attempts to stimulate gum exudation through chemical defoliation with 2,4-dichlorophenoxyacetic acid were made. In general these were not successful, but one *A. murrayana* tree did exude a small amount of gum from a stem crack after defoliation [J. R. Maconochie, personal communication, 1974]. The data for the natural exudate (NE) and defoliated (D) specimens of gum from *A. murrayana* are shown in Table 1. The differences in the specific rotations and in the nitrogen contents, which are in any event high compared with the values for other phyllodine species, are the most salient differences between these two gum specimens. Two further, very small, specimens of gum from *A. murrayana*, obtained subsequently by Mr. Maconochie in September 1974 and May 1975 from trees attacked by wood-boring insects were found to have nitrogen contents of 3.61% and 3.10% respectively. The gum from *A. murrayana* is therefore undoubtedly quite highly but variably nitrogenous. Nitrogen values ranging from 0.04% [24] to 6–8% [7] and even 9.4% have been reported for *Acacia* gums. It is feasible that the proportions of the nitrogenous components of a gum exudate may be more sensitive to change than the composition of the polysaccharide components when different stimuli to gum exudation are involved. Differences in nitrogen content were observed in earlier studies [29, 30] of different forms of the exudate from *A. senegal*.

Of the species studied here, only the samples from *A. salicifórmis* and *A. xanthina* were large enough, and their nitrogen contents sufficiently favourable, to permit amino acid analyses to be undertaken. The data obtained (Table 2) are compared with the data reported [23] for *A. polyacantha* gum. There are indications that the proteinaceous components of *Acacia* gums contain large proportions of hydroxyproline, serine and aspartic acid, and that very small proportions of the sulphur-containing amino acids are present. It is hoped to obtain the data for the gums from a much wider range of *Acacia* species in due course. The importance of the proteinaceous component remains central to a more complete understanding of the properties, tertiary structure, and mechanism of biosynthesis of gum exudates [17, 19–22]. The additional analytical parameters derived from amino acid analyses may also assist chemotaxonomic considerations in cases where the analytical parameters for the polysaccharide components of a gum exudate do not act as useful indicators of botanical affinities.

EXPERIMENTAL

Origin of gum specimens. Gum from *A. salicifórmis* Tindale was collected 19.7 km NNW of Colo Heights, New South Wales, by Messrs. R. Coveny and P. Hind on 14th December 1976 (NSW 108003). Gum from *A. implexa* Benth. was collected at Black Mountain, Canberra, by Mr. R. D. Croll on 8th April 1980. Gum from *A georginae* F. M. Bail. was collected 32 miles ESE of Alice Springs in 1974 (Nelson 2245; NT 37065). Gum from *A. murrayana* F Muell ex Benth. was collected 5 miles south of Alice Springs in 1974 (Nelson 2242; NT 37062); this sample (NE, Table 1) was the result of natural exudation. The sample D (Table 1) was obtained as a result of defoliation with 2,4-dichlorophenoxyacetic acid; the tree (Airport Road, 9 km south of Arid Zone Research Institute gate) was sprayed on 11/12/1973, gum collected 8/1/1974. Two other very small samples of gum from *A murrayana* were secured by Mr J. R. Maconochie from trees suffering from insect borer damage (a) at Arid Zone R I, 16/9/1974, (b) 5 km west of Alice Springs, 1/5/1975.

Table 2. The amino acid composition (residues per 1000 residues) of the proteinaceous components of the gums from *Acacia salicifórmis* and *A. xanthina*

	A. salicifórmis	*A. xanthina*	*A. polyacantha**
%N	1.2	0.67	0.31
Alanine	38	66	42
Arginine	3	0	12
Aspartic acid	83	96	111
Cystine	1	0	0
Glutamic acid	17	45	64
Glycine	19	50	59
Histidine	42	51	42
Hydroxyproline	287	174	n.d.
Isoleucine	14	24	31
Leucine	41	58	76
Lysine	9	32	47
Methionine	—	—	4
Phenylalanine	11	26	46
Proline	72	79	102
Serine	171	142	169
Threonine	85	61	88
Tyrosine	30	28	31
Valine	78	70	67

*Data from ref. [23].

All other gum specimens studied were collected in Western Australia in September 1975 by Mr. B. R. Maslin. Voucher specimens are lodged at Perth as follows:- *A. xanthina* Benth., BRM 4291; *A. rostellifera* Benth., BRM 3817; *A. cyclops* A. Cunn. ex G. Don, BRM 3893; and a new *Acacia* species 'P31', BRM4013, described as "an undescribed species which is doubtfully allied to *A. dentifera*; it has racemes consisting of two flower heads and phyllodes predominantly uninerved but with minor secondary parallel nerves—these two characters tend to be reminiscent of *A. cyclops* but the legumes of this taxon are not like those of *A. cyclops*" [B. R Maslin, personal communication].

Preparation of samples for analysis. The amounts of gum available for analysis were very small (1–3 g) except from *A. saliciformis* and *A. xanthina* The gums were dissolved in distilled water (100 ml) with occasional stirring for 48 hr. The solutions were filtered (muslin, then Whatman No. 1 and No. 42 papers), dialysed (2 days *vs* tap water; 1 day *vs* distilled water) then recovered by freeze-drying. The gum from *A. salicıformis* required mild treatment with alkaline borohydride [31] to facilitate dissolution. The gums from *A. saliciformis*, *A. cyclops*, *A. murrayana*, *A. georginae* and *A. implexa* gave pale yellow solutions; the gums from *A. xanthina*, *A. rostellifera*, and new species 'P31' gave brown solutions.

Analytical methods. The standard analytical methods used for the polysaccharide components have been described [9]. Amino acid analyses were carried out on an automated analyser (Rank–Hilger Chromaspek) after hydrolysis of the samples in boiling 6 M HCl for 20 hr in an atmosphere of nitrogen.

Acknowledgments—We thank Rowntree–Mackintosh plc (York) for financial support (to M.C.L.G.); and Dr. Mary Tindale (Royal Botanic Gardens, Sydney), Mr. L. Pedley (Queensland Herbarium, Brisbane), Mr. B. R. Maslin (Western Australian Herbarium, Perth) and the late Mr. J. R. Maconochie (Department of Primary Production, Division of Agriculture, Alice Springs) for helpful comments on the systematic relationships of phyllodine species and for arranging the collection of specimens.

REFERENCES

1. Anderson, D. M. W., Ashby, P., Busuttil, A., Kempson, S. A. and Lawson, M. E. (1984) *Toxicol. Letters* **21**, 83.
2. Anderson, D. M. W. and Dea, I C M. (1969) *Phytochemistry* **8**, 167.
3 Bentham, G (1875) *Trans. Linn. Soc.* (*London*) **30**, 444.
4. Anderson, D. M. W and Farquhar, J. G. K. (1979) *Phytochemistry* **18**, 609.
5. Anderson, D. M. W., Bridgeman, M. M. E. and Pinto, G. (1984) *Phytochemistry* **23**, 575.
6. Anderson, D. M. W., Farquhar, J. G. K. and Gill, M. C. L. (1980) *Bot. J. Linn. Soc* (*London*) **80**, 79.
7. Anderson, D. M. W., Farquhar, J. G. K. and McNab, C. G. A. (1983) *Phytochemistry* **22**, 2481.
8. Anderson, D. M. W., Farquhar, J. G. K. and McNab, C. G. A. (1984) *Phytochemistry* **23**, 579.
9. Anderson, D. M. W., Bell, P. C. and McNab, C G. A. (1972) *Phytochemistry* **11**, 1751.
10. Anderson, D. M. W. and Bell, P. C. (1976) *Phytochemistry* **15**, 301.
11. Anderson, D. M. W. (1978) *Kew Bull.* **32**, 529.
12. Anderson, D. M. W., Bridgeman, M. M. E., Farquhar, J. G. K. and McNab, C. G. A. (1983) *Int. Tree. Crops J.* **2**, 245
13. Eastwood, M. A., Brydon, W. G. and Anderson, D. M. W. (1983) *Toxicol. Letters* **17**, 159.
14. Churms, S. C., Merrifield, E. H., Miller, C. L. and Stephen, A. M. (1979) *S. Afr. J. Chem.* **32**, 103.
15. Churms, S. C., Merrifield, E. H. and Stephen, A. M. (1980) *S. Afr. J. Chem.* **33**, 39.
16. Churms, S. C., Merrifield, E. H. and Stephen, A. M. (1981) *S. Afr. J. Chem.* **34**, 68.
17. Anderson, D M. W. and Karamalla, K. A. (1966) *Carbohydr Res.* **2**, 403.
18. Anderson, D. M. W. and Dea, I. C. M. (1968) *Carbohydr. Res* **6**, 104.
19. Anderson, D. M. W. and Stoddart, J. F. (1966) *Carbohydr Res.* **2**, 104.
20. Anderson, D. M. W. and Dea, I. C. M. (1967) *Carbohydr. Res.* **5**, 461.
21. Anderson, D. M. W. and Hendrie, A. (1971) *Carbohydr. Res.* **20**, 259.
22. Anderson, D. M. W. and Herbich, M. A. (1963) *J. Chem. Soc.* 1.
23. Anderson, D. M. W., Hendrie, A. and Munro, A. C (1972) *Phytochemistry* **11**, 733.
24 Charlson, A. J., Nunn, J. R. and Stephen, A. M (1955) *J. Chem. Soc.* 269.
25. Churms, S. C., Merrifield, E. H. and Stephen, A. M. (1981) *S. Afr J. Chem.* **34**, 8.
26. Pettigrew, C. J. and Watson, L. (1975) *Aust. J Botany* **23**, 833
27. Maslin, B. R. (1978) *Aust. J. Botany* **26**, 5.
28. Anderson, D. M W., Bell, P. C., Conant, G H. and McNab, C. G. A. (1973) *Carbohydr. Res.* **26**, 99.
29. Anderson, D. M. W., Bell, P. C. and McNab, C. G. A. (1971) *Carbohydr. Res.* **20**, 269.
30. Anderson, D. M. W., Dea, I. C. M., Karamalla, K. A. and Smith, J. F. (1968) *Carbohydr. Res.* **6**, 97.
31. Anderson, D. M. W., Bell, P. C. and King, H A. R. (1972) *Carbohydr Res.* **22**, 453