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SHORT COMMUNICATION

THE AMINO ACID AND AMINO SUGAR COMPOSITION OF SOME PLANT GUMS*

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Abstract—The amino acid compositions of typical plant gum exudates from the genera *Acacia*, *Araucaria*, *Azadirachta* and *Lannea* are reported. The proteins involved contain large amounts of serine, threonine and aspartic acid; in addition, those of *Lannea* species contain major amounts of proline and leucine. There is evidence that gums from different species in a genus have very similar amino acid compositions. Glucosamine also occurs in exudates of the genera named above, but was not detected in *Khaya*, *Fagara* or *Boswellia*.

INTRODUCTION

CONSIDERABLE attention has been given to identifying the characteristic amino acids present in the seeds and other tissues of gum-forming genera, e.g. *Acacia*[1] and *Albizia*,[2] but the nitrogenous matter associated with the acidic polysaccharides in gum exudates has not yet been studied.

Although other investigators have frequently ignored, or failed to analyse for, the presence of nitrogen in plant gums, all those studied in this laboratory over the past 12 yr have contained nitrogen. In some species the amount is small, e.g. 0·08% (*Acacia nilotica*[3]), 0·07% (*Acacia arabica*[4]), and 0·04% (*Acacia leucoclada*[5]), but it can be as high as 1·2% (*Acacia drepanolobium*[6]), 1·55% (*Acacia paramattensis*[5]), 2·5% (solubilised *Araucaria araucana*[7]) and 5·6% (*Azadirachta indica*[8]). In the gums from *Acacia seyal*[9] and *Acacia nubica*,[10] the nitrogen content was not reduced on electrodialysis or attempted purification by precipitation methods; in other species, however, a partial separation of protein was obtained, e.g. in *Acacia senegal*[11] (from 0·37 to 0·29%) and in *Acacia nilotica*[3] (from 0·08 to 0·02%). With *Albizia*[12] species, precipitation of nitrogen-rich material occurred during electrodialysis, but attempts[8] to separate the nitrogenous material from carbohydrate in *Azadirachta indica* gum failed.

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The tendency of some plant gums to form water-insoluble gels is of commercial importance, and it was reported[13] that the insoluble jelly from *Acacia pycnantha* gum contained three times as much nitrogen as the water-soluble gum. In contrast, the insoluble gel given by *Acacia drepanolobium*[6] contained only half as much nitrogen as the water-soluble fraction, and recently it has been found[14] that the nitrogen content of each of 4 separate water-insoluble nodules of *Acacia senegal* gum was only 25–30% of the average value (0·37%) established[11] as typical of that species.

Clearly the nitrogen-containing material present in plant gum exudates merited examination; the results of chromatographic analyses of the amino acid composition of a number of plant gums are reported in this paper, together with the results of analyses that sought to establish whether amino sugars are also present, as has been claimed[15] for one species (*Azadirachta indica*).

RESULTS AND DISCUSSION

The amino acid compositions shown in Table 1 indicate that plant gums from at least the *Araucaria*, *Acacia*, *Azadirachta* and *Lannea* genera contain proteinaceous material. Molecular-sieve chromatography experiments, which will be the subject of a separate communication, have indicated that high molecular weight protein is involved, not short-chain polypeptides, and frequently more than one component appears to be present. The results in Table 1 are interesting from several points of view. The two *Araucaria* spp. studied are remarkably similar in amino acid composition, as are the four *Lannea* samples and the two specimens of *Azadirachta indica* gum, that came from different geographical locations. Yet all are characterised by the high proportions of serine, threonine and aspartic acid involved; the *Lannea* spp. are also high in proline and leucine.

The comparison of *Acacia campylacantha* gum before and after treatment with $NaBH_4$–NaOH, a procedure[16] that has proved to be invaluable for the solubilisation of difficulty

TABLE 1. THE AMINO ACID COMPOSITION* OF SOME PLANT GUM EXUDATES

	Araucaria araucana	*Araucaria columnaris*	*Acacia campylacantha*	*Acacia campylacantha* + BH_4'-OH'	*Lannea coromandelica*	*Lannea humilis* A	*Lannea humilis* B	*Lannea schimperi*	*Azadirachta indica* A	*Azadirachta indica* B
Lysine	84	96	47	45	21	31	33	13	44	59
Histidine	32	14	42	42	6	5	0	6	17	18
Arginine	38	87	12	11	18	21	25	15	27	28
Aspartic acid	99	102	111	111	†	†	†	†	138	145
Threonine	42	56	88	94	161	134	135	210	66	64
Serine	194	155	169	170	206	128	132	260	75	79
Glutamic acid	71	64	64	69	79	73	78	34	78	81
Proline	61	51	102	108	145	220	203	148	73	57
Glycine	62	78	59	57	33	34	34	28	73	71
Alanine	67	62	42	39	56	69	83	50	53	55
Cystine	—	—	—	—	—	—	—	—	18	14
Valine	50	49	67	66	39	54	45	30	75	76
Methionine	8	4	4	4	—	—	—	—	3	0
Isoleucine	30	36	31	27	39	42	45	17	51	51
Leucine	83	65	76	75	138	110	115	130	84	80
Tyrosine	31	32	31	29	37	42	40	37	30	31
Phenylalanine	35	34	46	43	12	24	21	14	57	57
Glucosamine	13	15	9	10	10	14	11	9	38	34

* Expressed as μmoles amino acid 1000 μmoles total.

† This amino acid partly obscured by peak for hydroxyproline.

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soluble and gel-forming species of gum, also gave an interesting result. Whilst the precise way in which the borohydride-alkali treatment improves solubility remains obscure, there is no doubt that the composition of the proteinaceous material present is not changed.

In glycoproteins and proteoglycans, linkage of protein to carbohydrate has, in the cases where it has been proved,[17] usually involved acetylated amino sugars and either serine, threonine, or aspartic acid although Italian workers[18] have recently identified a linkage involving a hexosamine and a hydroxyamino acid. The chromatographic evidence presented here for the presence of significant amounts of serine, threonine, aspartic acid and hydroxyproline (in the *Lannea* spp.), together with the evidence for the previously unsuspected presence of amino sugars, now makes it desirable that attempts be made to isolate the molecular species actually involved in the carbohydrate-protein linkages.

The amount of hexosamine found in both specimens of *Azadirachta indica* gum is similar to the value reported by Lakshmi and Pattabiraman,[15] who also examined 8 other species of gum and found no hexosamine. Although we have detected glucosamine in 7 different species of gum, tests made on the gums from *Boswellia papyrifera*, *Fagara macrophylla*, *F. xanthoxyloides*, *Khaya nyasica* and *K. senegalensis* all proved to be negative. A logical explanation for the fact that at least a partial separation from protein is easier to achieve in some plant gums than in others may therefore be forthcoming in due course.

At the present stage it is clear that the proportions of glucosamine indicated in Table 1 are under-estimates. Nolan and Smith[32] observed that hydrolysis of mixtures of amino acids, sugars, and amino sugars for 12 hr with 6 N HCl at 97–100° resulted in the loss of 47% of the glucosamine. If degradation to this extent did indeed occur, then calculation shows that the gums listed in Table 1 would contain approx. 1 glucosamine residue per polysaccharide molecule. Studies of amino sugars in plants has so far been restricted to a few species,[19] but rapid, automated methods[20] for hexosamine determination and for the estimation of glucosamine and galactosamine in mixtures[21] using anion-exchange chromatography have been described; GLC[22] and mass spectroscopy[23] can also be used. It appears to be opportune for broader surveys of the occurrence of amino sugars to be undertaken, and for ways of isolating amino sugars by less destructive methods to be devised.

EXPERIMENTAL

Origin of samples. The origins of the following specimens have been described: *Acacia campylacantha*,[24] *Araucaria araucana*,[7] *Araucaria columnaris*,[7] *Lannea coromandelica*,[25] *Lannea humilis* A and B,[25] *Lannea schimperi*,[25] *Azadirachta indica*,[8] *Boswellia papyrifera*.[26] Specimens of the gum exudates from *Fagara macrophylla*, *Fagara xanthoxyloides*, *Khaya nyasica* and *Khaya senegalensis* were all obtained from Mr. A. A. Enti, Curator, The Herbarium, Legon-Accra.

Amino acid analyses. Samples of polysaccharides, containing 1–2 mg protein (as calculated from the N content determined by a semi-micro Kjeldahl method) were weighed into Pyrex tubes and were dissolved in re-distilled constant boiling (19%, w/v) HCl (10 ml). The tubes were placed in liquid N_2, and after evacuation

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to < 0·1 mm Hg, the tubes were sealed then allowed to thaw. Hydrolysis was effected at 105 ± 0·5° for 24 hr. The tubes, after cooling, were placed in liquid N_2, then opened. The contents were filtered then taken just to dryness on a rotary evaporator. The residual HCl was removed by repeated addition of de-ionised water followed by concentration to dryness. The final hydrolysis product was allowed to stand over NaOH, then P_2O_5. The amino acid analysis described by Spackman *et al.*,[27] as modified by Benson and Patterson,[28] was used; two commercial amino acid analysers were used.

(*a*) *Beckman model* 120 *C amino acid analyser.* The prepared hydrolysates (approx. 0·75 mg, in 0·5 ml. buffer) were added to columns at 56°; (i) Beckman Custom Research Resin, type PA-35 (6 × 0·9 cm) using 0·2 M citrate buffer at pH 5·2 as eluent, and L-2-amino-3-guanidinopropionic acid[29] as an internal standard. This column separated basic amino acids and ammonia, (ii) Beckman Spherical Resin, Type UR-30 (50 × 0·9 cm) using 0·2 M citrate buffer at pH 3·28, with change of buffer, after the elution of valine, to 0·2 M citrate at pH 4·30. DL-norleucine[29] was used as an internal standard, and this column separated the neutral and acidic amino acids.

(*b*) *Technicon automatic amino acid analyser.* The prepared hydrolysates (approx. 0·2 mg in 0·5 ml buffer) were added to columns of; (i) Amberlite CG 120 resin (8 × 0·636 cm) using 0·35 M sodium citrate buffer at pH 5·22 as eluent at 55°; (ii) Technicon *A* resin (58 × 0·636 cm) using 0·2 M sodium citrate buffer at pH 3·28 at 52°. After the elution of valine, the buffer was changed to 0·2 M sodium citrate at pH 4·25. The internal standards used were as for the Beckman instrument.

In the earliest runs carried out, it was observed that two components were eluted on column (ii) after the other amino acids. The major, slower-eluting, of these components was shown to have the same elution characteristics as glucosamine, which could be differentiated from galactosamine. All the samples studied also showed several components, in varying amounts, which were eluted faster than the amino acids on column (ii). These components are probably ninhydrin-positive degradation products of amino acids and sugars that can arise during the hydrolysis. In the hydrolyses of the *Lannea* spp., a medium-sized peak and a large peak that were eluted between these acid-degradation products and the first of the amino acid peaks were identified as methionine sulphoxide and hydroxyproline; the latter peak partly obscured the peak due to aspartic acid.

Determination of the hexosamine content. Two procedures, based on the Elson and Morgan colorimetric method,[30] were used. The first procedure was essentially that of Belcher *et al.*[31] *Azadirachta indica* gum sample A (30–50 mg) was hydrolysed with 2 N HCl for 6 hr on a boiling water-bath. After colour development with *p*-dimethylaminobenzaldehyde, the hexosamine content, indicated by a calibration curve prepared with reference to different weights of glucosamine, was 2·1%. To test the belief[15,32] that the hydrolysis conditions used gave complete hydrolysis of hexosamine without significant degradation, a duplicate experiment with 4 N HCl gave a concordant result, but degradation occurred when 6 N HCl was used.

The second analytical procedure used was that of Lakshmi and Pattabiraman,[15] based on the method of Levvy and McAllan.[33] Values of 2·0 and 2·2% were found for the hexosamine content of *Azadirachta indica* samples A and B respectively.

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Key Word Index—Acacia; Leguminosae; *Araucaria;* Araucariaceae; *Azadirachta;* Meliaceae; *Lannea*; Anacardiaceae; gum exudates; amino acids; glucosamine.