ELSEVIER

Food Hydrocolloids 16 (2002) 599–603

FOOD HYDROCOLLOIDS

www.elsevier.com/locate/foodhyd

Structural elucidation of proteic fraction isolated from *Acacia glomerosa* gum

G. León de Pinto[a,*], L. Sanabria[a], M. Martínez[a], O. Beltrán[a], J. Manuel Igartuburu[b]

[a]*Centro de Investigaciones en Química de los Productos Naturales, Facultad de Humanidades y Educación, La Universidad del Zulia, Apartado 526, Maracaibo, Venezuela*

[b]*Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, Apartado 40, 11510-Puerto Real, Cádiz, Spain*

Received 3 August 2001; revised 24 October 2001; accepted 17 December 2001

Abstract

Acacia glomerosa Benth., Venezuelan Vulgares species, exudates a clear gum, which is able to produce a gel easily. Physico-chemical data of this gum, except the nitrogen content, are very close to those reported for *Acacia senegal* gum. The gum samples (untreated and treated with alkali) were studied by size exclusion chromatography on a packed column with Fractogel S-60, using 0.1 M NaCl as eluent. The elution profiles of protein and carbohydrate fractions were determined at 220 and 490 nm, respectively. The elution profiles of the original gum (untreated gum) showed a high molecular weight fraction, rich in protein and carbohydrate, which may correspond to an arabinogalactan–protein (AGP) complex. Other peaks were also observed of higher elution volume that may probably be peptides. The presence of the AGP was also observed in the elution profile of the gum after basic treatment. Sugar and amino acid composition of five fractions, containing carbohydrate and protein, were studied. On the other hand, sugar and amino acid composition were determined on the *A. glomerosa* gum and its degraded products. The results obtained so far suggest that the gum from *A. glomerosa* is a mixture of polysaccharide and arabinogalactan–protein. Approximately 18% of this gum is protein, which is characteristically rich in hydroxyproline, leucine, serine and threonine.

Keywords: Acacia glomerosa; Vulgares species; Proteic fraction; Amino acid composition; Structural elucidation

1. Introduction

Analytical data and structural features of gums from *Acacia macracantha* have been reported (Martínez, León de Pinto, & Rivas, 1992; Martínez, León de Pinto, Rivas, & Ocando, 1996); *Acacia tortuosa* (León de Pinto, Martínez, Ortega, Villavicencio, & Borjas, 1993; León de Pinto, Martínez, Bolaño, Rivas, & Ocando, 1998), and *Acacia glomerosa* (León de Pinto, Martínez, & Sanabria, 2001). The overall composition of these Venezuelan *Acacia* gums, having high nitrogen content, is predominantly carbohydrate; galactosyl, arabinosyl, rhamnosyl and glucuronosyl with its 4-*O*-methyl derivative are the major residues.

The finding that gum arabic was a kind of arabinogalactan–protein (AGP), supported by many studies (Akiyama, Eda, & Kato, 1984; Connolly, Fenyo, & Vandevelde, 1987; Osman et al., 1993, 1995; Randall, Phillips, & Williams, 1989; Yariv, Lis, & Kalachalski, 1967), suggests that the elucidation of the protein components of the Venezuelan *Acacia* gums may be a contribution to the structural characterization of AGPs. This work deals with the structural elucidation of protein fraction isolated from *A. glomerosa*.

2. Materials and methods

2.1. Origin and purification of gum samples

Gum from *A. glomerosa* Benth. (Vulgares Series), known as tiamo, was collected from trees located in a county, 65 Km SW of Maracaibo City, Zulia State, Venezuela, South America. The identification of the voucher specimen was confirmed by Prof. Carmen Clamens, botanical taxonomist of La Universidad del Zulia, Maracaibo, Venezuela.

The gum exudates, collected by the authors, two weeks after incision was made at the trunk level, during January–March 1999, was dissolved partly in H_2O, and the solution was filtered through muslin and then through Whatman No. 1 paper. The solution was dialyzed against running tap water for two days, and the gum was recovered by freeze-drying.

* Corresponding author.
E-mail address: gleon@luz.ve (G. León de Pinto).

0268-005X/02/$ - see front matter
PII: S0268-005X(02)00022-X

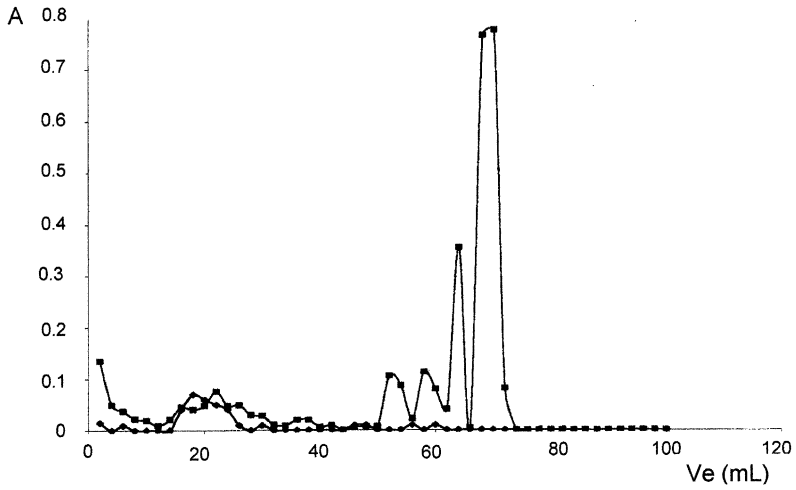

Fig. 1. Size-exclusion chromatography of the original gum from *A. glomerosa* on Fractogel S-60. Injection of sample (2 ml, $C = 15$ mg/1 ml), Column: 37×1.5 cm; NaCl 0.1 M as eluent; detected at 220 and 490 nm.

2.2. General methods

The standard methods of gum analysis were used. Paper chromatography was carried out on Whatman No. 1 and 3 MM papers with the following solvent systems (v/v): (a) benzene, butan-1-ol, pyridine, water (1:5:3:3, upper layer); (b) acetic acid, ethyl acetate, formic acid, water (3:18:1:4) and (c) butan-1-ol, ethanol, 0.1 M hydrochloric acid (1:10:5). Before solvent (c) was used, the paper was pretreated with 0.3 M sodium dihydrogen phosphate solution and allowed to dry. Neutral sugars were determined by the phenol–sulphuric acid (Dubois, Gilles, Hamilton, Rebers, & Smith, 1996) and HPLC methods. Uronic acids were determined by direct titration with standard sodium hydroxide solution on exhaustively electrodialyzed samples and by the *m*-hydroxydiphenyl-sulfuric acid method. The nitrogen content was determined by semimicrokjeldahl analysis. Optical rotation was determined by a Perkin–Elmer 343 Polarimeter at 589 nm.

2.3. Amino acid determination

The samples (5 mg) were digested, in a nitrogen atmosphere, in 6 M HCl (1 ml) for 12 h. The solution was filtered and reduced in volume to dryness in a nitrogen atmosphere. The residue was dissolved in a buffer solution (sodium citrate, pH 2.20) and injected to a post column derivatizer (Pickering Laboratories), adapted to a Perkin–Elmer 785 UV/vis detector.

2.4. Basic hydrolysis of the gum

The original gum (5 g) was hydrolyzed with a saturated barium hydroxide solution (200 ml) at 100 °C for 8 h. The hydrolyzed gum was neutralized with sulphuric acid (1 M), filtrated and then the gum, after basic treatment, was freeze-dried.

2.5. Profiles elution of the original gum and that of the gum, after basic hydrolysis

The sample (150 mg), original gum untreated and treated with barium hydroxide was dissolved in deionized water (10 ml). The elution profile was determined on a glass column (3.7×1.5 cm), packed with Fractogel S-60, NaCl 0.1 M solution was used as eluent. The carbohydrate profiles were obtained, after the application of phenol–sulfuric method at 490 nm, while the protein contents were measured at 220 nm.

2.6. Hydrolysis with trifluoracetic acid

The samples chosen, having carbohydrate and protein, were treated with trifluoracetic acid (TFA) (0.1 M) at 100 °C for 1 h, and then the acid concentration was increased (2 M). Aliquots were withdrawn at different intervals (1, 6, 15, 24 h) and analyzed by p.c.

3. Results and discussion

The analytical data of the gum from *A. glomerosa*, Vulgares species, except nitrogen content, are quite similar to those reported for *Acacia senegal* gum, Table 1. Structural features of the polysaccharides from both gums are very similar (León de Pinto et al., 2001).

The elution profiles of the carbohydrate and protein components from the original gum (Fig. 1) indicates a polydisperse system. It showed a relatively high molecular weight fraction (Ve 18 ml), which may correspond to an AGP complex. Others fractions were also observed (Ve 52, 58, 64, 70 ml) which belong probably to peptides and free amino acids.

The gum, after basic treatment, showed an elution profile (Fig. 2) which corroborates the presence of the AGP complex as was observed in Fig. 1. Comparison of the

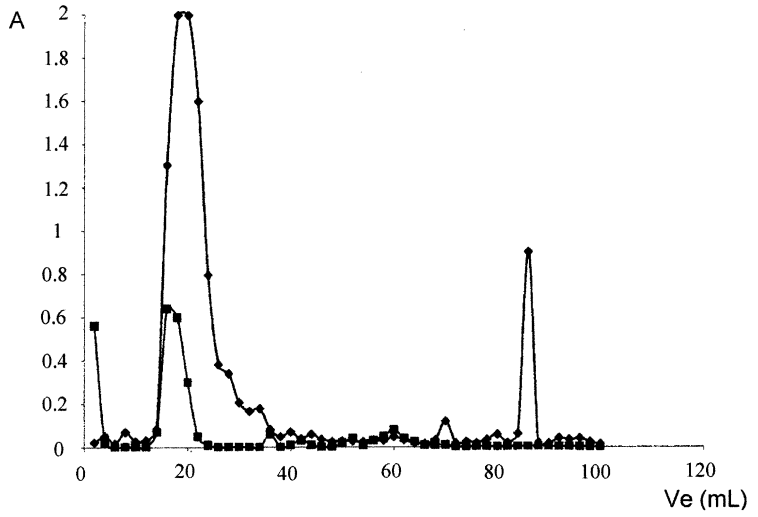

Fig. 2. Size-exclusion chromatography of the hydrolyzed basic *A. glomerosa* gum on Fractogel S-60. Injection of sample (2 ml, $C = 15$ mg/1 ml), Column: 37 × 1.5 cm; NaCl 0.1 M as eluent; detected at 220 and 490 nm.

proteic fractions of the original gum and the gum after basic treatment (Fig. 3) indicates that basic treatment led to hydrolysis of the mentioned peptides (Fig. 1) as was shown by the absence of some peaks (Ve 52, 58, 64, 70 ml) and the presence of a new peak (Ve 90 ml), related to a relatively low molecular weight compound, which may be a free amino acid. The elution profiles of the carbohydrate component, present in the original gum and in the gum after basic treatment (Fig. 4) showed the high molecular weight fraction (AGP), Ve 18 ml, and other fractions of lower molecular weight (Ve 36, 42, 52, 60 ml), which may, probably correspond to carbohydrates, attached to the polypeptide backbone, removed during the basic treatment.

The elution profile of the original gum, after basic treatment (Fig. 2) yielded eleven fractions. Five of them, containing both carbohydrate and protein, were chosen to be isolated and hydrolyzed with TFA. The main fraction (145.2 mg) corresponds probably to an AGP complex and the other four fractions were recovered as minor components (16–38 mg), Table 2. Monitoring of the hydrolytic process of these fractions (Table 2) showed that rhamnose and glucuronic acid residues were removed from the highest molecular weight fraction, after 24 h. The easy removal of rhamnose (0.1 M TFA, 1 h), is according to the presence of this sugar as a terminal residue in the structure of *A. glomerosa* gum (León de Pinto et al., 2001); glucuronic acid residues were removed after more severe conditions (2 M TFA, 15 h). Hydrolysis of the second fraction, F_2, showed only glucuronic acid and galactose. The major fraction, constituted by glucuronic acid, galactose and arabinose residues, is probably the arabinogalactan–protein as was demonstrated by its amino acid composition, Table 4. The lowest molecular weight fractions (F_4, F_5) showed glucuronic acid, galactose

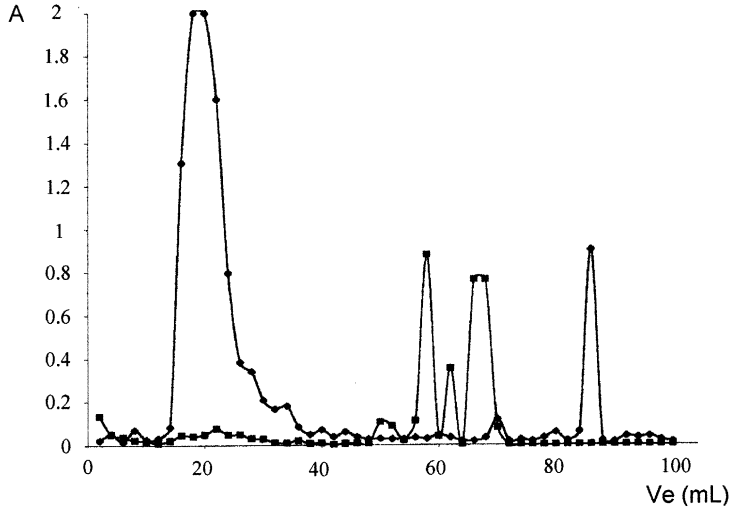

Fig. 3. Comparison of the elution profiles from proteic fractions from *A. glomerosa*: original gum and the gum after basic treatment.

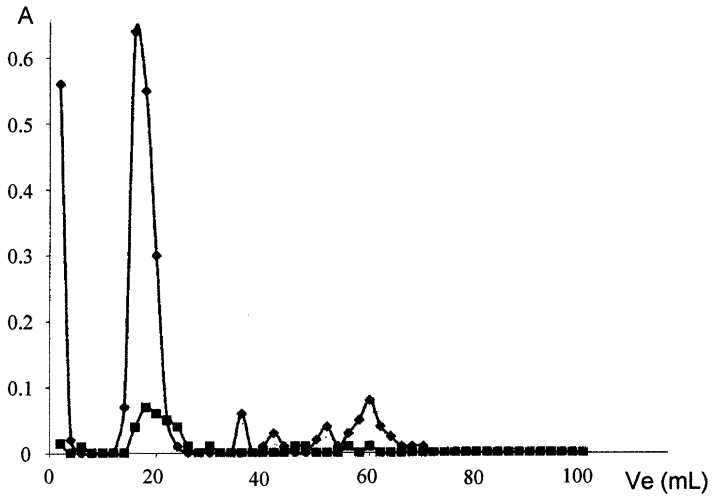

Fig. 4. Comparison of the elution profiles carbohydrate fractions from *A. glomerosa*: original gum from and the gum after basic treatment.

Table 1
Comparison of analytical data of *A. glomerosa* and *A. senegal* gums

Parameter	Species	
	A. glomerosa	*A. senegal*
Moisture (%)	11.29	–
Ash (%)	7.98	0.07
Tannin (%)	0	–
Molecular weight	0.9×10^5	2×10^5
Intrinsic viscosity (ml g^{-1})	27	20
$[\alpha]_D$ in H_2O (°)	− 15	− 31
Nitrogen (%)	2.82	0.33
Hence protein (%) ($N \times 6.25$)	17.63	2.06
Equivalent weight (g)	1475	1085
Hence uronic acid (%)	12	18
Neutral sugar composition after hydrolysis (%)		
Galactose	46	40
Arabinose	27	28
Rhamnose	15	14

Table 2
Monitoring of the acid hydrolysis of five fractions, containing carbohydrate and protein, isolated by size-exclusion chromatography, after basic treatment of *A. glomerosa* gum. (Hydrolysis with trifluoracetic acid (TFA, 0.1–2 M, 100 °C))

Fraction	Weight (mg)	Ve (ml)	Time (h)	Sugars detected
1	16.2	4	1–6	Rha
			15–24	Rha, GlcA
2	23.6	8	1–6	GlcA
			15–24	GlcA, Gal
3	145.2	18	1	GlucA, Gal, Ara
			6–24	Idem
4	38.0	40	1–6	GlcA
			15–24	GlcA, Gal
5	34.3	44	1–6	GlcA
			15–24	GlcA, Rha

and rhamnose. These fractions may correspond to oligosaccharides, which have been removed from the structure of the gum, after basic treatment.

The original gum and all of degraded products have similar amino acid composition (Table 3) hydroxyproline, leucine, serine and threonine, remained the major amino acid present; while phenylalanine, removed in the first degradation, proline and tyrosine, represented the minor amounts of amino acids.

The chromatography conditions were not adequate to resolve the overlapping of histidine and arginine, which together account 20%. Aspartic acid, glutamine, cystine and methionine were not detected in the samples studied.

Amino acid composition of the high molecular weight fraction (AGP), isolated from size-exclusion chromatography from the original gum, after basic treatment (Fig. 2,

Table 3
Amino acid compositions of the original gum from *A. glomerosa* and its degraded products (I and III represents polysaccharides I and III. They were not observed aspartic acid, glutamine, cystine and methionine. Histidine and arginine were overlapped)

Amino acid	Polymer		
	Original gum	I	III
Alanine	7	3	9
Glycine	9	6	9
Hydroxyproline	11	13	12
Isoleucine	6	9	7
Leucine	10	11	9
Lysine	6	2	1
Phenylalanine	1	–	–
Proline	4	2	3
Serine	11	10	11
Threonine	8	8	9
Tyrosine	0.2	1	0.15
Valine	7	9	9
Arg + His	20	26	21
Total	100	100	100

Table 4
Amino acid composition of the fraction of high molecular weight (AGP) isolated *A. glomerosa* gum after basic treatment. (They were not observed aspartic acid, glutamine, cystine and methionine. Histidine and arginine were overlapped)

Amino acid	*A. glomerosa*
Alanine	4
Glycine	3
Hydroxyproline	16
Isoleucine	6
Leucine	9
Lysine	5
Proline	3
Serine	18
Threonine	12
Tyrosine	4
Valine	4
Hist + Arg	16
Total	100

Table 4) showed the same amino acids, described previously, in the original gum and its degraded products. Hydroxyproline, leucine, serine and threonine are the major amino acids.

The results obtained so far suggest that the gum from *A. glomerosa* is a mixture of polysaccharide and arabinogalactan–protein. Approximately 18% of this gum is protein, which is characteristically rich in hydroxyproline, leucine, serine and threonine. Some *O*-galactosyl-hydroxyprolyl junctions and also some seryl residues have been reported for gum arabic (Akiyama et al., 1984). Although linkages between carbohydrate and protein have not yet been confirmed in *A. glomerosa* gum.

Acknowledgements

The authors greatly appreciate the financial support of the University of Zulia (CONDES),Venezuela.

References

Akiyama, Y., Eda, S., & Kato, K. (1984). Gum arabic is a kind of arabinogalactan-protein. *Agricultural and Biological Chemistry*, *48*, 235–237.

Connolly, S., Fenyo, J. C., & Vandevelde, M. C. (1987). Effect of a proteinase on the macromolecular distribution of *Acacia Senegal* gum. *Carbohydrate Polymers*, *8*, 23–32.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1996). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

León de Pinto, G., Martínez, M., Ortega, S., Villavicencio, N., & Borjas, L. (1993). Comparison of gum specimens from *Acacia tortuosa* and other Gummiferae species. *Biochemical Systematics and Ecolology*, *21*, 795–797.

León de Pinto, G., Martínez, M., Bolaño, L., Rivas, C., & Ocando, E. (1998). The molecular characterization of the polysaccharide gum from *Acacia tortuosa*. *Phytochemistry*, *47* (1), 53–56.

León de Pinto, G., Martínez, M., & Sanabria, L. (2001). Structural features of the polysaccharide gum from *Acacia glomerosa*. *Food Hydrocolloids*, in press.

Martínez, M., León de Pinto, G., & Rivas, C. (1992). Composition of *Acacia macracantha* gum exudates. *Phytochemistry*, *31*, 535–536.

Martínez, M., León de Pinto, G., Rivas, C., & Ocando, E. (1996). Chemical and spectroscopic studies of the gum polysaccharide from *Acacia macracantha*. *Carbohydrate Polymers*, *29*, 247–252.

Osman, M. E., Menzies, A. R., Williams, P. A., Phillips, G. O., & Baldwin, T. C. (1993). The molecular characterization of the polysaccharide gum from *Acacia senegal*. *Carbohydrate Research*, *246*, 303–318.

Osman, M. E., Menzies, A. R., Marlin, B. A., Williams, P. A., Phillips, G. O., & Baldwin, T. C. (1995). Characterization of gum arabic fractions obtained by anion-exchange chromatography. *Phytochemistry*, *38*, 409–417.

Randall, R. C., Phillips, G. O., & Williams, P. A. (1989). Fractionation and characterization of gum from *Acacia senegal*. *Food Hydrocolloids*, *3*, 65–75.

Yariv, J., Lis, H., & Kalachalski, E. (1967). Precipitation of arabic acid and some seed polysaccharides by glycosylphenylazo dyes. *Biochemical Journal*, *105*, 1C–2C.