Characterization of Commercial Samples of Gum Arabic

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Six commercial gum arabic samples and authenticated samples of gum from *Acacia senegal* and *Acacia seyal* have been characterized using a range of chemical and physicochemical techniques including determination of specific rotation, sugar composition, nitrogen and amino acid content, and molecular mass distribution. Although some of the gums have slightly different chemical and physicochemical characteristics, gel permeation chromatography shows that each consists of essentially three molecular mass fractions classified as an arabinogalactan, an arabinogalactan–protein complex, and a glycoprotein. The proportions of each varied for the individual samples. The gums were tested using a specifically developed enzyme-linked immunosorbent assay which can identify gum from *A. senegal* and chemically related species. Their interaction with the antibody could be correlated with differences observed in their molecular compositions. Such evidence will assist in their chemotaxonomic classification.

INTRODUCTION

Gum arabic is an approved food additive which is used principally in confectionery, where it inhibits sugar crystallization, and in flavor oil concentrates, where it functions as an emulsifier. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) defines the gum as "a dried exudation obtained from the stems and branches of *Acacia senegal* (L) Willdenow or related species of Acacia (Fam. Leguminosae)" (FAO, 1986) which grow naturally throughout the Sahelian regions of Africa. However, there are over 1000 *Acacia* species and subspecies known, and hence to more clearly define the gum and to minimize potential adulteration, JECFA has recently proposed new specifications (FAO, 1990) introducing three significant additional criteria: (i) gum arabic should include gum from *Acacia senegal* and only "*closely*" related species; (ii) optical rotation limits (−26° to −34°) should be adopted; (iii) nitrogen content should be set between 0.27 and 0.39%.

Work carried out by Anderson and Stoddart (1966), Street and Anderson (1983), and Churms et al. (1983) on *A. senegal* gum using Smith degradation procedures indicated that the gum was a highly branched polysaccharide consisting of a main chain of β-1,3-linked galactose residues with 1,6-linked ramified side chains containing galactose, arabinose, rhamnose, and glucuronic acids. The heterogeneous nature of the gum is well-known (Anderson and Stoddart, 1966; Jermyn, 1962), and recent work by Vandevelde and Fenyo (1985) has shown that the gum contains a high molecular mass protein-rich fraction and a lower molecular mass protein-deficient fraction. They described the high molecular mass fraction as an arabinogalactan protein complex (AGP) following the work of Akiyama et al. (1984). Connolly et al. (1987, 1988) undertook enzyme degradation studies and provided evidence that the structure of the gum conformed to the "wattle blossom model" proposed by Fincher et al. (1983) for AGPs generally and concluded that the high molecular mass fraction of the gum consists of a number of carbohydrate blocks of molecular mass ca. 2×10^5, linked individually to a main polypeptide chain. Randall et al. (1989) used hydrophobic affinity chromatography to fractionate the gum and, in addition to the two fractions identified by Vandevelde and Fenyo (1985), isolated a third fraction. Analysis of the fractions showed that each contained similar proportions of the various sugars and differed principally in their molecular masses and nitrogen contents. The bulk of the gum (88.4% of the total) was shown to have a molecular mass of 2.79×10^5 from light scattering measurements and was low in protein (0.44% w/w). For discussion purposes here we have designated this the arabinogalactan (AG) fraction. The second major fraction (10.4% of the total) had a higher molecular mass of 1.45×10^6 and contained a greater proportion of protein (9.18% w/w). This we refer to as the arabinogalactan–protein (AGP), which corresponds to the AGP fraction reported by Vandevelde and Fenyo (1985). The third minor fraction (ca. 1% of the total) had a molecular mass of 2.5×10^5, and because of its high protein content (ca. 50% w/w) we refer to it as a glycoprotein (GP). The proteinaceous component of the AG and AGP fractions had similar amino acid distributions, with hydroxyproline and serine being the most abundant. The amino acid distribution of the GP fraction was very different, with aspartic acid predominating. The AGP fraction was degraded by Pronase to yield a component similar in molecular mass to the bulk of the gum, providing further support for the wattle blossom model. Subsequent work by Williams et al. (1990) using ^{1}H and ^{13}C NMR spectroscopy and methylation analysis showed that there were no major differences in the structures of the AG, AGP, and GP fractions each comprising a D-galactan core with 3- and 6-linkages with L-Rha*p*, L-Ara*f*, L-Ara*p*, D-Gal*p*, and D-Glc*p*A (or the methyl ether) occurring peripherally. A recent paper by Qi et al. (1991) described the isolation of the AGP complex (which they referred to as a glycoprotein) by size exclusion chromatography. Subsequent characterization implied a rodlike molecule consisting of a polypeptide backbone of some 400 amino acids with numerous small polysaccharide substituents attached (ca. 30 sugar residues), linked through hydroxyproline. They described the molecule as a "twisted hairy rope".

In this paper we characterize a number of commercial gum arabic samples reported to be *A. senegal*, originating from different sources, and compare their characteristics to those of authenticated samples of gum from *A. senegal* and *Acacia seyal*. The latter is probably the most commercially abundant acacia gum after *A. senegal*.

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Table I. Commercial Gum Arabic Samples

sample	origin	form
1[a]	Sudan	kibbled
2	Sudan	kibbled
3	Sudan	kibbled
4	Chad	nodules of varying sizes
5	Senegal	nodules of varying sizes
6	Senegal	nodules of varying sizes
7	Ethiopia	nodules of varying sizes
8[b]	Sudan	nodules of varying sizes

[a] Authentic sample of *A. senegal*. [b] Authentic sample of *A. seyal*.

In addition to chemical and physicochemical characterization procedures, we have also analyzed the gums using an immunoassay recently developed in our laboratory which can differentiate *A. senegal* gum from gums originating from other acacia species.

EXPERIMENTAL PROCEDURES

Materials. Six commercial samples of gum arabic reported to be from *A. senegal* and authenticated samples of *A. senegal* and *A. seyal*, hand-picked by personnel of the Gum Arabic Co. Ltd. (Khartoum, Sudan), were kindly supplied by Agrisales Ltd. (London, U.K.). Details of their origin and nature are given in Table I.

Methods. Determination of optical rotation, neutral sugars, and nitrogen content were carried out as described previously by Randall et al. (1988, 1989). The acid equivalent weight (and hence glucuronic acid content) was determined by potentiometric titration after the gum was converted to the acid form using a cation-exchange column packed with R1-120H$^+$ Amberlite resin. The sample (50 cm^3 of 2–3%) of test gum was passed down the column, and the eluent and washings (ca. 300 cm^3) were titrated using 0.1 mol dm^{-3} NaOH.

The molecular mass distribution of the samples was monitored by gel permeation chromatography (GPC) using the Pharmacia FPLC system. Gum (1% w/v dry wt) solutions in 0.5 mol dm^{-3} NaCl were filtered through 0.45-μm Millipore filters, injected through a 100-μL loop into a Superose 6 column (Pharmacia), and were eluted using 0.5 mol dm^{-3} NaCl which had been filtered and degassed before use. The flow rate was maintained at 0.5 cm^3 min^{-1}, and the eluent was monitored by UV at a wavelength of 206 nm (LKB 2238 Uvicord SII detector) by differential refractometry (differential refractometer, R401 Waters Millipore) and by "on-line" photon correlation spectrometry (PCS) using the Oros 801 molecular size detector. This latter technique gives the equivalent sphere hydrodynamic radius, R_H, of the polymer molecules as they elute from the column.

An enzyme-linked immunosorbent assay (ELISA) previously described by Williams et al. (1992) and Menzies et al. (1992) can identify gums related to *A. senegal*. The technique is based on the use of antibodies that have been raised in rabbits against gum arabic which recognize and interact with a specific binding site in the gum molecule. Such sites, generally referred to as epitopes, often consist of a number of chemical groups linked in a specific arrangement.

In the procedure (Figure 1) solutions of the test gum and then the antibody are added to microwell plates previously coated with antigen (*A. senegal* gum; step 1). If the test gum is not from *A. senegal*, then the antibody can recognize and interact only with the gum already adsorbed on the plate. If the test gum has the chemical features of *A. senegal*, then competition occurs and the antibody can interact with either the molecules in solution or those adsorbed on the plate. The procedure is so designed that in this situation the antibody does not interact with all of the adsorbed gum moleclues and so there are fewer attached to the plate (step 2). The solution in the well is then removed, and a second antibody (anti-rabbit IgG) conjugated to an enzyme (peroxidase) label is added which recognizes and interacts with the gum arabic antibody now attached to the plate. A chromogenic reagent is then introduced which interacts with the second antibody, producing a blue-green coloration. The intensity of color is proportional to the amount of the second antibody attached to the plate (step 3). Little or no color

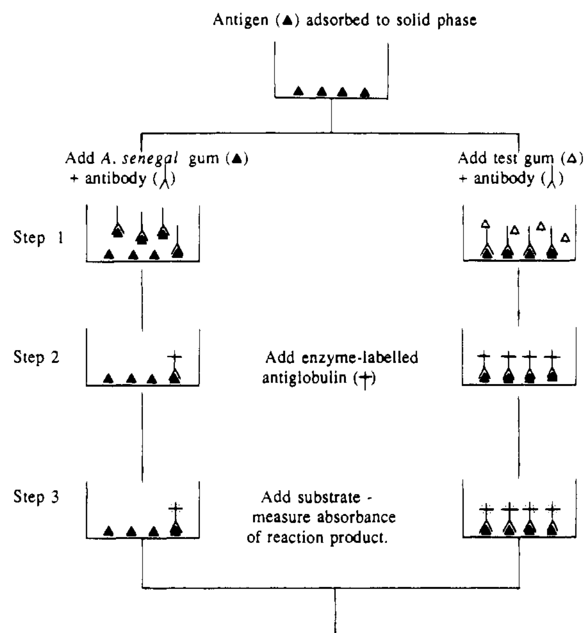

Figure 1. Schematic diagram illustrating the procedure for the ELISA.

indicates, therefore, that the test gum is from *A. senegal*; an intense color indicates it is not. Details of the experimental procedure have been reported previously by Williams et al. (1992) and Menzies et al. (1992). The absorbances of the solutions were measured at 405 nm and plotted as a percentage of the maximum absorbance obtained when the procedure was carried out with antiserum in the absence of gum.

RESULTS AND DISCUSSION

The chemical and physical chemical characteristics of the various gums are given in Table II. The specific rotation values for samples 1–6 are all very similar and are in accordance with literature values for gum from *A. senegal* (Anderson et al., 1990). Sample 7 gave a slightly lower value of −23°, which is outside the proposed range set by JECFA, and sample 8 (*A. seyal*) was found to be dextrorotatory having a value of +54°, which is consistent with values quoted by Stephen (1987). All of the gums contained galactose, arabinose, and rhamnose. The sugar compositions for samples 1–6 are similar and within the range reported for gums from *A. senegal*. The equivalent weights of samples 2–5 are consistent with literature values for *A. senegal* gum. The value for sample 7, however, is a little higher. Sample 7 also shows higher rhamnose and galactose contents than the others. The *A. seyal* exudate (sample 8) has a lower rhamnose content and a higher arabinose content, in keeping with values obtained by Stephen (1987).

The nitrogen and protein contents for the various samples are given in Table III. The protein content was calculated using the nitrogen conversion factor (NCF) of 6.60 as proposed by Anderson (1986). Samples 1–5 and 7 have similar nitrogen values which are consistent with the literature for gum from *A. senegal*. The nitrogen content was found to be much higher for sample 6 and was well above the proposed values set by JECFA. The *A. seyal* gum (sample 8) has a lower nitrogen value in accordance with values obtained by Anderson and Herbich (1963).

The amino acid analyses of certain gum samples are presented in Table IV. *A. senegal* gum derives from the *Acacia* subgenus Aculeiferum (series Vulgares Benth.), whereas *A. seyal* derives from the subgenus *Acacia* (series

Table II. Chemical Analysis of the Acacia Gums

	sample							
	1	2	3	4	5	6	7	8
$[\alpha]^{20}_D$	−29.4	−29.0	−30.0	−29.5	−29.0	−31.0	−23.0	+54.0
% rhamnose	14 ± 1	14 ± 1	12 ± 1	14 ± 1	12 ± 1	15 ± 1	18 ± 1	3 ± 1
% arabinose	29 ± 2	26 ± 2	24 ± 1	27 ± 1	27 ± 1	26 ± 1	26 ± 1	41 ± 2
% galactose	36 ± 2	37 ± 1	38 ± 2	35 ± 1	38 ± 2	32 ± 2	41 ± 1	32 ± 2
equivalent wt	nd[a]	1040	1119	1040	1095	nd	1270	nd
uronic acid, %	nd	17	16	17	16	nd	14	nd

[a] nd, not determined.

Table III. Nitrogen and Protein Content of Gum Samples

	sample							
	1	2	3	4	5	6	7	8
% nitrogen	0.365	0.351	0.327	0.393	0.318	1.06	0.384	0.147
% protein[a]	2.41	2.32	2.16	2.59	2.10	7.00	2.53	0.97

[a] Using a NCF of 6.60 as suggested by Anderson (1986) for gum from *A. senegal.*

Gummiferae Benth.). For comparison we have included two additional gums from each species, namely *Acacia gerrardii* and *Acacia goetzii* (Anderson and McDougall, 1987), and the values obtained for samples 1 and 3 are similar to those reported in the literature for *A. senegal* by Anderson et al. (1983, 1985). For all of the samples hydroxyproline and serine are the major amino acids present, with the hydroxyproline contents being particularly high in samples 6 and 7. These two samples are also seen to have slightly higher threonine and leucine contents than the other samples. It is interesting to note that the amino acid profile for sample 8 (*A. seyal*) is similar to that for sample 1 (*A. senegal*). Indeed, the amino acid analyses would not appear to be significantly different between the two subgenera, despite the fact that the overall amount of protein differs. Anderson et al. (1985) have also reported similar amino acid profiles for a number of commercial gum arabic samples and for *A. senegal* specimens originating from various countries in the Sahelian gum belt.

The nitrogen conversion factors (NCF) were determined for each sample from the amino acid analysis (Table IV), and the values obtained even for sample 8 (*A. seyal*) are reasonably close to the value of 6.60 proposed by Anderson (1986) for gum from *A. senegal.*

The GPC chromatograms showing the molar mass distributions of the various gums are given in Figure 2. The chromatograms shown in Figure 2a,b were obtained by monitoring the eluent by refractive index (RI) and those in Figure 2c,d by UV. The reason the elution profiles are different using the two techniques is that RI is essentially sensitive to the total concentration of material present, whereas UV is more sensitive to the chemical nature of the various molecular mass fractions. At 206 nm UV detects the carboxyl groups associated with the polysaccharide and also the amino acids which make up the proteinaceous component. Although the latter is present in very small quantities (ca. 2% of the total mass), it has a very high molar extinction coefficient by Randall et al. (1989) and, therefore, gives an intense absorption peak.

Scrutiny of Figure 2 allows the assignment of the various elution profiles to the three fractions which have previously been reported by Randall et al. (1989), namely the AG, AGP, and GP fractions. The assignments are shown on the figure and can be readily understood if it is recognized that the UV profiles are mainly indicative of the protein distribution within the gum, whereas the RI profiles reflect more accurately the weight distribution of total material present. Compare, for example, parts a and c of Figure 2 for samples 1–5. The AGP fraction (corresponding to an average distribution coefficient, K_{av}, of 0.04) shows its presence in Figure 2a (RI) but due to its high protein content is the major peak in Figure 2c (UV). The bulk of the material, i.e., the AG fraction (K_{av} = 0.33), is the main peak in Figure 2a (RI) but due to its low protein composition shows only as a shoulder in Figure 2c. The GP fraction does not show on Figure 2a (RI), because it represents less than 1% of the gums, but shows clearly in Figure 2c (UV) (K_{av} = 0.46). All of the profiles for samples 1–5 are similar, indicating comparable molecular characteristics. All contain the AGP, AG, and GP fractions and in approximately the same proportions. This is not so for samples 6–8. Their profiles are different. However, it is significant that in each of the samples all three fractions can also be recognized, although they are present in different proportions. For *A. seyal* (sample 8) there is yet another fraction that can be observed only on the UV elution profile close to Vt (K_{av} = 0.53), indicating that it may contain some protein. It would not, however, appear to be a major fraction, but it is significant that it is characteristic of the gum *A. seyal.* Although we are unable to supply quantitative data, the elution profiles show clearly the difference in proportion of the various components in the different samples examined. The findings are summarized in Table V.

The molecular mass distribution of the various gums is also illustrated by the equivalent sphere hydrodynamic radii (R_H) obtained by monitoring the eluent by on-line photon correlation spectroscopy as illustrated in Figure 3. These again show that samples 1–5 are very similar with the bulk of the material, i.e., the AG fraction, having an R_H of between 8 and 12 nm with a small proportion, i.e., the AGP fraction, having R_H values up to about 30 nm. These results are in very good agreement with R_H values obtained previously for the isolated gum fractions by Randall et al. (1989), which showed that the AG fraction had an R_H of 9.2 ± 0.5 nm, corresponding to an average molecular mass of 2.79×10^5 by light scattering, and that the AGP fraction had an R_H of 22.8 ± 0.5 nm, corresponding to a molecular mass of 1.45×10^6. The data for samples 6–8 again illustrate that these three samples are very different from samples 1–5, with the R_H values for samples 6 and 7 in particular being much higher.

The immunoassay results are presented in Figure 4 and show that the percent maximum absorbance decreases with increasing gum concentration, indicating increased inhibition of the gum arabic–antibody interaction with gum adsorbed on the plate. The curves for samples 1–6 are very similar and show that 50% maximum absorbance corresponds to gum concentrations of 6–14 $\mu g\ cm^{-3}$. The curves for samples 7 and 8 are significantly different, with 50% maximum absorbance corresponding to concentrations of 80 and >100 $\mu g\ cm^{-3}$, respectively. The fact that samples 1–6 interact with the gum arabic antibody to very similar extents further confirms the previously observed closeness in their molecular characteristics as indicated

Table IV. Amino Acid Compositions of Acacia Subgenera and Commercial Gum Arabic (Residues/1000 Residues of Gum)

	subseries Vulgares		commercial gum arabic				subseries Gummiferae	
				sample				
	sample 1 (*A. senegal*)	*A. goetzii*[a]	*A. senegal*[b]	3	6	7	sample 8 (*A. seyal*)	*A. gerrardii*[a]
Asp	91	91	60	58	39	22	65	72
hydroxy-Pro	256	215	274	252	298	351	240	320
Thr	72	62	77	78	109	109	62	55
Ser	144	121	137	162	174	178	170	80
Glu	36	56	36	40	23	15	38	49
Pro	64	64	77	71	67	64	73	58
Gly	53	41	49	54	49	42	51	59
Ala	28	56	31	28	17	11	38	42
Cys	3		1					
Val	35	88	45	36	21	15	42	60
Met	2							3
Ile	11	15	14	12	6	5	16	36
Leu	70	59	75	78	105	99	85	52
Tyr	13	26	11	10	4	5	13	30
Phe	30	35	29	30	23	14	24	18
His	52	33	51	52	47	54	51	25
Lys	27	31	26	26	14	11	18	23
Arg	15	5	7	9	4	4	11	12
% N	0.365	0.89	0.35	0.327	1.06	0.384	0.147	1.86
nitrogen conversion factor	6.77	6.85	6.43	6.67	6.43	6.70	6.60	7.0

[a] Data from Anderson and McDougall (1987). [b] Data from Anderson et al. (1985).

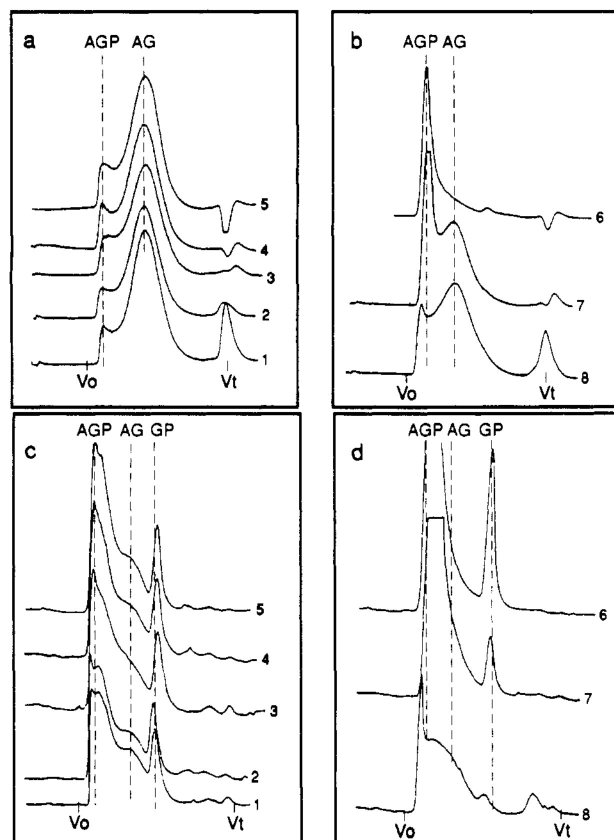

Figure 2. GPC elution profiles of 1% w/v gum solutions: (a) samples 1–5 as monitored by RI; (b) samples 6–8 as monitored by RI; (c) samples 1–5 as monitored by UV at 206 nm; (d) samples 6–8 as monitored by UV at 206 nm. The eluent was 0.5 mol dm^{-3} NaCl, and the flow rate was set at 0.5 cm^3 min^{-1}.

by the distributions in proportions of AG, AGP, and GP. This is despite the fact that sample 6 has a much higher protein content. We have previously shown (Williams et al., 1992) that the antibody has a strong affinity for the GP fraction, a lesser affinity for the AGP fraction, and very little, if any, affinity for the AG fraction. Yet we have shown that the carbohydrate structures of all three fractions are very similar (Williams et al., 1990). This

Table V. Relative Proportions of the Various Fractions in Each Gum Sample

	sample[a]				
fraction	1 (*A. senegal*)	2–5	6	7	8 (*A. seyal*)
AG	+++	+++	+	++	+++
K_{av}	0.33	0.33		0.23	0.33
AGP	++	++	+++	+++	++
K_{av}	0.04	0.04	0.04	0.04	0.04
GP	+	+	++	+	+
K_{av}	0.46	0.46	0.46	0.46	0.46
4					+
K_{av}					0.5

[a] +++, ++, and + refer to the peaks as major, secondary, or minor, respectively.

suggests, but cannot be taken as proof, that the amino acids present may be an integral part of the antibody binding site. This conclusion does not accord with Pazur et al. (1986, 1991) and Miskiel and Pazur (1991), who reported that the purified antibody interacts with the carbohydrate moieties of gum arabic; however, these workers did not consider the possible contribution from the presence of the protein associated with the gum arabic molecule. This aspect requires further investigation. Nevertheless, in this context it is significant that sample 6, which contains the highest proportion of the GP and AGP components, also shows a slightly enhanced interaction with the antibody compared to samples 1–5.

The fact that samples 7 and 8 interact to a much lesser extent in the ELISA is indicative of a much reduced interaction of the antibody with the gum molecules. Nevertheless, some interaction does occur, in keeping with the presence of a certain amount of the GP and AGP fractions in these samples also. The indication is that there is some moiety which interacts with the antibody which is common to all samples, including *A. seyal*, but that its overall concentration varies significantly.

Using ^{13}C NMR spectra, significant differences have been reported by Anderson et al. (1991) between gum arabic samples from various geographical locations. Despite having the overall characteristics of *A. senegal*, major spectroscopic differences were found. It was concluded by Anderson et al. (1991) that *A. senegal* could be a variable species, with recognized subspecies and varieties, which

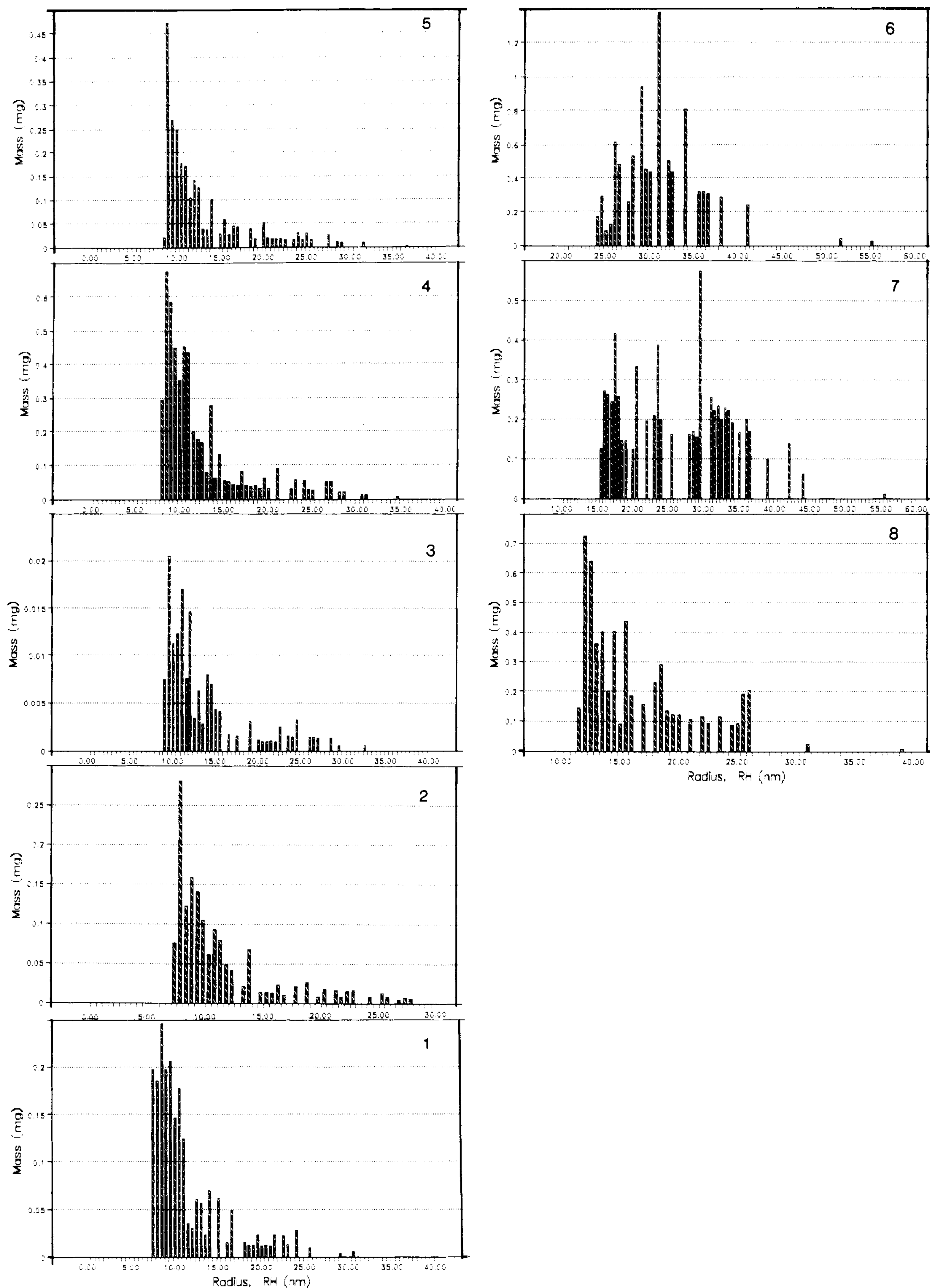

Figure 3. R_H distributions for the various gum samples. The experimental conditions are given in Figure 2.

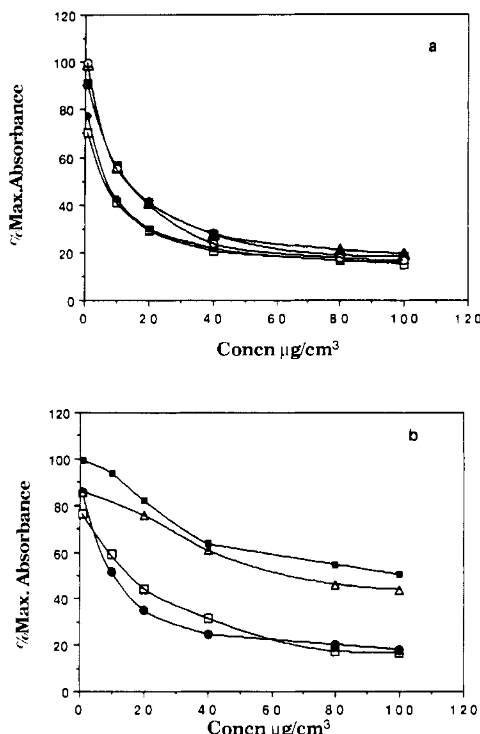

Figure 4. Interaction of commercial gums in the ELISA: (a) sample 1 (□); sample 2 (●); sample 3 (△); sample 4 (■); sample 5 (○); (b) sample 1 (□); sample 6 (●); sample 7 (△); sample 8 (■).

might be influenced by differences in climate and soil. Our findings support a broad similarity in the components within the molecularly disperse gum arabic samples, but the proportions are characteristic of the sample, and probably the nature of the subgenus. Thus, the chemical approach to studying the differences in molecular composition could provide a better definition of the various subgenus sections within the *Acacia* species than the aggregate quantities of specific optical rotation and nitrogen content which have recently been proposed by the Joint FAO/WHO Expert Committee on Food Additives (FAO 1990). As is evident from our results, *A. senegal* and *A. seyal* are different; however, their overall amino acid distribution is not unduly different, nor is this true for the other two representative gums from each of the Vulgares (*A. goetzii*) and Gummiferae (*A. gerrardii*) series of Bentham's botanical classification of the genus.

In summary, therefore, the GPC procedure can identify the individual AG, AGP, and GP components and their overall distribution within individual gum samples. Allied with the immunoassay, this information can be harnessed to provide a practical method to identify the composition of gum from the *Acacia* species. It is significant that even between two different subseries of *Acacia*, such as Gummiferae (*A. seyal*) and Vulgares (*A. senegal*), there are significant points of chemical similarity. In particular, both appear to contain similar and discrete molecular mass fractions indicative of a common taxonomic origin. Our objective is to relate these botanical classifications with chemical composition to further the gum industry and regulatory bodies in their efforts to provide effective quality control systems in the producer countries and for the subsequent user of gum arabic.

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