Chemical and Physical Properties and the Enzymatic Degradation of Some Tropical Plant Gums

J. Y. MORIMOTO, IRENE C. J. UNRAU, and A. M. UNRAU[1]

Department of Agricultural Biochemistry, Hawaii Agricultural Experiment Station, University of Hawaii, Honolulu 14, Hawaii

The seed of five of the six tropical legumes studied contained more than 23% water-soluble gum with one, *Caesalpinia pulcherima*, containing over 30% of galactomannan in the seed. The galactan from *Centrosema plumari* contained 8% sugars other than galactose, while the other galactomannans were essentially similar in composition to guar gum. Mucilages from *Samanea saman* and *Leucaena glauca* leaves were acidic and their compositions were more complex. Undegraded hexose residues after periodate degradation indicated the presence of some unusual linkages. Relatively large quantities of erythritol formed indicated that the main polymeric linkage was of the 1,4 type, while the glycerol formed was believed to originate mainly from single unit galactopyranose branches. The gums were degraded to varying extents by three commercial mold enzyme preparations.

A CLASS of polysaccharides referred to as gums and mucilages are used in large quantities in a variety of industries. Although the composition of many galactomannans from various legume seeds is quite similar, their physical properties vary to the extent that the individual gums may serve in rather specialized systems. In a recent monograph (*23*), Smith and Montgomery enumerated many of the different uses of gums and mucilages.

During World War II, the dwindling supply of some of the industrially important gums, particularly locust bean gum from the carob tree, prompted an investigation (*4*) of the gum content of various legume seeds. A visual estimation of the amount of endosperm in the seed was made on 163 species, and the ratio of galactose to mannose was determined chemically in about 12 of the 163 species. The survey by Anderson (*4*) showed that very few of the large number of different species of legumes contained as much endosperm as did guar seeds.

Galactomannans from legume seeds usually contain higher proportions of mannose than galactose. A notable exception is the polysaccharide isolated from alfalfa seed by alkaline extraction where the galactose:mannose ratio is 2:1 (*16*). Hot water extraction of the same seed yields a structurally different galactomannan which has a galactose:mannose ratio of 4:5 (*5*). A few galactans have been obtained from legume seeds. Relatively pure galactans have been isolated from the seed of *Lupinus albus* (*17*) and *Strychnos nux vomica* (*6*).

[1] Present address, Department of Chemistry, University of British Columbia, Vancouver 8, Canada.

The viscosity of solutions of neutral gums can in many instances be increased by the addition of borax (*15*, *28*). The borate complex is stable in the presence of borax, but removal of borate by dialysis causes liquefaction of the borax gel (*12*). The property of forming borate complexes appears to be dependent on the presence of *cis*-hydroxyls and has been used for classification purposes.

Degradation of gums by enzymes has been used with some success in structural and other types of investigations. Germinating seeds of a number of legumes (*7*) and certain molds (*11*) elaborate enzymes that hydrolyze galactomannans.

For an extensive discussion of the chemistry of gums and mucilages, the reader is referred to the recent monograph by Smith and Montgomery (*23*) and an earlier review by Von Wiesner (*27*) concerning investigations on species of 10 legume genera which contain gum in the endosperm of the seed.

Experimental Methods

The following solvent systems were used in paper partition chromatography of the gum hydrolyzates: *A*, BuOH:EtOH:H_2O (4:1:5) (*21*); *B*, BuOH:HOAc:H_2O (2:1:1) (*21*); and *C*, BuOH:pyridine:H_2O (6:4:3) (*18*). Generally, a *p*-anisidine trichloroacetate spray reagent (*10*) was used for development of irrigated chromatograms, although in some instances, ammoniacal silver nitrate solution (*20*) was also used. Unless otherwise stated, all chromatographic separations were carried out using Whatman No. 1 filter paper. Melting points reported for compounds are uncorrected.

Isolation of the Gums. Coarsely ground seed (5 grams) was extracted several times with boiling hot water during 8 hours until no further gum could be precipitated from the aqueous extract by pouring it into three volumes of ethanol. The products were treated for 2 hours with hot (80° to 90° C.) 10% alkali to remove protein and after cooling and acidification with acetic acid, they were reprecipitated with alcohol. The gums, usually in a white, fibrous form, were dried to constant weight at 65° C. and kept under vacuum over anhydrous calcium chloride.

Hydrolysis of Gums. Suitable quantities (2 to 3 grams) of the gums were hydrolyzed with 1*N* H_2SO_4 (25 ml.) by refluxing the solutions for 8 to 10 hours. The solutions were neutralized ($BaCO_3$), decolorized with carbon, filtered, deionized, and evaporated under reduced pressure to a thick sirup. The sirups were chromatographed together with known sugars and the components tentatively identified. Aliquots of the hydrolyzates were chromatographed in solvent *C* and the components located by excision of guide strips followed by spraying these with *p*-anisidine trichloroacetate reagent (*10*). The sugars were eluted from the paper and the quantity of each determined by the phenol–sulfuric acid method (*13*). Recovery of the sugars from the hydrolyzates ranged from 93 to 96% of that expected.

Identification of Components. Galactose and mannose in the gum hydrolyzates were resolved by sheet paper chromatography (solvent *C*) using Whatman No. 3MM paper. The sugars were located on developed chromatograms as mentioned in the previous section and eluted. Mannose was treated with sodium acetate and phenylhydrazine

hydrochloride in the usual manner. The mannose phenylhydrazones from the various gums had m.p. and mixed m.p. at 198° to 200° C. Galactose was transformed to mucic acid which, after washing and drying, had m.p. and mixed m.p. at 217° to 219° C.

Viscosity. The relative viscosity of the different gums was determined in a modified Ostwald (Ostwald-Fenske) viscosimeter. A quantity of gum (100 mg.) was dissolved in hot water (70 ml.) and filtered while hot. The filter paper was washed with boiling hot water and the volume of the filtrate adjusted to 100 ml. The solutions were cooled to the water bath temperature (20° C.) at which the flow time of the 0.1% gum solution was determined. In a series of tests, it was determined that less than 0.75 mg. of the solute was retained by fine-sintered glass filters and by filter paper.

The effect of borate on viscosity was determined by adding to a series of gum solutions (0.1% concentration) the following amounts of borax (sodium tetraborate) per 100 ml. of solution: 50, 100, 200, 350, 500, 750, and 1000 mg.

Degradation of the Gums with Periodic Acid. The gums were subjected to periodate oxidation in order to determine the extent of periodate attack on the hexose units in the polymeric chain. To a solution (25 ml.) of the gum (250 mg.) was added a 25% excess of periodic acid, on the assumption that the hexose unit would consume 1 mole of periodate. The solutions were left at room temperature and in the dark until no further periodate was consumed. Upon completion of the oxidation, the solutions were neutralized ($BaCO_3$) and filtered. The clear filtrates containing the polyaldehyde were treated with potassium borohydride (350 mg.) (*1*, *2*); the reduction was allowed to proceed for 10 hours after which time the solutions were acidified with sulfuric acid and refluxed for 6 hours. After neutralization ($BaCO_3$) and filtration, the solutions were evaporated to dryness. Methanol (containing 1% hydrogen chloride) was added several times and evaporated to effect removal of borate (*9*). The residue was finally dissolved in water, the solution deionized, and evaporated. The residue was chromatographed and the developed paper chromatograms sprayed with ammoniacal silver nitrate to identify the components. The quantity of each alcohol (erythritol and glycerol) was determined by a periodate–chromatropic acid procedure (*8*, *19*, *25*, *26*) and sugars, if present, were determined by the phenol–sulfuric acid method (*13*).

Enzymatic Degradation of Gums. The enzymatic degradation of the gums was carried out as follows: To a solution (50 ml. citrate-phosphate buffer, pH 3.6) of the gum (150 mg.) dry enzyme (10 mg.) was added and the contents were thoroughly mixed. A few drops of toluene were added and the Erlenmeyer flasks placed in a water bath (37° to 38° C.). After 70 hours of incubation, the solutions were passed through Amberlite IR-120 cation exchange resin (Rohm and Haas Chemical Co.) and Duolite A-4 anion exchange resin (Chemical Process Co.) in that order and the effluent was evaporated to a sirup. The enzyme hydrolyzates were chromatographed and the components located by spraying developed paper chromatograms with *p*-anisidine trichloroacetate. The amount of each component was determined by the phenol–sulfuric acid method in the following classification: mannose, galactose, di- and trisaccharide, and tetra- and higher oligosaccharides. Each gum was treated with three enzyme preparations referred to by their trade names, Cellulase 36, Kleerzyme 200 and Pectinol 10M (all from Rohm and Haas Chemical Co.).

Table I. Gum Content of Seeds of Some Tropical Legumes and Composition of the Gums

Species	Gum Content of Seed, %	Composition of Gum, %					
		Galactose	Mannose	Arabinose	Rhamnose	Xylose	Uronic acid
Leucaena glauca	25	42	56	...	...	...	...
Caesalpinia pulcherima	31	34	66	...	...	...	...
Cassia fistula	27	23	75	...	...	...	...
Crotalaria mucronata	23	30	68	(trace)	...	...	...
Centrosema plumari	25	92	4	3	...	...	...
Samanea saman	13	47	...	18	10	8	14[a]
Leucaena glauca (leaves)	2.5	36	...	17	19	8	20[a]

[a] Expressed as galacturonic acid.

Table II. Relative Viscosity, Viscosity of Borate Complexes, and Degree of Polymerization of Gums

Species	Mg. Borax Added/100 Ml. 0.1% Gum Solution (Relative Viscosity 20° C.)							
	0.0	50	100	200	350	500	750	1000
Leucaena glauca	2.52	2.55	2.60	2.64	2.90	2.58	2.56	2.37
Caesalpinia pulcherima	2.89	3.14	3.56	4.09	4.20	3.52	3.45	2.75
Cassia fistula	2.11	2.79	2.98	3.10	3.59	2.76	2.63	2.71
Crotalaria mucronata	1.25	1.35	1.40	1.38	1.31	1.30	1.26	1.21
Centrosema plumari	1.25	1.24	1.27	1.27	1.31	1.31	1.37	1.34
Leucaena glauca (leaves)	2.12	2.18	2.10	2.02	1.95	1.85	1.95	1.95
Guar gum	2.70	3.02	3.38	7.0	10.0	9.8	8.4	8.0
Gum arabic	1.23	1.24	1.28	1.26	1.23	1.32	1.30	1.28
Gum tragacanth	1.42	1.45	1.43	1.40	1.40	1.49	1.38	1.39

Results and Discussion

The gum content of the different seeds and the composition of the gums are summarized in Table I.

Except in one instance (*Caesalpinia pulcherima*), the amount of hot water-soluble gum in the seeds was below 30%. In comparison, guar seed contains 34 to 40% water-soluble galactomannan. Considering the growth characteristics of the species examined, seeds from Caesalpinia, Cassia, and Samanea are mechanically difficult to obtain. Although the pods of *Caesalpinia pulcherima* are easily opened and the seeds relatively large, the thorny and brushy character of the plant would make harvesting difficult. Because of the more herbaceous nature of Centrosema, Crotalaria, and the younger plants of *Leucaena glauca*, the seed from these species could possibly be harvested mechanically. The seeds of *Centrosema plumari* and *Leucaena glauca* are sufficiently large so they could be milled, whereas the seed of *Crotalaria mucronata* is rather small.

The composition (mannose:galactose ratio) of some of the gums was similar to that of guar in that the amount of mannose was greater than galactose. An interesting exception was the composition of the polysaccharide from *Centrosema plumari* which might be referred to as a galactan on the basis that only a minor part (8%) of the molecule was made up of mannose and arabinose. The possibility exists that a small amount of arabogalactan was associated with the galactan. Only one species of Centrosema has been investigated and the possibility exists that other species of this genus may also contain galactans.

The gums from *Samanea saman* seed and green leaves of *Leucaena glauca* are similar to gum arabic and gum tragacanth in that they contained no mannose and in addition to galactose, the major constituent, arabinose, rhammose,

xylose (*Samanea saman*), and hexuronic acid tentatively identified as galacturonic acid were present.

A considerable range in the viscosity of the various gums was observed (Table II). The viscosity of the galactomannan from *Crotalaria mucronata* was rather low compared to that of Leucaena seed gum. The galactan from Centrosema had a low viscosity similar to the viscosities of gum arabic and gum tragacanth.

None of the gums isolated showed the tendency to complex with borate to the extent that was shown by guar gum. The galactomannan from Crotalaria showed only a small tendency to complex with borate. Centrosema galactan and Leucaena leaf mucilage showed no complexing and were similar in this respect to gum arabic and gum tragacanth. The results with guar gum and a few other gums indicated, as shown previously (*12*), that formation of the borate complex is a function of concentration of $B(OH)_4^-$ ion. Optimum viscosity was found when approximately 350 mg. of borax was added per 100 ml. of gum solution. In the case of the Centrosema galactan and other gums not containing mannose, complexing with borate would not be expected due to the absence of *cis*-hydroxyl groups such as are found in 1,4- or 1,6-linked mannose residues.

Periodate oxidation followed by reduction of the polyaldehyde with potassium borohydride and hydrolysis of the product (*1, 2*) gave information regarding the principal glycosidic linkages in the polysaccharides. Results of such experiments are summarized in Table III.

By application of the usual periodate degradation procedures and the newer modification (*14*), evidence was obtained which pointed to the presence of some unusual linkages. In the case of the Centrosema galactan, about 15% of the galactose residues were not degraded. This suggests that they may be linked through C_2 and C_4 or involved in 1,3-linkages. Similarly about 15% of the hexose residues in Leucaena gum were not attacked by periodate. In this case, the undegraded hexose residues were composed of about 95% mannose and 5% galactose. From this result, it would appear that a few galactose residues occupy internal positions in the polymer. In experiments with gum from *Crotalaria mucronata* seed, 13% of the hexose residues were not attacked by periodate. In this case, the hexose component consisted of 97% mannose and 3% galactose. The gum from *Cassia fistula* seed gave similar results in that mannose, but no galactose could be detected in the undegraded hexose fraction.

The large amounts of glycerol produced in the periodate degradations suggest that the galactose residues occupy single unit-branch positions as has been found for guar gum (*3, 22, 24*). From the relatively large quantities of erythritol formed, it may be concluded that the major glycosidic linkage is of the 1,4-type. Evidence for the presence of unusual linkages in some galactomannans has been reported (*22*) and this structural aspect of gums could well be investigated more closely. In general, the gums investigated do not appear to differ basically with respect to their probable structure from such polysaccharides as guar gum or locust bean gum.

It has been shown that enzymes that will hydrolyze galactomannans are gen-

Table III. Components of Periodate-Degraded Gums

Species	Mannose	Galactose, %	Erythritol	Glycerol
Leucaena glauca	14.3	0.8	30.5	54.2
Crotalaria mucronata	12.6	0.6	38.5	48.2
Centrosema plumari	...	15.0	78.3	6.0
Cassia fistula	12.5	...	48.8	37.8

Table IV. Enzymatic Degradation of Gums

Species	Enzyme	Components in Hydrolyzates, % Mannose	Galactose	Arabinose	Rhamnose	Xylose	Di + Trisaccharides	Oligosaccharides
Leucaena glauca (seed)	Pectinol	36	29	...	...	...	15	20
	Kleerzyme	27	18	...	...	...	15	40
	Cellulase	28	15	...	...	...	9	47
Caesalpinia pulcherima	Pectinol	40	12	...	...	...	28	20
	Kleerzyme	32	20	...	...	...	24	20
	Cellulase	43	16	...	...	...	11	30
Cassia fistula	Pectinol	47	21	...	...	...	19	12
	Kleerzyme	16	24	...	...	...	22	38
	Cellulase	48	12	...	...	...	12	28
Crotalaria mucronata	Pectinol	50	21	...	...	...	17	12
	Kleerzyme	34	17	...	...	...	17	32
	Cellulase	54	20	...	...	...	4	22
Centrosema plumari	Pectinol	3	82	1	...	...	5	9
	Kleerzyme	2	69	Trace	...	...	10	18
	Cellulase	2	83	Trace	...	...	2	12
Leucaena glauca (leaves)	Pectinol	...	7	Trace	6	Trace	Trace	85[a]
	Kleerzyme	...	3	1	15	Trace	1	78[a]
	Cellulase	...	5	Trace	17	Trace	2	74[a]
Guar gum	Pectinol	9	6	...	...	...	15	70
	Kleerzyme	9	8	...	...	...	11	72
	Cellulase	15	14	...	...	...	16	55
Gum tragacanth	Pectinol	...	7	13	3	1	3	73[a]
	Kleerzyme	...	10	12	4	Trace	5	68[a]
	Cellulase	...	8	14	3	Trace	Trace	73[a]
Gum arabic	Pectinol	...	12	11	5	...	10	62[a]
	Kleerzyme	...	14	12	5	...	12	57[a]
	Cellulase	...	16	10	4	...	10	60[a]

[a] Figures include acidic fraction eluted from anion exchange resin column.

erated in molds (*11*) and in germinating legume seeds (*7*). The ease whereby the galactomannans as well as gum guar, tragacanth, and arabic were hydrolyzed by some commercial mold enzyme preparations was determined. Experimental results are summarized in Table IV.

Except in a few instances, the enzyme preparation referred to as Cellulase 36 degraded the galactomannans to monosaccharide units to the greatest extent (42 to 75%). Kleerzyme was the least effective (30 to 52%). The galactan from Centrosema seed was degraded to galactose extensively by all three enzyme preparations. Guar gum was degraded to monosaccharide units only to the extent of 15 to 17% by Pectinol 10M and Kleerzyme, respectively. Cellulase 36 degraded the gum to monosaccharide units to an extent of about 30%. Why guar gum was so much more resistant to the hydrolytic action of the enzymes was not investigated.

Mucilage from the leaves of *Leucaena glauca* was degraded by the enzymes in an interesting fashion. Both Kleerzyme and Cellulase 36 removed almost all of the rhammose units. Only a small proportion of the galactose residues were removed from the polymer, while 74 to 78% of the polymer was left in the form of larger oligosaccharide fragments. Pectinol 10M removed a smaller quantity of rhammose and 85% of the polysaccharide remained as larger oligosaccharide fragments. In the case of gum arabic and gum tragacanth, considerable arabinose was removed, but in either case, the specific removal of a particular constituent was not as evident. The possibility exists that in the case of the mucilage from the leaves of *Leucaena glauca*, the rhammose units may be terminal and the presence of a rhammosidase accounted for the somewhat specific removal of these sugar residues.

Acknowledgment

The authors thank J. C. Moomaw, University of Hawaii, for supplying seed of some of the legume species. Samples of the commercial mold enzyme preparations were kindly supplied by G. G. Dull, Pineapple Research Institute, Honolulu, Hawaii.

Literature Cited

(1) Abdel-Akher, M., Hamilton, J. K., Montgomery, R., Smith, F., *J. Am. Chem. Soc.* **74,** 4970 (1952).
(2) Abdel-Akher, M., Hamilton, J. K., Smith, F., *Ibid.*, **73,** 4691 (1951).
(3) Ahmed, Z. F., Whistler, R. L., *Ibid.*, **72,** 2524 (1950).
(4) Anderson, E., *Ind. Eng. Chem.* **41,** 2887 (1949).
(5) Andrews, P., Hough, L., Jones, J. K. N., *J. Am. Chem. Soc.* **74,** 4029 (1952).
(6) Andrews, P., Hough, L., Jones, J. K. N., *J. Chem. Soc.* **1954,** 806.
(7) Bourquelot, E., Herissey, H., *Compt. rend.* **129,** 614 (1899); **130,** 42 (1900).
(8) Boyd, M. J., Logan, M. A., *J. Biol. Chem.* **146,** 279 (1922).
(9) Briggs, D. R., Garner, E. F., Montgomery, R., Smith, F., *Anal. Chem.* **28,** 1333 (1956).
(10) Christensen, G. M., Smith, F., *J. Am. Chem. Soc.* **79,** 4209 (1957).
(11) Deuel, H., Leuenberger, R., Huber, G. *Helv. Chim. Acta* **33,** 942 (1950).
(12) Deuel, H., Neukom, H., *Makromol. Chem.* **3,** 13 (1949).
(13) Dubois, M., Hamilton, J. K., Gilles, K. A., Rebers, P. A., Smith, F., *Anal. Chem.* **28,** 350 (1956).
(14) Goldstein, I. J., Hay, G. W., Lewis, Bertha A., Smith, F., Abstracts, 135th Meeting, ACS, Boston, Mass., April 1959.
(15) Hart, R., *Ind. Eng. Chem., Anal. Ed.* **2,** 329 (1930).
(16) Hirst, E. L., Jones, J. K. N., Walder, W. O., *J. Chem. Soc.* **1947,** 1443.
(17) *Ibid.*, p. 1225.
(18) Jeanes, A., Wise, C. S., Dimler, R. J., *Anal. Chem.* **23,** 415 (1951).
(19) Lambert, Marguerite, Neish, A. C., *Can. J. Research* **28B,** 83 (1950).
(20) Partridge, S. M., *Nature* **158,** 270 (1946).
(21) Partridge, S. M., Westall, R. G., *Biochem. J.* **42,** 238 (1948).
(22) Rafique, C. M., Smith, F., *J. Am. Chem. Soc.* **72,** 4634 (1950).
(23) Smith, F., Montgomery, R., "Chemistry of Plant Gums and Mucilages," ACS Monograph No. 141, Reinhold, New York, 1959.
(24) Swanson, J., *J. Am. Chem. Soc.* **71,** 1510 (1949).
(25) Unrau, A. M., Ph.D. thesis (Part II) University of Minnesota, 1960.
(26) Unrau, A. M., Smith, F., *Chem. & Ind. (London)* 330 (1957).
(27) Von Wiesner, J., "Die Rohstoffe des Pflanzenreiches," 4th ed., W. Engelman, Leipzig, 1928.
(28) Williams, A. L., *Analyst* **53,** 411 (1928).

Received for review January 18, 1961. Accepted June 12, 1961. Published with the approval of the Director, Hawaii Agricultural Experiment Station, as Technical Paper, Paper No. 501.

Correction

Chelometric Titration of Calcium and Magnesium in Plant Tissue. Method for Elimination of Interfering Ions

In this article by R. M. Carlson and C. M. Johnson [J. AGR. FOOD CHEM. **9,** 460 (1961)], the following corrections should be made:

On page 460, column 2, line 24, the order: Mg < Ca < Mm < Al < Zn should read the order: Mg < Ca < Mn < Al < Zn. On page 461, column 1, line 19 of the paragraph titled "Anion Exchange Columns": CyDTA and washing with 2 ml. of should read CyDTA and washing with 20 ml. of. On page 462, column 2, the first sentence should read: Five plant tissue samples were analyzed for calcium and magnesium in 11 replicates each, using the dry-washing technique. (The comma should come after the word "each" instead of before it.)