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Immunochemical Properties of Hualtaco Gum

M. HEIDELBERGER AND C. V. N. RAO*

Department of Pathology, New York University School of Medicine, New York, and Institute of Microbiology, Rutgers, The State University, New Brunswick, New Jersey

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Summary. Hualtaco gum, from the South American plant *Loxopterygium huasango*, yields D-galactose, D-glucuronic acid, L-rhamnose and small quantities of arabinose and aldobiuronic acid on hydrolysis with strong acid. Like gum arabic, hualtaco yields a highly immunologically reactive product after mild degradation, while stronger hydrolysis furnishes an aldobiuronic acid, 6-*O*-(β-D-glucuronopyranosyl)-D-galactose, identical with that isolated similarly from gum arabic. Quantitative data are given on some of the precipitin reactions of the gum in antipneumococcal sera and the chemical basis for the reactivity in each system is discussed insofar as present knowledge permits.

INTRODUCTION

A South American gum designated 'hualtaco', available in large quantities and said to originate in the plant *Loxopterygium huasango*, family Anacardiaceae (we are indebted to Professor Juan Ibañez, botanist, of the University of Chile, Santiago, Chile, for this information), was recently shown to give cross-reactions in a number of antipneumococcal sera (Heidelberger, 1960a). As these reactivities of the gum showed a striking similarity to those of gum arabic (cf. Heidelberger, 1960a, b), a more detailed chemical and immunological study of hualtaco gum was undertaken and the results are presented herewith.

MATERIALS AND METHODS

Antipneumococcal (anti-Pn) sera were supplied by the Division of Laboratories and Research, Department of Health, State of New York, through the kindness of Miss Jessie L. Hendry and by the Bureau of Laboratories, New York City, Department of Health, by courtesy of Miss Annabel W. Walter.

Quantitative precipitin reactions were set up as in earlier papers (Heidelberger and Kendall, 1935) at 0° and kept in a 0° bath for 48 hours in the case of homologous reactions and 7–15 days or more for cross-reacting systems. A cold box (Heidelberger and Rebers, 1958) was used during drainage of the tubes. Nitrogen was estimated by the Markham (1942) method.

Whatman No. 1 papers were used for identification of sugars by paper chromatography and Whatman 3 MM papers for their isolation. Solvent-systems were: (a) Pyridine–ethyl-acetate–acetic acid–water, 5 : 5 : 1 : 3 (Fischer and Dörfel, 1955); (b) n-butanol–pyridine–water–benzene, 5 : 3 : 1 : 3 (upper layer) (Laidlaw and Reid, 1952); (c) n-

* Present address: Department of Macromolecules, Indian Association for the Cultivation of Science, Calcutta 32, India.

butanol–pyridine–water, 6 : 4 : 3 (Chargaff, Levine and Green, 1948); (d) n-butanol–ethanol–water–benzene, 5 : 3 : 3 : 1 (Jeanes, Wise and Dimler, 1951); and (e) n-butanol–ethanol–water–ammonia, 40 : 10 : 49 : 1 (Block, 1952).

Reducing sugars were located on paper chromatograms with spray-reagents: (1) aqueous saturated aniline oxalate; and (2) alkaline silver nitrate (cf. Kabat and Mayer, 1961).

Individual sugars were estimated by the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers and Smith, 1951, 1956) after separation by quantitative paper chromatography. Galactose and rhamnose were determined simultaneously by their reaction with L-cysteine (Dische and Shettles, 1948) and glucuronic acid with carbazole and sulphuric acid (Dische, 1947). Consumption of periodate during oxidation of polysaccharide was estimated by the method of Fleury and Lange (1933a, b). All evaporations were carried out at 35–40° *in vacuo*.

RESULTS

ATTEMPTED FRACTIONATION

The gray-brown lumps of hualtaco gum were powdered and extracted with benzene to remove resins and coloring material. The white product (15 g) was dissolved in water (1500 ml), centrifuged and fractionally precipitated with ethanol containing 1 per cent LiCl. Three fractions were collected: Fraction I, at 1500 ml ethanol, 6·5 g after reprecipitation in the same way; Fraction II with 1500 ml more ethanol, 1·9 g; Fraction III by centrifugation of the liquid decanted from Fraction II and reprecipitation as before, 1·3 g. Analyses showed all fractions to have essentially the same composition (Table 1). On hydrolysis and chromatographic examination all were found to contain galactose, rhamnose, glucuronic acid and small quantities of arabinose and an aldobiuronic acid. Subsequent studies were accordingly carried out with Fraction I.

TABLE 1

PROPERTIES AND COMPOSITION OF FRACTIONS OF HUALTACO GUM

	Fraction I	Fraction II	Fraction III
$[\alpha]_D$, water	−14°	−13°	−14°
Ash as Li (%)	0·44	0·40	0·44
D-Galactose (%)*	56·7	57·8	58·7
L-Rhamnose (%)*	20·2	20·6	20·6
D-Glucuronic acid (%)*	10·9	10·4	9·9
Arabinose (%)*	2·9	2·8	3·1
Aldobiuronic acid (%)*	5·6†	5·4†	5·1†

* Ash-free.

† 2× phenol–sulphuric acid colour-value as galactose.

A portion of its hydrolysate was separated into the component sugars on thick filter papers and individual, chromatographically homogeneous, substances were isolated. Their specific rotations at 23° were: galactose, +79° in H_2O (conc. 1·2); glucuronic acid, +33° in H_2O (conc. 0·3); rhamnose, +12·6° in H_2O (conc. 0·3). The corresponding values from the literature are: D-galactose, +80°; D-glucuronic acid, +36°; L-rhamnose, +9·1°.

On oxidation by sodium metaperiodate at 4°, hualtaco gum consumed 0·9 moles of oxidant for each anhydrohexose residue and 1 mole of formic acid was formed per 3·5 anhydrohexose residues. Excess periodate was destroyed with ethylene glycol. The

dialysed product gave 37 per cent galactose and 11·5 per cent glucuronic acid on hydrolysis. Rhamnose and arabinose had disappeared.

DEGRADATION OF THE GUM

Four hundred milligrams of Fraction I were heated 1·5 hours with 40 ml of 0·1 N oxalic acid in boiling water. The degraded polysaccharide was precipitated by addition to 120 ml of ethanol containing 1 per cent LiCl. The supernatant fluid, neutralized with $CaCl_2$, filtered, concentrated, and chromatographed, gave spots corresponding to rhamnose and arabinose. On further hydrolysis with 2 N H_2SO_4 the degraded gum yielded galactose, 68 per cent, glucuronic acid, 19 per cent and an aldobiuronic acid, as did the original gum, together with rhamnose, 6 per cent, and a small quantity of arabinose.

During oxidation of the degraded gum by periodate 1 mole of oxidant was consumed per anhydrohexose unit and 1 mole of formic acid was liberated per 2·2 such residues. The periodate-oxidized degraded gum still contained 38 per cent galactose and 11 per cent glucuronic acid.

Two grams of Fraction I were dissolved in 200 ml of 0·1 N sulphuric acid and heated at 100° for 40 hours. The hydrolysate was neutralized with $BaCO_3$ and the filtrate and washings were de-ionized on a column of IR-120 (H+) resin. The solution contained galactose, rhamnose, arabinose, aldobiuronic acid and spots which moved more slowly on paper chromatograms. The acidic fraction was absorbed on a column of Dowex-1x-4 (OH) and the neutral sugars were washed out with water. The acids were eluted with 0·5 N NH_4OH and the aldobiuronic was isolated as its NH_4 salt by separation on thick filter papers. It had the same mobility in solvent (a) as that from gum arabic; $[\alpha]_D^{23}$ − 1·4°→ −6·9°. Hydrolysis of the acid and chromatography of the hydrolysate gave spots corresponding to galactose and glucuronic acid. On reduction and hydrolysis the methyl ester methyl glycoside of the aldobiuronic acid yielded galactose and glucose. The reduced glycoside consumed 4 moles of periodate with liberation of 1·9 moles of formic acid. Reduction and hydrolysis of the product of oxidation, followed by chromatography, gave spots corresponding to glycerol and glycollic aldehyde. Quantitative estimation of the former gave a value within a few percent of that calculated for the methyl ester methyl glycoside. The aldobiuronic acid was crystallized from water–acetone: melting point 114–115° with effervescence at 125–128°; mixed melting point with the aldobiuronic acid from gum arabic, 114–115° with effervescence at 125–128°. Therefore, the aldobiuronic acid of hualtaco gum is 6-*O*-(β-D-glucuronopyranosyl)-D-galactose (Heidelberger and Kendall, 1929; Challinor, Haworth and Hirst, 1931; Hotchkiss and Goebel, 1936).

IMMUNOLOGICAL REACTIVITY OF HUALTACO GUM

Hualtaco gum precipitates antipneumococcal horse sera of Types I, II, VIII, XIV, XIX, XXII and XXIII (cf. also Heidelberger, 1960a). Three-quarters of the antibody in the Type XXIII serum used was precipitated by the gum. The degraded and oxidized gums also precipitate certain antibodies. Quantitative data on many of these reactions are given in Table 2.

DISCUSSION

Hualtaco gum greatly resembles gum arabic and other gums of the family *Anacardiaceae*, both with respect to the sugars of which it is constituted and with regard to at least a portion of its D-glucuronic acid and D-galactose residues, which are linked as the aldobiuronic acid, 6-*O*-(β-D-glucuronopyranosyl)-D-galactose, in both hualtaco gum and gum

TABLE 2

CROSS-REACTIONS OF HUALTACO GUM, ITS DERIVATIVES, AND CERTAIN OTHER POLYSACCHARIDES IN ANTIPNEUMOCOCCAL SERA OF TYPES I, II, XXII AND XXIII

Polysaccharide	Amount (mg)	Antibody nitrogen precipitated at 0°, calculated to 1·0 ml, from serum, type and No.					
		I 884 C[a] (μg)	I 1057 C[a] (μg)	I 1057FC[b] (μg)	II 513 (μg)	XXII 566[c] (μg)	XXIII 912 (μg)
Homologous	At maximum pptn	980	1024	*ca.* 1000	3600	374	275
Hualtaco, Fraction I	0·15					35	
	0·20			33			192[d]
	0·30	33	19	30		36	200[d]
	0·60	36	20	36	29[e]		
	10·0				256		
	25·0				319		
Degraded Fraction I	0·15					30	
	0·20						175
	0·30	15		44	369	28	185
	0·50				487		
	0·60	16		31			
	1·0				483		
Oxidized Fraction I	0·1	0					
	0·25	0			16		
Oxidized degraded Fraction I	0·5			+++	523[f]		
	1·0				561[f]		
Gum arabic	0·6						234
	2·0						229
	100·0				1197[g]		
Degraded gum arabic	0·2						197[h]
	0·3						209[h]
	0·5				626[g]		
Aerobacter aerogenes 8172	0·05						71[i]
	0·15						66[i]

[a]Absorbed with pneumococcal C-substance. Sera II 513 and XXIII 912 contained only traces of anti-C.
[b]Felton solution from I 1057, absorbed with C-substance. Fraction II precipitated the same amount of antibody from this as did Fraction I, but required larger amounts.
[c]Absorbed with C-substance and partially with glycogen.
[d]Supernatants+S XXIII at the 13 μg level gave 51 μg N. Oxidized Fraction I gave little or no precipitate in anti-Pn XXIII.
[e]From precipitin curve.
[f]Precipitates formed very slowly. Supernatants of tubes containing 1 mg were opalescent.
[g]From Heidelberger (1960b). After absorption with degraded gum arabic, II 513 gave 11 μg N with degraded hualtaco gum; after absorption with ketha gum (cf. Heidelberger, Tyler and Mukherjee, 1962) 64 μg N.
[h]Supernatants+hualtaco, Fraction I, at the 160 μg level, gave 9 μg N.
[i]Supernatants+hualtaco, Fraction I at the 400 μg level gave 114 μg N.

arabic. Like gum arabic (Heidelberger Avery and Goebel, 1929), hualtaco gum is readily degraded to a derivative which shows much greater avidity in its combination with antibodies to the capsular polysaccharide of pneumococcal type II (S II). Since nothing was known of the chemistry of hualtaco gum or of its source at the time the present studies were initiated, it was deemed worthy of further study.

Crystallization and characterization of the aldobiuronic acid showed this portion of the glucuronic acid and galactose to be the D-isomers. Larger fractions of the two sugars were also separated chromatographically from the products of more drastic hydrolysis of the gum and were found to have optical rotations close to those established for the D-forms. The isolated rhamnose was similarly indicated to be the L-isomer.

Oxidation of intact hualtaco gum with periodate destroys the arabinose and L-rhamnose residues, roughly two-fifths of the D-galactose, and relatively little of the D-glucuronic acid, indicating that much of the D-galactose and most of the acid residues carry substituents. Oxidation of the degraded gum, however, removes about one-half of each of these two sugars, which would imply that a large proportion of the pentose and methylpentose residues are attached to D-glucuronic acid in the intact gum and are lost during degradation by acid, with formation of unsubstituted non-reducing end-groups of D-glucuronic acid. An approximately equal number of D-glucuronic acid residues in the degraded gum survive oxidation and are therefore presumably substituted in position 3. This applies also to the D-galactose.

The cross-reaction of hualtaco gum with antipneumococcal (anti-Pn) type I horse sera is a relatively minor one and an understanding of its basis must await further studies, now in progress, on the chemistry of the capsular polysaccharide of type I pneumococcus.

Precipitation of anti-Pn type II sera is entirely analogous to that of gum arabic. Large amounts of unhydrolysed hualtaco gum are required to approach maximal precipitation, while this is achieved with the degraded gum with one-fiftieth to one-hundredth as much. Presumably this is due to the splitting off of pentose and/or methylpentose, with exteriorization of non-reducing end-groups of D-glucuronic acid such as are characteristic of degraded gum arabic and of S II (Butler and Stacey, 1955).

Comment is necessary on the unexpected reactivity of the periodate-oxidized degraded gum in antiserum II 513. The supernatants failed to precipitate readily with degraded gum arabic, showing that antibodies had indeed reacted. However, the opalescence of the supernatants and the slow deposition of insoluble material in the control tube, to which no degraded gum arabic had been added, indicated that the aldehyde groups of the periodate-oxidized degraded hualtaco gum might have combined covalently and not entirely specifically with some of the protein, perhaps macroglobulin, in this serum of unusually high antibody content. This seems the more likely since another anti-Pn II horse serum, 1054C, with one-third as much antibody, failed to precipitate with the oxidized product, giving only a faint opalescence at the 1 mg level, although the unoxidized degraded gum, with its non-reducing end-groups of D-glucuronic acid intact, precipitated the antiserum as expected. Moreover, the degraded gum reacted with the Felton antibody solution I 1057FC (Table 2) (Felton, 1925), as did also the oxidized degraded gum, but the latter precipitate was powdery in an opalescent solution and failed to dissolve on warming to room temperature, while the precipitate of the unoxidized degraded gum dissolved.

The cross-precipitations of hualtaco gum in anti-Pn VIII, XIV and XIX are relatively minor and were not studied quantitatively. The rather weak anti-Pn XXII available had been partially absorbed with glycogen, yet about 10 per cent of the residual antibody was precipitated by hualtaco gum and almost as much by its degraded derivative, with no difference in the reacting proportions of gum and antibody. It is possible, therefore, that similarly linked residues of D-galactose are responsible for the precipitation, although S XXII is not definitely known to contain galactose.

Hualtaco gum and its product of degradation as well as the corresponding gum arabic products, precipitate about three-quarters of the antibody in the single anti-Pn XXIII serum available (Table 2). An appreciable portion of the presumed repeating units of the gums must therefore approximate that of S XXIII. Unfortunately, all that is known of this capsular polysaccharide is that a preparation (Brown, 1939) contained D-glucose 32

per cent, D-galactose, 31 per cent, easily split L-rhamnose, 35 per cent, phosphorus, 3·1 per cent, and no uronic acid (unpublished data, J. K. N. Jones and M. B. Perry). The extensive cross-reactivity with the gums might be due to the presence in each presumed repeating unit of either similarly linked L-rhamnosyl-D-galactose, D-galactosyl-L-rhamnose combinations, or of repetitions of single residues of one or both of these sugars similarly located in space on the macromolecules of each substance. It is hoped that further work will supply the chemical reason for this unusual degree of immunological correspondence.

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