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# The Anionic Glycan from the Cactus *Cereus peruvianus*

## Structural Features and Potential Uses

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## ABSTRACT

The columnar cactus *Cereus peruvianus* provides various compounds of interest that account for most of its 10% dry wt content. Included are acidic gum and cellulose as the highly polymerized carbohydrate components, and a complex waxy lipid fraction. The major gum fraction (1.5 g% of the fresh phytobiomass on single aqueous extraction) is an uronylated rhamnoarabinogalactan whose intrinsic viscosity may exceed 1000 mL/g. Its rheological behavior is, in part, influenced by the native *o*-acetyl and cation components, mainly $Ca^{2+}$. A pigment-free powdered gum was obtained by precipitating and washing the fresh mucilage with 2-3 vol of ethanol. The almost protein-free polysaccharide forms viscous solution upon redissolution. The possible uses to be investigated for the pretreated cactus gum will be as an adjuvant in the flocculation of water impurities and in formulation of cosmetics.

**Index Entries:** Cactus gum; *Cereus;* viscosity.

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## INTRODUCTION

Plants belonging to the Cactaceae family are efficient in retaining water, which corresponds to the occurrence of the mucilage mass in the parenchyma (*1*) and of an epicuticular wax layer (*2*), thus ensuring low transpiration as a result of their adaptation to arid climates. Despite the ready extraction of gummy materials that can form viscous solutions, very few studies were carried out on their sugar composition, fine chemical structure, and applications. The basic structure of the gum from *Opuntia ficus-indica* (a palm cactus) has been studied (*3,4*) and contains xylose as one of the side-chain components, linked to the glycan backbone. The gum from *Cereus peruvianus* (a columnar cactus whose cladodes may exceed several meters in length) was partially characterized (*1*). Correlation between the structure of cactus polymer and properties, such as rheology, also deserves attention if one considers its abilities for absorption/flocculation for several compounds and impurities (*5,6*), food thickening, and application in cosmetics. Our current investigation focuses on determination of structural features for a better understanding of properties and uses of the polymer.

## MATERIALS AND METHODS

### Polysaccharide Source and Processing

A relatively thorn-free variety of the cactus *Cereus peruvianus* (>3 m high adult plants) was collected in the Experimental Farm of the Maringa State University, Parana, Brasil. The cladodes (modified stems), following addition of 1 vol of tap water, were minced in a knife mill and pressed in a screw-based dewatering machine. Recovery of the viscous polysaccharide, free of low-mol-wt components and pigments, was carried out via precipitation with 2–3 vol ethanol. The precipitate was washed further with solvent to provide a powdery product.

### Whole Polymer Analyses

A $^{13}$C-NMR (nuclear magnetic ressonance) spectrum of the gum suspension in $d_6$-DMSO was obtained with a Brucker AC-300 P machine (in a 5-mm diameter tube) at 70°C. Molecular weight estimation was carried out by combining GPC-LS (gel permeation chromatography—light scattering) with the following facilities: TSK PW5000+PW4000 SEC-columns; 50 m*M* NaCl solvent; 0.2-mm cellulose acetate in-line filter; the sample (c=0.39 mg/mL) was separated at a flow rate of 0.82 mL/min at room temperature (20°C); scattered laser-light (HeNe: 632 nm) was detected at an angle of 4.8°; the detection section consisted of an Optilab interferometric DRI-detector (630 nm) from Wyatt Technology (California) and the low

angle laser light scattering instrument KMX-6 from LDC Analytical; data acquisition/processing was performed with the PCLALLS software package also from the latter firm. Routine viscosity measurements were determined with an Ostwald viscometer at 25°C or in a Brookfield model DV-II viscometer, with an SC4-18 spindle and the 8-mL cylindric vessel. Rotational speed was usually in the rpm range. The optical rotation was obtained in an ACATEC-500 instrument. Protein was determined with the Coomassie-based reagent purchased from Bio-Rad (Richmond, CA). Colorimetric procedures for estimating *O*-Acetyl and uronic acid(s) contents were those using the hydroxylamine (*7*) and 3-*O*-phenyl-phenol (*8*) reagents, respectively.

### Polymer Fragmentation Analyses

Acid hydrolysis of the gum was performed with 2*M* TFA (trifluoroacetic acid) at 100°C from 15 min to 12 h. Snail gastric juice (ex-*Megalobulimus paranaguensis*) and *A. niger* pectinase (Sigma Co., St. Louis, MO) were used for gum enzymatic depolymerization in 50 m*M* sodium acetate buffer at 40°C for 12 h. Hydrolysis products were analyzed by means of the TLC, GLC, and electrophoretic procedures previously described (*9*). Investigation of bound phenol-carboxylic acids was carried out on ethyl acetate extracts of a gum boiled for 10 min with 1*M* TFA or 1*M* KOH, the extracted material being previously derivatized with hot methanolic-HCl. A capillary OV-225 column inserted in a Varian-Finnigan GLC-MS unit was used for the analysis and resolution of the resulting methyl ester derivatives.

## RESULTS AND DISCUSSION

*Cereus peruvianus* (seven-winged cladodes; Fig. 1) was cultivated either by seeding or with cuttings in the fertile soil of Parana State, Brazil, reaching full growth within 1 yr. One-meter segments of the seven-wing cladode (13–16 cm diameter) weighed about 5 kg. Its dry matter corresponded to 10 g% of the fresh biomass as evaluated by oven-drying or lyophilization. Careful dewatering of cactus slices by means of lyophilization (Fig. 2) facilitated an interesting dissection of the anatomical parts and their contribution to the whole plant, which consisted of epicuticular wax (1.2%), pecto-cellulosic cuticle (18.1%), gummy aqueous parenchyma (58.2%), cellulosic core ring (9.1%), and gummy marrow (13.4%). The viscous mucilage is concentrated in the parenchyma and marrow. Taking into account these values and a 1.5% yield obtained from nonexhaustive squeezing of the fresh cladodes, a yield of gum around 12 t per ha can be estimated. Cellulose and wax are also valuable byproducts. The gummy polysaccharide was easily recovered from its native and highly viscous solution by organic solvent precipitation. The resulting powder had:

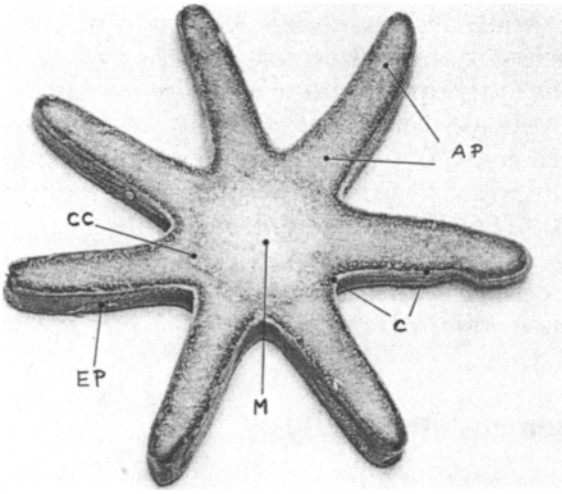


Fig. 1. *Cereus peruvianus* cladode (transversal section of a seven-winged specimen). EP = epicuticular wax; C = cuticle; AP = aqueous parenchyma; CC = core cylinder; M = marrow.

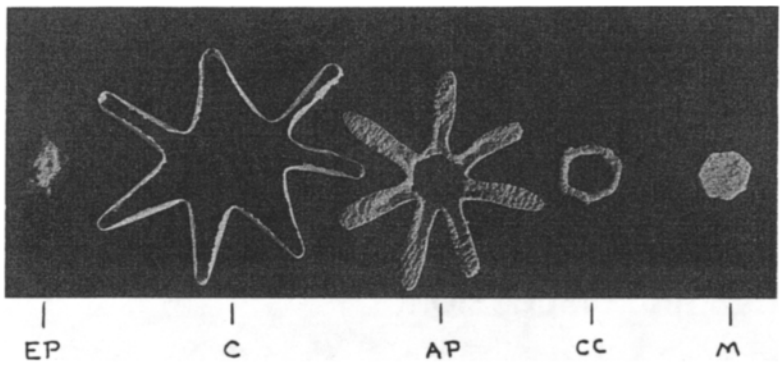


Fig. 2. The anatomic parts from a dissected cladode of *C. peruvianus*. (Parts of a lyophilized slice were separated and weighed: EP = 1.2 g% of wax; C = 18.1 g% of pecto-cellulose partially impregnated with wax; AP = 58.2 g% of gum; CC = 9.1 g% of (ligno) cellulosics; M = 13.4 g% of gum.)

A. A very high Mw (weight average mol wt) in the range $4.1 \times 10^6$ and an Mn (number weight mol wt) of about $1.2 \times 10^6$ as evaluated by GPC-LS. A variety of chromatographic columns also indicated a marked degree of polydispersity with some polysaccharide material being excluded even from a TSK PW6000 column whose $V_o = 20.0 \times 10^6$. Work on the clarification of polymer interchain interactions is in progress. Since side-chain substitution of pectins and hemicelluloses with single phenol-carboxylic acids may lead to an appreciable increase in mol wt as a result of individual polysaccharide chain cross-

linkage (e.g., $P_1$-(diferulate)—$P_2$; *10*), a search for these compounds was performed by GLC-MS in the material extracted with ethyl acetate after medium-strength treatment of *Cereus* gum with acid or alkali. Full chromatographic separation of the methyl esters of vanillic, *p*-coumaric, syringic, and ferulic acids (as standards) was rapidly attained on an OV-225 capillary column ($R_T$=8.04, 9.46, 10.16, and 11.25, respectively) giving their molecular ions ($m/z$=182, 178, 212, and 208). Gum extracts showed some fluorescence under UV light, but despite the similarity of the chromatographic profiles with the standard mixture (results not shown; six peaks arising from alkaline treatment of the gum and about 12 from the corresponding acid-treated one), the distinct mass fragmentation patterns of gum phenol derivatives did not allow correspondence with the standards, and hence, if some crosslinkage does occur in *Cereus* gum, this probably does not result from any of the assayed phenol-carboxylic acids.

B. $Ca^{2+}$ and $K^+$ appeared as the major cations, followed by lesser amounts for $Na^+$ and $Mg^{2+}$.
C. A relatively low protein content was found (1.5 g%).
D. There were 13–15 mol% of bound *O*-acetyl groups.
E. No clear indication for methoxyl groups was indicated by $^{13}C$-NMR spectroscopy (peak at $\delta$ 55 ppm for the uronyl R-COOMe was barely resolvable from the baseline noise; Fig. 3). Using this spectroscopic technique, several signals could be assigned. These were: acetate ($\delta$ 169.72 and $\delta$ 20.25 for the C=O and $CH_3$ groups of acetic acid); $\delta$ 17.45 for the $CH_3$ from $\alpha$-L-rhamnose; strong ones at $\delta$ 105.1 and $\delta$ 107.0 for the anomeric carbons (C-1) of $\beta$-D-galactopyranosyl and $\alpha$-L-arabinofuranosyl units and at $\delta$ 61.2 and $\delta$ 60.2 for their (C-6)- and (C-5)-$CH_2OH$ groups, respectively. Also readily identified were three additional signals for C-2 to C-4 of the nonreducing $\alpha$-L-arabinofuranosyl units ($\delta$ 77.0–84.0); these were superimposed on other $\beta$-D-galactopyranose sequences (*11*). Unexpectedly, there was no indication for a high content in (galact)uronic acid ($\delta$ 174.0–176.0; COOH groups) and at $\delta$ 100.0–101.0 for C-1 of the $\alpha$-anomer. Repeated permeation of the native gum with strong cationic resin (Dowex-50-$H^+$) in order to reduce viscosity and thus obtain better spectra on one hand caused extensive arabinose removal and hence diminution of branching, but on the other hand, enhanced the uronic acid signals (results not shown).

A high galacturonic acid content (44%) was reported for *Cereus* mucilage (*1*) using colorimetry with carbazole, although this uronic acid moiety

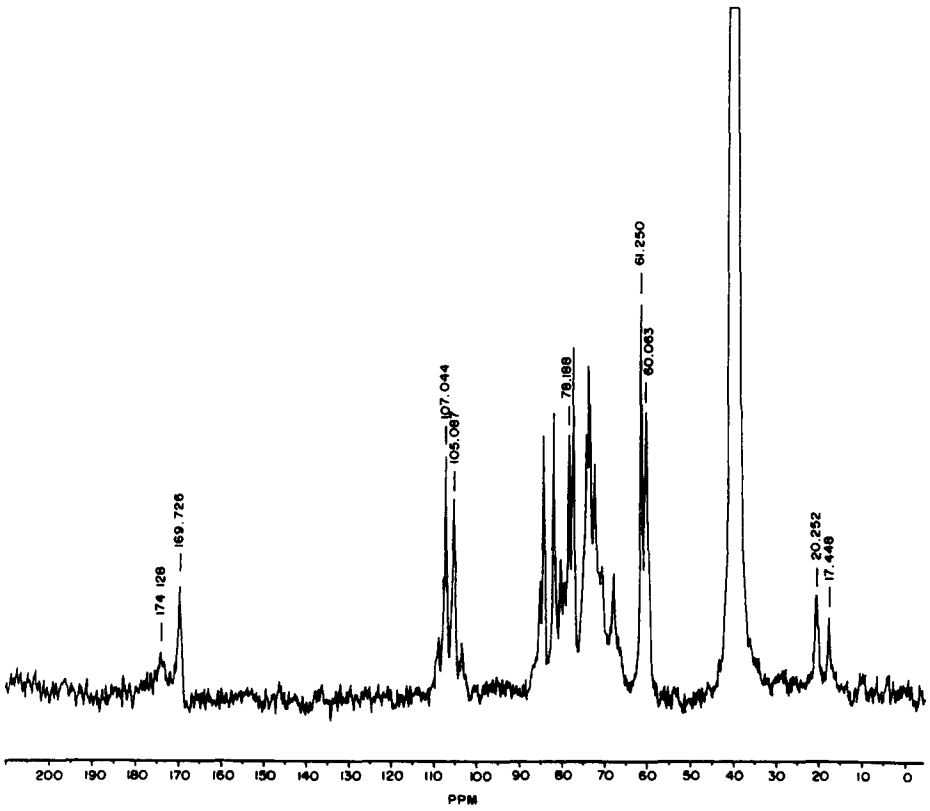


Fig. 3. $^{13}$C-NMR spectrum of *Cereus peruvianus* acidic glycan. (The largest peak at $\delta$ 40 is the DMSO-$d_6$ reference.)

was not found in the sulfuric acid hydrolyzates. Since the uronyl content of the gum, despite its exact value, may play an important role in polysaccharide rheology (e.g., interchain linkage through di- or polyvalent cations), and taking into account previous conflicting reports on the acidic nature of *Opauntia* gum (*3,12*), the difficulty in releasing uronic acid from *Cereus* mucilage was partially overcome with parallel acid and enzymatic hydrolyses, the first examined in detail following a time kinetics. Careful TLC analysis (Fig. 4) (after use of a five-component solvent and graded heating with the orcinol-sulfuric spray, which generated different colors for each of the four monomeric units of the cactus gum) revealed arabinose and galactose as the major, peripheral, and more easily hydrolyzed components. The free pentose is the only component detected after 15 min of 2*M* TFA hydrolysis. Rhamnose was detected only after 4 h of heating with the same acid, or by the use of snail enzymes and *Aspergillus* pectinase. The latter were capable of causing a limited cleavage of uronic acid(s). The hydrolysis-resistant acidic units were better detected on paper electrophoresis (Fig. 5), which allowed a sample overload prohibitive for TLC,

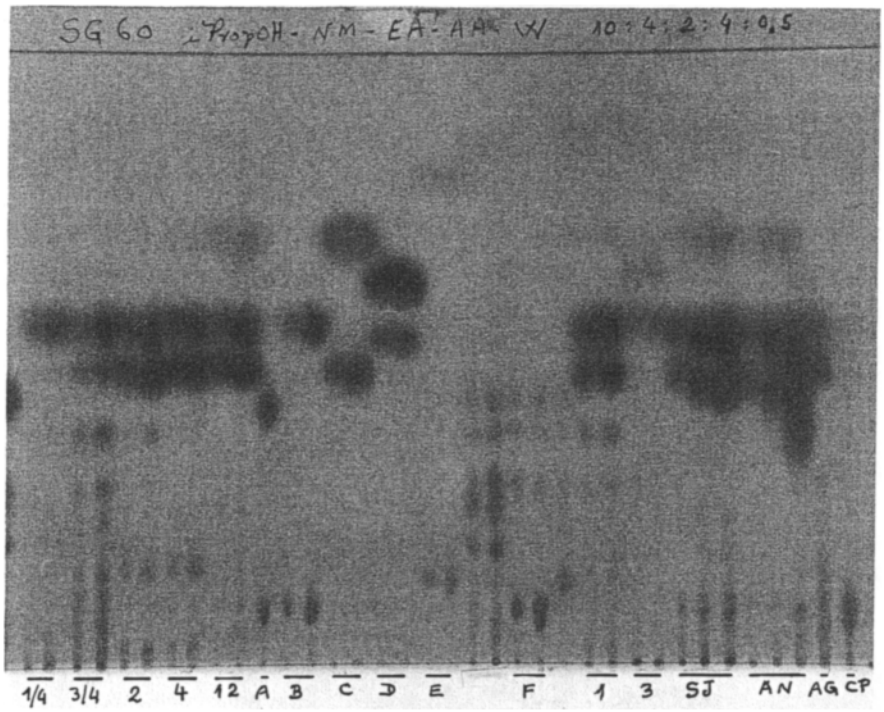


Fig. 4. TLC (thin-layer chromatogram) of the acid and enzymatic hydrolysis of *C. peruvianus* gum. (Chromatoplate = silica gel 60, Merck [Darmstadt, Germany]; solvent = isopropanol:nitromethane:ethyl acetate:acetic acid:water, 10:4:2:4:0.5; visualization = 0.5 g% orcinol in sulfuric acid: methanol 5:95, 100°C for 5 min.) Samples: 1/4, 3/4, 2, 4, 12 = time (h) of hydrolysis with 2*M* TFA at 100°C; 1, 3 = hydrolysis at pH 1 or 3 with TFA for 12 h at 100°C; SJ and AN = enzymatic hydrolyses with snail gastric juice or *A. niger* pectinase for 12 h at 40°C; AG and CP = control hydrolyses for snail and *A. niger* enzymes with larch arabinogalactan and citric pectin, respectively. Standards (in order of decreasing $R_f$): A = arabinoslyl-galactose dissaccharide + D-galacturonic acid ($Na^+$ salt); B = L-arabinose + D-galacturonic acid; C = L-rhamnose + D-galactose; D = D-xylose + D-glucose; E = D-glucuronolactone + D-glucuronic acid; F = 4-O-methyl-D-glucuronic acid (impure; $R_f$ 0.29) + D-galacturonic acid.

and this analysis indicated the occurrence of two different and internal uronic acids in the *Cereus* gum structure. One was galacturonic acid, confirming the previous TLC indication (enzyme incubations).

Quantitation of the hydrolytic products by GLC of oxime-silyl derivatives on a capillary SE-30 column (Fig. 6) showed that the 12-h hydrolysis with 2*M* TFA was the more efficient depolymerization and indicated a relative proportion of 6.2:3.0:1.0 for the neutral components D-galactose, L-arabinose, and L-rhamnose. The underestimated uronic acid content accounted for only 1/4 of that observed for rhamnose (no signal of uronic

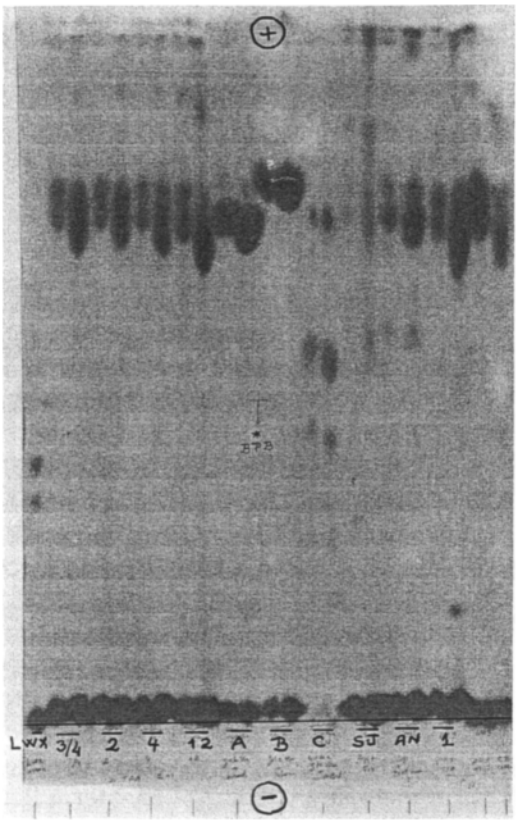


Fig. 5. Paper electrophoretogram of acid and enzymatic hydrolyses of *C. peruvianus* gum. (Paper = Whatman 2-chr; volatile buffer = 10.0% pyridine—0.4% acetic acid; run conditions: 1.5 kV, 20 mA, 65 min.; visualization = alkaline silver nitrate). Samples (as in Fig. 4). Standards: LWX = larchwood xylan *O*-acetylated uronylated oligosaccahrides; A = D-galacturonic acid + L-arabinose; B = D-glucuronic acid + L-rhamnose; C = 4-*O*-methyl-D-glucuronic acid + aldobio- and aldotriouronic acids.

acid lactonization was detected in the TLC plates). Drawbacks arising from stronger acid conditions could be free pentose/hexose destruction or even uronic acid decarboxylation. Since the colorimetric procedure with the phenyl-phenol reagent in polysaccharide solutions indicated only 7.8–12.9% of uronic acid(s), cactus gum samples were treated with $NaBH_4$ containing 2% KOH at 25°C overnight or at 60°C for 1 h. The de-*O*-acetylated polymer, free of the reagents, was passed through Dowex-2-$H^+$ resin with or without cooling, reprecipitated with ethanol, and 100-mg samples titrated with 20 m*M* freshly prepared KOH solution to pH 7.0. Titration values proved quite variable (tentative content of uronic acid[s] from 12 to 33%), depending on the degree of *O*-deacetylation, and mainly on gum partial depolymerization by the $H^+$-resin (confirming the arabinose loss as seen by $^{13}C$-NMR and, hence, an artifactual uronic acid enrichment

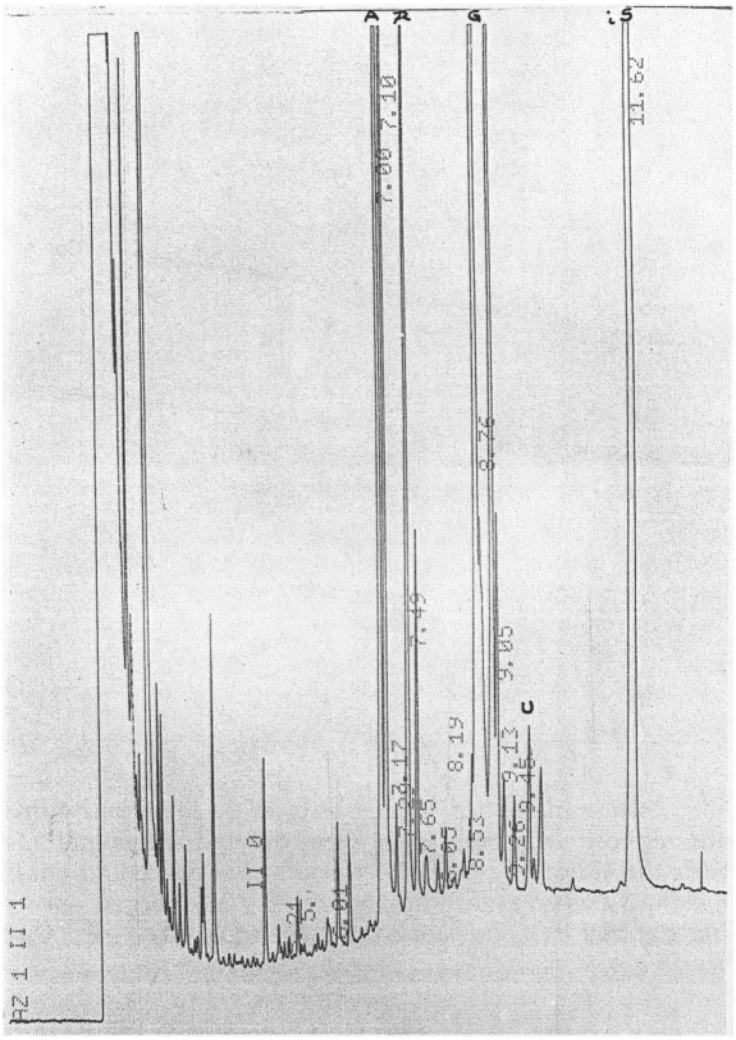


Fig. 6. GLC (gas liquid chromatogram) of the oxime-silyl derivatives of hydrolysis products from *C. peruvianus* gum with 2M TFA for 12 h at 100°C. (Column: capillary Se-30 methyl-silicone; apparatus and conditions: Varian 3330 GC at 155°C [2-min hold], then 20°C/min until 255°C, with $N_2$ as flow gas (20 psi at gage). A = L-arabinose; R = L-rhamnose; G = D-galactose: U = D-galacturonic acid; iS = internal standard (β-phenyl-D-galactopyranoside.)

owing to decrease of the gum mol wt). The methodology, obviously, claims for optimization taking into account the importance of a more accurate estimation of uronic acid content and its role. Pectin is present, at least, in the cactus cuticle layer. At this time, the possible contribution of true pectin in the gum preparations, cannot be discarded as a factor complicating the uronic acid quantitation in *Cactaceae* mucilages. Incidentally,

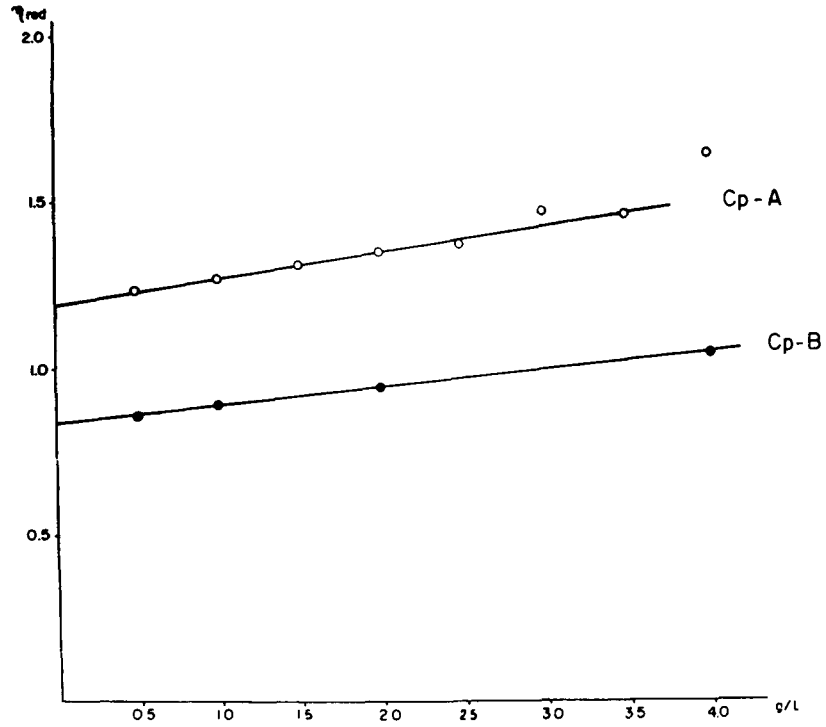


Fig. 7. Determination of the viscosity of *C. peruvianus* gum. Measurements of the reduced viscosities ($\eta_{red}$) were obtained utilizing an Ostwald viscometer using the indicated range of polysaccharide concentration. Intercept of each curve at the *Y* axis gives the intrinsic viscosity [$\eta$], mL/g. (○——○): Cp-A = pH 5.3 gum clarified by centrifugation at 18 krpm; (●——●): Cp-B = pH 6.8 crude gum.

*Cereus* gum was strongly bound on DEAE-Biogel or cellulose ($Cl^-$ form), and complete recovery was not achieved as determined by gravimetry of the fractions eluted successively with water, 1*M* LiCl, 0.5*M* HCl, and 0.5*M* NaOH (results not shown). Polarimetry indicated an $[\alpha]^{25}_D + 24°$ ($c = 0.1$ g%, water) for *Cereus* gum, and this was much lower when compared with the range for pectin (+220–240°). The data, combined with a low galacturonic acid content, are consistent with contributions of α-L-arabinofurabnose (strongly negative optical activity) and of β-D-galactopyranose (slightly positive) in the polymer.

For the anionic glycan, viscosity varied with the harvesting time, extraction conditions, mol-wt range, counterions, *O*-acetyl content, and pH, among other factors. Viscosity measurements (Ostwald apparatus) on two different preparations gave values of intrinsic viscosity [$\eta$] = 1150 mL/g (cps) for an 18-krpm clarified pH 5.3 preparation and 850 mg/L for a noncentrifuged pH 6.8 preparation (Fig. 7). Different effects resulted from

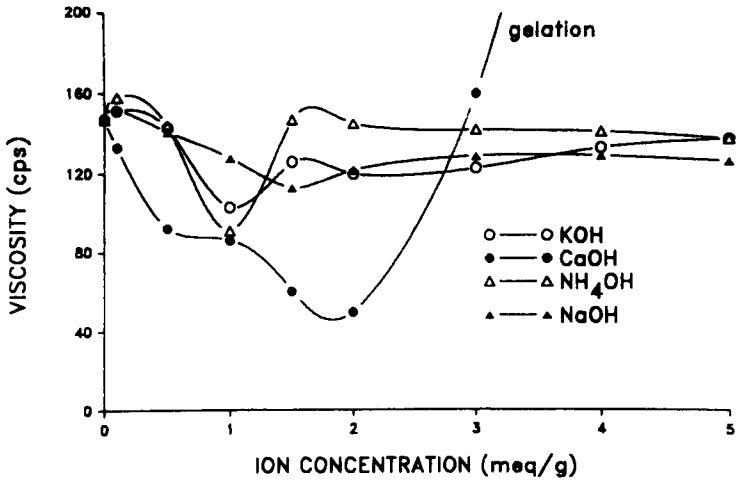


Fig. 8. Effect of different alkali additions on the rheological behavior of *C. peruvianus* gum. (Viscosity measurements were determined using a Brookfield DV-II apparatus. Ion concentration refers to the amount of each alkali added per gram of gum.)

the nature of alkali addition on less viscous solutions (1–5 meq $OH^-$/g of polysaccharide) (Fig. 8). In relation to the strong and weak monovalent hydroxides, an oscillation in the 1–2 meq/g range is followed by a plateau in the 2–5 range. Differently, the divalent $Ca^{2+}$ hydroxide (allowing electrostatic interchain bridging) resulted in a more marked oscillation, the final result being gellation at 3 meq/g (pH 8.5–10.5 range). The temperature effect on viscosity, heating the cactus gum from 10 to 80°C (measured with the Brookfield apparatus), resulted in a graded drop of viscosity to 60% (comparing the extreme points and taking the lowest temperatures as 100%). There was an almost complete correspondence between viscosity readings when the opposite operational order was carried out, namely cooling the same solution from 80 to 10°C, an interesting property to be considered in any technological use of this gum.

The gum obtained from *Cereus peruvianus* was proposed as an adjuvant to aluminium sulfate in the flocculation of water impurities for drinking purposes (*6*). The same author also recommended the polyelectrolyte for lime-based paints and for chromium precipitation in alkaline effluents from tanneries. If advantage is taken of the present data on gum structure and properties, its use in cosmetic preparations also seems feasible.

The possible negative contribution of any endogenous hydrolase activity on *Cereus* gum viscosity stability was not examined yet, but in this case, steaming of fresh mucilage could be used since boiled solutions of the gum remained viscous. One of the advantages of wet milling/extrusion (besides the light green color arising from chlorophyll to fresh gum) is that the centrifugation at >15 krpm allowed separate recovery of both cellulose populations (from the waxy cuticle as the precipitate and from

the core ring by flotation) and wax extraction can then be carried out on the precipitated fraction using hot chloroform. The residual cuticle consisted of cellulose and a pectic substance (*13*). In terms of the wax, the brown-colored and solid material obtained after solvent evaporation (mp 109.6°C) showed five main fractions on silica gel TLC developed with petroleum ether:ethyl ether:acetic acid (70:29:1) and revealed by charring with potassium dichromate/sulfuric acid. Part of the darkest matter could be removed via fractionation on a filtering layer of silica gel. After applying a solvent gradient of increasing polarity from hexane to methanol in the filtering layer, the resulting fractions of this procedure showed lower mp(s) of 71.2–106°C. These may correspond to the previously reported $C_{31\text{-}27}$-hydrocarbons, esters of $C_{20\text{-}16}$-fatty acids with $C_{30\text{-}26}$-alkanols, and free $C_{18:1\text{-}16}$-fatty acids, in decreasing order of their percentage proportion (*14*).

## CONCLUSIONS

Fine chemical structural features of the viscous gum from *Cereus peruvianus*, a columnar cactus, were determined. These data provide a better understanding of the properties, mainly the rheological ones, of this partially *O*-acetylated and uronic acid-containing rhamnoarabinogalactan, thus facilitating the optimization of its indicated and potential uses, such as water clarification and those concerning cosmetology. The byproducts of processing the whole cactus phytobiomass, wax and cellulose, may also find useful applications, thus increasing the economic feasibility of the process.

## ACKNOWLEDGMENTS

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