[CONTRIBUTION FROM THE ORGANIC CHEMISTRY LABORATORY, INDIAN INSTITUTE OF SUGAR TECHNOLOGY]

The Structure of Neem Gum

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Neem gum on hydrolysis yields L-arabinose, L-fucose, D-galactose and D-glucuronic acid. The aldobiuronic acid component of the gum obtained by graded hydrolysis is shown to be 4-*O*-(D-glucopyranosyluronic acid)-D-galactopyranose. Information on the structure of the gum has also been obtained by periodate oxidation studies.

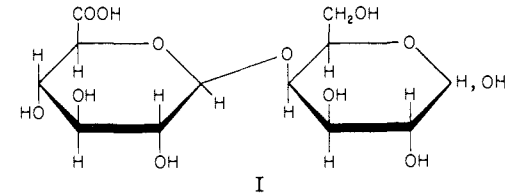
Neem gum, a typical plant gum exudate from the tree (*Melia azadirachta, Meliaceae*) is the salt of a complex polysaccharide acid. It has been in pharmaceutical use in India for many centuries.[2]

The gum acid, obtained from acidified aqueous solutions of the gum by precipitation with alcohol, is a white amorphous powder which has an equivalent weight of 1080.

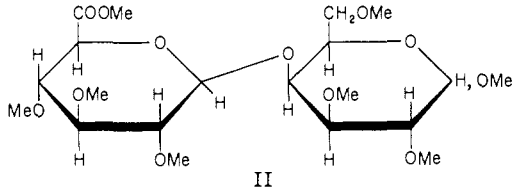
This communication is concerned with the composition of neem gum and with the determination of the structure of an aldobiuronic (I) produced when the gum is hydrolyzed with acid.

Complete hydrolysis of the gum followed by partition chromatography and the preparation of crystalline derivatives has shown that the gum contains L-arabinose, L-fucose, D-galactose, D-glucuronic acid and traces of D-xylose. The ratio of D-galactose to L-arabinose proved to be 3:2.

By mild acid hydrolysis the arabinose and fucose units were removed first. The ease of removal of the arabinose units indicated that they were present in the gum in the furanose form.[3]



I



II

More drastic hydrolysis of the gum affords D-galactose and an aldobiuronic acid I composed of a unit of D-glucuronic acid and one of D-galactose.

The structure of the aldobiuronic acid (I) was established as follows. Upon methylation of the barium salt of I an octa-*O*-methyl derivative II was produced. Hydrolysis of II afforded equal amounts of 2,3,6-tri-*O*-methyl-D-galactose and 2,-3,4-tri-*O*-methyl-D-glucuronic acid, the former being identified as the crystalline γ-lactone[4] and the latter as the 1,5-lactone 6-methyl ester.[5] These facts prove that the structure I assigned to the aldobiuronic acid is correct, a view supported by the further observation that oxidation with periodate resulted in formation of 2–3 moles of formic acid and 0.5 mole of formaldehyde per mole of the aldobiuronic acid. It is of interest to note that both lemon gum and grapefruit gum[6] afford the aldobiuronic acid (I) on hydrolysis.

When the gum itself was oxidized with periodate 2 moles of formic acid was produced per equivalent weight of the gum and chromatographic analysis of the oxidized gum, after hydrolysis, showed that certain of the galactose units had survived periodate oxidation. This evidence demonstrated the highly branched chain character of the gum and that the branching in the molecular complex is located at those galactose units of the gum which are not affected during periodate oxidation.

Experimental[7]

The neem gum as obtained from the tree consisted of pale-yellow to light-brown nodules (ash content, 5.4%). The gum dissolved in cold water giving light brown viscous solutions. The gum acid was precipitated by pouring a solution of the gum acidified with dilute hydrochloric acid into 95% ethanol with stirring. The precipitated gum was filtered, washed successively with ethanol and ether and dried *in vacuo* over phosphorus pentoxide. The gum acid thus obtained was a white powder having an ash content of 0.92%; equivalent weight (by direct titration with 0.1 *N* sodium hydroxide using phenolphthalein as indicator), 1080; $[\alpha]^{21}D$ −70° (*c* 0.3, water). It had no effect on Fehling solution.

Hydrolysis of the Gum and Characterization of L-Arabinose, L-Fucose and D-Galactose.—A solution of the gum (50 g.) in hot sulfuric acid (2 l., 0.05 *N*) was heated on a boiling water-bath for 22 hours, the course of hydrolysis being followed polarimetrically and by iodometric titrations.[8] The solution was neutralized ($BaCO_3$), filtered and evaporated. The residue was extracted with boiling methanol and the filtered extract concentrated to a sirup A. The solid amorphous residue B left after methanol extraction was the barium salt of a polysaccharide. Paper chromatography of the sirup A using 1-butanol–ethanol–water (5:1:4) as the irrigating solvent and a silver nitrate spray reagent[9] revealed the presence of arabinose, galactose and fucose.

Resolution of sirup A into its components on a cellulose column[10] using 1-butanol–water as the solvent yielded (a) L-arabinose, m.p. and mixed m.p. 156°, $[\alpha]^{18}D$ +105° equilibrium value in water (*c* 1) (after recrystallization from aqueous methanol); diphenylhydrazone, m.p. and mixed m.p. 196°; (b) D-galactose, m.p. 165°; $[\alpha]^{36}D$ +82° equilibrium value in water (*c* 0.7) (after recrystallization from aqueous methanol); methylphenylhydrazone, m.p. and mixed m.p. 185–186°; (c) L-fucose, this fraction did not crystallize probably because of the presence of a trace of

(1) Department of Agricultural Biochemistry, University of Minnesota, St. Paul 1.

(2) K. M. Nadkarni, "The Indian Materia Medica," 1927, p. 584.

(3) J. K. N. Jones and F. Smith, "Advances in Carbohydrate Chemistry," Vol. 4, Academic Press, Inc., New York, N. Y., 1949, p. 243.

(4) R. E. Gill, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1025 (1946).

(5) A. Robertson and R. B. Waters, *ibid.*, 1709 (1931).

(6) J. J. Connell, Ruth M. Hainsworth, E. L. Hirst and J. K. N. Jones, *ibid.*, 1696 (1950).

(7) All evaporations were done under reduced pressure at 40–50° unless otherwise stated.

(8) J. L. Baker and H. F. E. Hulton, *Biochem. J.*, **14**, 754 (1920).

(9) W. E. Trevelyan, D. P. Procter and J. S. Harrison, *Nature*, **166**, 444 (1950).

(10) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 2511 (1949).

xylose but it readily gave a methylphenylhydrazone, m.p. and mixed m.p. 175° (after recrystallization from 50% ethanol). *Anal.* Calcd. for $C_{13}H_{20}O_4N_2$: C, 58.3; H, 7.5. Found: C, 58.2; H, 7.0.

Periodate Oxidation of the Gum. (a).—To the purified gum (50–100 mg.) dissolved in water (10 ml.) was added sodium periodate (5 ml., 0.228 *M*). The solutions were kept in dark at room temperature (33°) for seven days, the excess periodate destroyed by adding ethylene glycol and the formic acid evolved titrated against 0.1 *N* sodium hydroxide. In duplicate experiments, the formic acid produced per equivalent of the gum was found to be 1.7 and 2.0 moles, respectively, after correcting for the titratable acidity of the gum.

(b) To a solution of the gum (0.45 g.) dissolved in water (75 ml.) was added potassium chloride (3 g.) and sodium periodate (20 ml., 0.25 *M*). The solution was kept under conditions similar to the above experiment; 0.5-ml. portions were removed periodically and after destruction of the excess periodate titrated against 0.01 *N* sodium hydroxide. After the oxidation was complete (144–164 hours), the excess of periodate was destroyed by adding ethylene glycol and the inorganic ions removed by dialysis. The dialyzed solution was made 1 *N* with sulfuric acid and heated on a boiling water-bath for 5 hours. The hydrolyzate after neutralization, filtration and concentration of the filtrate, was chromatographed. A strong spot corresponding to galactose was noted. A trace of arabinose also was detected.

Quantitative Hydrolysis of the Gum.—The purified gum (0.127 g.) was heated in a sealed tube with 2 *N* sulfuric acid (10 ml.) for 24 hours. The tube was opened in a flask containing rhamnose (69.0 mg.) and the resulting solution was neutralized ($BaCO_3$) and filtered. The sirup obtained after concentration of the filtrate was chromatographed and the individual sugars extracted from the paper. The values of the sugars estimated by oxidation with periodate and titration of the formic acid liberated[11] indicated a galactose to arabinose ratio of 3:2.

Hydrolysis of the Barium Salt B and Isolation of the Aldobiuronic Acid (I).—The barium salt **B** (22 g.) which had an $[\alpha]^{35}D$ −8° (*c* 0.5, water) and a Ba content of 6.4% corresponding to a hexasaccharide uronic acid, was dissolved in sulfuric acid (500 ml., 1 *N*) and heated on a boiling water-bath till the iodine titration was constant (7 hr.). The solution was neutralized ($BaCO_3$), filtered, the residue washed with hot water and the combined filtrate evaporated. The residual sirup was exhaustively extracted with boiling methanol and the filtered extract evaporated to give a sirup C which upon seeding with D-galactose and keeping in cold gave crystalline D-galactose which was identified as already described. The methanol insoluble barium salt D had $[\alpha]^{37}D$ +67° (*c* 0.1, water). *Anal.* Calcd. for the barium salt of an aldobiuronic acid $(C_{12}H_{19}O_{12})_2Ba$: Ba, 16.2. Found: Ba, 16.5. The barium salt B was heated with 3 *N* sulfuric acid on a boiling water-bath for 24 hours. The neutralized ($BaCO_3$) hydrolyzate gave a negative basic lead acetate test[12] for galacturonic acid.

The barium salt of the aldobiuronic acid was also prepared in good yield by heating the gum (30 g.) with *N* sulfuric acid on a boiling water-bath for 40 hours after which time the iodine titration had become constant. After neutralization of the hydrolyzate with barium carbonate, the barium salt was isolated in the usual manner; yield 9.5 g.

The aldobiuronic acid was found to be chromatographically pure when tested with the solvent ethyl acetate–acetic acid–formic acid–water mixture[13] (9:1.5:0.5:2 by volume) and the *p*-anisidine spray reagent.[8]

(11) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1659 (1949).

(12) F. Ehrlich, *Ber.*, **65**, 352 (1932).

(13) Private communication from Dr. J. K. N. Jones.

Oxidation of the Aldobiuronic Acid with Periodate.—The barium salt of the aldobiuronic acid (114.6 mg.) was oxidized with sodium periodate (10 ml., 0.22 *M*) for 24 hours at room temperature (32°) and then treated with dimedone solution (5 ml.).[14] The crystalline dimedone derivative of formaldehyde separated, m.p. and mixed m.p. 185–186°. The yield (39.6 mg.) corresponded to 0.5 mole of formaldehyde per mole of the barium salt.

Methylation of the Aldobiuronic Acid.—To a solution of the barium aldobiuronate (4 g.) in water (15 ml.) methyl sulfate (25 ml.) was added followed by sodium hydroxide (50 ml., 40%), the sodium hydroxide being added dropwise during 8 hours with stirring. After stirring for another 12 hours, the solution, which was non-reducing to Fehling solution, was filtered and concentrated and the residue methylated by dissolving it in sodium hydroxide (100 ml., 40%) and adding methyl sulfate (70 ml.) in the previous manner. The reaction mixture was acidified with sulfuric acid (tested with congo red), filtered and the filtrate extracted with chloroform (in a liquid–liquid extractor). The sirup (4.4 g.) obtained on evaporation of the chloroform extract was methylated twice with the Purdie reagent to give a sirup (2.56 g.; OCH_3, 52.0) which upon fractional distillation gave: Fraction I 0.51 g., b.p. (bath temp.) 145–160°, 0.5–1 mm. Fraction II b.p. (bath temp.) 195–225°, 0.5–1 mm. (0.85 g.). This fraction consisted largely of 4-*O*-[methyl-(2,3,4-tri-*O*-methyl-D-glucopyranosyl)-uronate] methyl-2,3,6-tri-*O*-methyl-D-galactopyranoside (OCH_3, 51.8) and was examined further.

Hydrolysis of 4-*O*-[Methyl-(2,3,4-tri-*O*-methyl-D-glucopyranosyl)-uronate] Methyl-2,3,6-tri-*O*-methyl-D-galactopyranoside.—Fraction II (0.69 g.) was boiled under reflux with *N* sulfuric acid (20 ml.) for 16 hours and then neutralized with *N* sodium hydroxide solution. The solution was extracted with chloroform in a liquid extractor. The extract was dried (Na_2SO_4) and evaporated to give a sirup (0.32 g.). The latter was oxidized with bromine water for 5 days in the usual manner and after filtration and removal of the excess bromine by aeration it was neutralized (Ag_2CO_3), filtered before and after the passage of hydrogen sulfide and the resulting solution evaporated to give 2,3,6-tri-*O*-methyl-D-galactonic-1,4-lactone b.p. 160–200°, 1 mm., which crystallized spontaneously, m.p. 97–98° (after recrystallization from ether; a m.p. of 98° has been reported[4] for 2,3,6-tri-*O*-methyl-D-galactonic-1,4-lactone).

The aqueous layer after chloroform extraction was acidified (congo red) and extracted with chloroform. The extract was dried (Na_2SO_4) and evaporated to a sirup (0.32 g.) which was oxidized with bromine as described above. The product was refluxed with 1% methanolic hydrogen chloride (50 ml.) for 6 hours. The solution was neutralized (Ag_2CO_3), filtered and the filtrate evaporated to give a sirup. Distillation of the latter *in vacuo* gave 2,3,4-tri-*O*-methyl-D-glucaric 1,5-lactone-6-methyl ester, m.p. 106–107° (after recrystallization from ether. A m.p. of 106° has been recorded for this compound[5]).

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