pounds by about 18 cycles. It is not clear whether this shift is due to steric effects due to the additional crowding imposed by the two bromine atoms, inductive withdrawal of electrons by the additional halogen atom shielding due to magnetic anisotropy of the –C–Br bonds or all effects operating simultaneously.

We have also measured the NMR spectrum of camphor for comparison purposes and find that the peaks due to the methyl groups are at 221 and 225 c.p.s. while they have been appearing at 214 and 220 in the monochloro compounds, at 213 and 220 in the monobromo compound, and at 208, 212 and 217 in the dibromo compound. This difference may be due to a chemical shift resulting from the magnetic anisotropy of the halogens. If this is so, the magnetic anisotropy of the carbonyl group may result in a similar effect and one would predict the methyl peaks in camphane would fall at a higher field strength.

Compounds.—Cyclohexanone was fractionated through a one-meter column packed with glass helices. α-Chlorocyclohexanone was a portion of the sample described by Huitric and Kumler.[4] The other compounds were from samples on which the infrared spectra were reported in the paper by Brutcher, Roberts, Barr and Pearson.[5]

(4) W. D. Kumler and A. C. Huitric, THIS JOURNAL, **78**, 3369 (1956).

(5) F. V. Brutcher, Jr., T. Roberts, S. J. Barr and N. Pearson, *ibid.*, **78**, 1507 (1956).

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[CONTRIBUTION FROM THE ORGANIC CHEMISTRY LABORATORY, NATIONAL SUGAR INSTITUTE]

The Structure of *Acacia sundra* Gum. Part I. Nature of the Sugars Present and Structure of the Aldobiouronic Acid

BY S. MUKHERJEE AND A. N. SHRIVASTAVA

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Acacia sundra gum on hydrolysis yields D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid. The aldobiouronic acid component of the gum obtained by graded hydrolysis is shown to be 6-*O*-[β-D-glucopyranosyluronic acid]-D-galactose. Information on the structure of the gum also has been obtained by periodate oxidation studies.

Amongst the gums of the species of Acacia genus the studies on the structure of gum arabic have been most extensive.[1-6] Apart from gum arabic the other gums that have been studied are: *Acacia mollissima*[7] (black wattle gum), *A. pycantha*,[8] *A. cyanophylla*[9] and *A. karroo*.[10] Of the gums obtained from Indian species only *A. catechu*[11] appears to have been investigated. Though all of these contain the same monosaccharide units and the same aldobiouronic acid is produced on acid hydrolysis (with the exception of *A. karroo* which gives two aldobiouronic acids), these differ in the proportions of the different component sugars and equivalent weights. The present investigation deals with the structure of *Acacia sundra* gum and it was of interest to find out what relation it bears to other gums of the Acacia genus.

This communication deals with the composition of *A. sundra* gum and determination of the structure of an aldobiouronic acid produced on hydrolyzing the gum with acid.

Complete hydrolysis (bath temp. 95–98°) of the gum followed by partition chromatography, measurement of specific rotation and preparation of crystalline derivatives has shown that the gum contains D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid. A faint spot corresponding to R_f value of xylose also was detected on paper chromatogram. The molecular ratio of D-galactose, L-arabinose and L-rhamnose was found to be 3:2:1. This ratio agrees fairly well with the one found in the case of *A. verek*.[12]

Aqueous solutions of the gum are sufficiently acidic to undergo slow autohydrolysis when the solution is heated on a water-bath. On autohydrolysis all the rhamnose and part of the arabinose and some galactose is removed. More drastic hydrolysis of the gum affords D-galactose, L-arabinose and an aldobiouronic acid composed of a unit of D-glucuronic acid and D-galactose. Paper partition chromatography of the aldobiouronic acid showed two spots, one faint and the other strong, which is indicative of the probability of a small proportion of another aldobiouronic acid also being present.

Structure of the aldobiouronic acid was established with the help of methylation studies. The fully methylated derivative of the aldobiouronic acid on hydrolysis afforded equal amounts of 2,3,4-tri-*O*-methyl-D-galactose and 2,3,4-tri-*O*-methyl-D-glucuronic acid, the former being identified as the crystalline anilide and the latter as the 1,5-lactone 6-methyl ester. The methylated aldobiouronic acid is levorotatory. These facts suggest that the structure I assigned to the aldobiouronic acid is correct.

When the gum was oxidized with periodate 1.6 moles of formic acid was produced per equivalent

(1) C. O'Sullivan, *J. Chem. Soc.*, **45**, 41 (1884).

(2) C. L. Butler and L. H. Cretcher, THIS JOURNAL, **51**, 1519 (1929).

(3) S. W. Challinor, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 258 (1931).

(4) F. Smith, *ibid.*, 1724 (1939).

(5) J. Jackson and F. Smith, *ibid.*, 79 (1940).

(6) T. Dillon, D. F. O'Ceallachain and P. O'Colla, *Proc. Roy. Irish Acad.*, **55B**, 331 (1953).

(7) A. M. Stephen, *J. Chem. Soc.*, 646 (1951).

(8) E. L. Hirst and A. S. Perlin, *ibid.*, 2622 (1954).

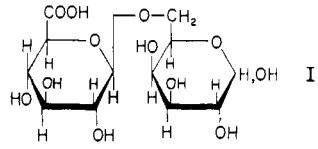
(9) A. J. Charlson, J. R. Nunn and A. M. Stephen, *ibid.*, 269 (1955).

(10) A. J. Charlson, J. R. Nunn and A. M. Stephen, *ibid.*, 1428 (1955).

(11) R. K. Hulyalkar, T. R. Ingle and B. V. Bhide, *J. Indian Chem. Soc.*, **33**, 861 (1956).

(12) W. W. Pigman and R. M. Goepp, Jr., "Chemistry of the Carbohydrates," Academic Press, Inc., New York, N. Y., 1948, p. 633.

weight of the gum, and for each equivalent weight 6.9 moles of periodate was consumed. Chromatographic analysis of the periodate-oxidized gum, after hydrolysis, showed that certain of the galactose units and some arabinose units also had



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survived periodate oxidation. This evidence demonstrates that the gum may be highly branched or may contain a proportion of 1:3 glycosidic linkages, or both, and that those galactose and arabinose residues in which branching occurs or which are 1:3 glycosidically linked are not affected during periodate oxidation.

Experimental

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

The *A. sundra* gum was in the form of clear nodules of yellowish orange color and was practically free from dirt and bark (sulfated ash 3.6%). The gum dissolved in water to give a clear pale brown viscous solution. The gum acid was precipitated by pouring a solution of the gum (100 g. in 600 ml. of water) acidified with hydrochloric acid (acidic to congo red paper) into 95% ethanol (2 l.) with stirring. This process was repeated three times and the gum acid was filtered, washed with ethanol, and then with ether, and dried *in vacuo* over phosphorus pentoxide. The gum acid thus obtained (yield 65 g.) was an almost white powder (sulfated ash 0.14%); having an equivalent weight (by direct titration with 0.1 *N* sodium hydroxide using phenolphthalein as indicator) 980, $[\alpha]^{30}D$ −29.6°, in water (*c* 1.49 as sodium salt).

The gum did not give an insoluble copper salt and did not reduce Fehling solution; nitrogen, sulfur and halogens were absent; pentosans, 23.3%; pentoses, 26.5% (calculated on the basis of pentosans); and furfural, 13.8% (estimated as phloroglucide by distilling the gum with 12% hydrochloric acid).

Hydrolysis of the Gum and Characterization of D-Galactose, L-Arabinose and L-Rhamnose. Graded Hydrolysis.—A solution of the gum (50 g.) in water (1 l.) was heated on a boiling water-bath for 80 hours, the course of hydrolysis being followed by iodometric titrations[13]; the solution was too dark for polarimetric observation. The hydrolyzed solution was cooled and neutralized (barium hydroxide), 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 (Ba, 12.3%). Paper chromatography of the sirup A using 1-butanol–ethanol–water (40:11:19, by volume) as the irrigating solvent and a silver nitrate spray reagent[14] revealed the presence of a strong spot of rhamnose, a fairly strong spot of arabinose, a faint spot of galactose and a faint trace of xylose.

Resolution of sirup A into its components on a cellulose column using 1-butanol half-saturated with water as the solvent yielded (a) L-rhamnose, identified as α-L-rhamnose hydrate, m.p. and mixed m.p. 93–94°, $[\alpha]^{31}D$ +8.6° (equilibrium value in water, *c* 1); (b) L-arabinose, after purification[15] gave m.p. and mixed m.p. 156°, $[\alpha]^{29}D$ +101° (equilibrium value in water, *c* 1), phenylosazone m.p. and mixed m.p. 163–164°; (C) D-galactose, m.p. and mixed m.p. 166–168°, $[\alpha]^{29}D$ +81.8° (equilibrium value in water, *c* 1), methyl phenylhydrazone m.p. and mixed m.p. 182–183°.

Periodate Oxidation of the Gum.—(a) To the purified gum (75 mg.) dissolved in water (50 ml.) was added sodium periodate (0.4 g.). The solution was kept in the dark at room temperature (31°) for five days, the excess periodate destroyed by adding ethylene glycol and the formic acid evolved titrated against 0.01 *N* sodium hydroxide using methyl red indicator. Formic acid produced per equivalent of the gum was found to be 1.6 moles after correcting for the titratable acidity of the gum; periodate consumption at this stage was 6.9 moles per equivalent of the gum.

(b) To a solution of the gum (0.53 g.) in water (75 ml.) was added potassium chloride (3 g.) and sodium periodate (25 ml., 0.25 *M*). The solution was kept under conditions similar to the above experiment; 1-ml. portions were removed periodically and after destruction of the excess periodate titrated against 0.01 *N* sodium hydroxide. After the oxidation was complete (120 hr.) the excess of periodate was destroyed by adding ethylene glycol. Inorganic ions were 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 (1.038 g.) was heated (95–98°) in a sealed tube with 2 *N* sulfuric acid (75 ml.) for 24 hours. The tube was then broken and its contents made up to 100 ml.; 30 ml. of this solution was added to a flask containing D-ribose (0.106 g.) and the resulting solution was neutralized (barium carbonate) and filtered. The component sugars of the sirup obtained after concentration of the filtrate were separated on a paper chromatogram and the individual sugars eluted from the paper strips with water. The eluted sugars were estimated by oxidizing them with periodate[16] and titrating of the liberated formic acid with standard alkali. Galactose, arabinose and rhamnose were found to be present in the ratio of 3:2:1.

Hydrolysis of the Barium Salt B and Isolation of the Aldobiouronic Acid.—The barium salt B (27 g.) (barium content of 12.3%) was dissolved in sulfuric acid (500 ml.), of such strength that the resulting solution was 0.1 *N*, and heated on a boiling water-bath until the iodine titration was constant (35 hr.). The solution was neutralized (barium carbonate), 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 on chromatographic examination showed strong spots of galactose and arabinose. These sugars were separated by column chromatography and identified as already described. The methanol-insoluble barium salt D contained 16.6% barium; the barium salt of an aldobiouronic acid $(C_{12}H_{19}O_{12})_2Ba$, requires 16.2% barium. The barium salt D was heated with *N* sulfuric acid on a boiling water-bath for 24 hours. The neutralized (barium carbonate) hydrolyzate, was concentrated and its methanolic extract gave a test for galactose only, which was identified as already described. The methanol-insoluble barium salt (Ba 29.2%); barium hexuronate: (Ba, 26.5%) was treated with the calculated amount of sulfuric acid and filtered. Its paper chromatogram, using 1-butanol–acetic acid–water solvent (4:1:5, by volume, upper layer), indicated the presence of glucuronic acid (strong spot) and glucurone (faint spot) with the *p*-anisidine phosphate[17] spray reagent.

The barium salt of aldobiouronic acid was refluxed with methanolic hydrogen chloride (2%) for 8 hours, neutralized (silver carbonate), filtered and evaporated to dryness. The resulting product was reduced with lithium aluminum hydride[18] and then hydrolyzed (*N* sulfuric acid). The hydrolyzate on a paper chromatogram gave spots of glucose and galactose.

The barium salt of aldobiouronic acid was deionized with Amberlite IR-120 and examined on a paper chromatogram using 1-butanol–acetic acid–water solvent system and *p*-anisidine phosphate spray reagent; it showed a main component and a small proportion of another.

Methylation of the Aldobiouronic Acid.—To a solution of barium aldobiouronate (10 g.) in water (50 ml.), methyl sulfate (75 ml.) was added followed by sodium hydroxide

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

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

(15) Ernest Anderson and Lila Sands, THIS JOURNAL, **48**, 3172 (1926).

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

(17) S. Mukherjee and H. C. Srivastava, *Nature*, **169**, 330 (1952).

(18) G. A. Adams and C. T. Bishop, THIS JOURNAL, **78**, 2842 (1956).

(225 ml., 30%), added dropwise during 8 hours with stirring. After stirring for another 12 hours, the solution, which was non-reducing to Fehling solution, was filtered, neutralized (sulfuric acid), concentrated and the residue methylated by dissolving it in sodium hydroxide (200 ml., 30%) and adding methyl sulfate (105 ml.) in the previous manner. The reaction mixture was acidified (sulfuric acid, acidic to congo red), filtered and the filtrate extracted with chloroform (in a liq.–liq. extractor). The sirup (6.0 g.) obtained on evaporation of the chloroform extract was methylated twice by the Purdie method[19] to give a sirup (4.2 g.) which upon fractional distillation gave: fraction I, 0.41 g. b.p. (bath temp.) 155–180° (0.2–0.3 mm.) (OCH_3 54.8%); fraction II, 0.41 g., b.p. (bath temp.) 180–210° (0.2–0.3 mm.) (OCH_3 46.0%); fraction III, 1.65 g., b.p. (bath temp.) 210–240° (0.1 mm.) (OCH_3 48.0%). The residue was extracted with chloroform and distilled in a short neck distillation unit to give a sirup (0.7 g.). Fraction III and this sirup were mixed and again methylated with Purdie reagent and distilled to give a sirup E, 1.6 g., b.p. (bath temp.) 210–240° (0.1 mm.) (OCH_3 48.0%). This sirup E was further examined; it consisted of methyl 6-*O*-[methyl (2,3,4-tri-*O*-methyl-β-D-glucopyranosyluronate]-2,3,4-tri-*O*-methyl-D-galactopyranoside.

Hydrolysis of Methyl 6-*O*-[Methyl (2,3,4-Tri-*O*-methyl-β-D-glucopyranosyluronate]-2,3,4-tri-*O*-methyl-D-galactopyranoside.—The sirup E, $[\alpha]^{35}D$ −19.6° (in chloroform, *c* 2) was boiled under reflux with methanolic hydrogen chloride (4%, 80 ml.) for 48 hours. Methanol was then distilled off and the bulk reduced to about 10 ml. Hydrochloric acid (*N*, 80 ml.) was added and the mixture was heated at 95–98° for 20 hours. The solution was neutralized (silver carbonate) and filtered, and silver ions were removed from the filtrate by passage of hydrogen sulfide and filtration of precipitated silver sulfide. The filtrate was evaporated to a sirup under reduced pressure and traces of moisutre were removed by adding absolute alcohol repeatedly and distilling the alcohol. The brown powder so obtained was exhaustively extracted with dry ether and the ether extract was evaporated to a pale yellow sirup (0.56 g.) (OCH_3 40.8%; calcd. for trimethyl galactose 41.9%). This sirup (0.24 g.) was refluxed for 2 hours with absolute alcohol (7.5 ml.) and freshly distilled aniline (0.13 g.); most of the alcohol was removed by distillation and on cooling crystals of the anilide separated out. On recrystallization with ethanol it had a m.p. 165–166°. (A m.p. of 164–165° has been reported for 2,3,4-tri-*O*-methyl-D-galactose anilide.[8])

The brown powder left after ether extraction was dissolved in water (40 ml.), acidified (hydrochloric acid) and extracted with chloroform in a liq.–liq. extractor. The extract was evaporated to a sirup and examined on a paper chromatogram with 1-butanol–acetic acid–water solvent system and *p*-anisidine spray reagent. It has an R_G value 0.82 (R_G value 0.84 has been reported for 2,3,4-tri-*O*-methyl-D-glucuronic acid).[20] The sirup (0.51 g.) was taken in water (10 ml.) and bromine added to it (0.5 ml.). It was kept in dark for four days during which time it was shaken occasionally. After filtration and removal of excess bromine by aeration the solution was neutralized (silver carbonate), filtered before and after the passage of hydrogen sulfide and evaporated to dryness. The product was refluxed with 1% methanolic hydrogen chloride (50 ml.) for 6 hours. The solution was neutralized (silver carbonate), filtered and filtrate evaporated to give a sirup. Distillation of the latter *in vacuo* (0.3 mm., bath temp. 160–180°) gave a sirup. Crystallization from ether–petroleum ether mixture afforded crystals of methyl 2,3,4-tri-*O*-methyl-D-glucarate 1,5-lactone, m.p. 106° (reported[21] m.p. 106°).

Acknowledgments.—The authors thank Professor S. N. Gundu Rao, Director, for his kind interest. Thanks are also due to the Forest Officer, Poona, for kindly supplying the gum.

(19) T. Purdie and J. C. Irvine, *J. Chem. Soc.*, **83**, 1021 (1903).

(20) R. A. Edington and E. Percival, *ibid.*, 3554 (1955).

(21) A. Rohertson and R. B. Waters, *ibid.*, 1709 (1931).

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[CONTRIBUTION FROM THE ORGANIC CHEMISTRY LABORATORY, NATIONAL SUGAR INSTITUTE]

The Structure of Tukhmalanga (*Salvia aegyptica*) Mucilage. Part I. Nature of Sugars Present and the Structure of the Aldobiouronic Acid

BY A. K. CHATTERJEE AND S. MUKHERJEE

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Salvia aegyptica mucilage on hydrolysis yields D-galactose, L-arabinose, L-rhamnose and D-galacturonic acid. An aldobiouronic acid, 2-*O*-(D-galactopyranosyluronic acid)-L-rhamnose, has been obtained by the partial hydrolysis of the mucilage which can be extracted from the outer coating of the Tukhmalanga seeds. Methylation of the aldobiouronic acid gave a fully methylated derivative which on hydrolysis gave 2,3,4-tri-*O*-methyl-D-galacturonic acid and 3,4-di-*O*-methyl-L-rhamnose in equimolecular proportion.

Tukhmalanga, (*Salvia aegyptica*) of the family Labiatae is a typical plant mucilage obtained from the seeds of a plant from the plains and hills. It has been in pharmaceutical use in India for a long time.[1]

This communication deals with the composition of *Salvia aegyptica* mucilage and determination of the structure of an aldobiouronic acid produced by hydrolyzing the mucilage with an acid.

Complete hydrolysis of the mucilage followed by partition chromatography, measurement of specific rotation and preparation of crystalline derivatives has shown that the mucilage contains L-rhamnose, L-arabinose, D-galactose and D-galacturonic acid. Quantitative hydrolysis of the mucilage showed that L-rhamnose, L-arabinose and D-galactose are present in the percentage of 62.8, 0.85 and 0.74, respectively. On partial hydrolysis of the mucilage using 0.1 *N* sulfuric acid, L-rhamnose, L-arabinose and D-galactose were obtained along with an aldobiouronic acid. The aldobiouronic acid on further hydrolysis with 1.0 *N* sulfuric acid gave L-rhamnose and a uronic acid. The uronic acid was found to be galacturonic acid, as it gave a positive basic lead acetate test for galacturonic acid and this conclusion was further confirmed by oxidation to mucic acid, m.p. 213°. Further information on the composition of aldobiouronic acid is obtained by reducing the esterified acid with potassium borohydride to the corresponding disaccharide. The disaccharide on hydrolysis gave L-rhamnose and D-galactose, confirming thereby that the aldobiouronic acid is made up of L-rhamnose and D-galacturonic acid.

(1) A. K. Nadkarni, "The Indian Materia Medica," Vol. I, Popular Book Depot, Bombay 7, India, 1954, p. 1093.