(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.

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KANPUR, INDIA

[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]

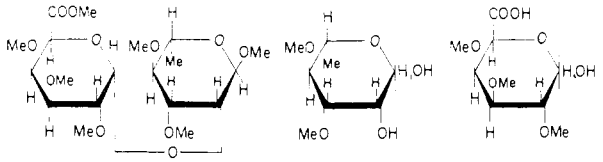
This communication deals with the composition of *Salvia aegyptica* mucilage and determination of the structure of an aldobiouronic acid produced by hydrolyzing the mucilage with an acid.

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.

The aldobiouronic acid consists of a pyranose galacturonic acid residue united by its reducing group to the hydroxyl group situated at C_2 of the rhamnopyranose portion. This conclusion follows from the identification of the hydrolysis products obtained from the fully methylated derivative (I) of the aldobiouronic acid. These were (a) 3,4-di-*O*-methylrhamnose (II), recognized after oxidation with bromine water, which gave crystalline 3,4-di-*O*-methyl-L-rhamnonolactone[1a] and (b) 2,3,4-tri-*O*-methyl D-galacturonopyranose (III), identified after oxidation with bromine water and esterification of the product, as the crystalline methyl ester of 2,3,4-tri-*O*-methylmucic acid.[2]

Further confirmation of the structure of the aldobiouronic acid was obtained when the fully methylated aldobiouronic acid was reduced with lithium aluminum hydride[3] to the corresponding methylated disaccharide. This methylated disaccharide was hydrolyzed and treated with aniline in alcohol to give the anilide of 2,3,4-*O*-methylgalactose[4,5] which separated out in fine crystals, m.p. 169–170°, proving that the aldobiouronic acid is 2-*O*-(D-galactopyranosyluronic acid)-L-rhamnose. The aldobiouronic acid has high positive rotation. These facts show that the structure (A) assigned to the aldobiouronic acid is correct. This aldobiouronic acid is identical with the acid obtained from flax seed,[6] slippery elm mucilage[7] and okra mucilage.[8]



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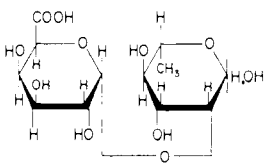

A

Experimental

Unless otherwise stated, solutions were concentrated at 40–50° (20 mm.); specific rotations were measured in aqueous solutions; paper chromatographic separations were carried out by descending method on Whatman No. 1 paper using the following solvents (A) butanol:ethanol:water[9] (40:11:19), (B) butanol:acetic acid:water (5:1:4—upper layer), (C) butanol:ethanol:water:concd. ammonium hydroxide (100:25:122.5:2.5), (D) methyl ethyl ketone:water azeotrope,[10] (E) benzene:ethanol:water[11] (200:47:15—upper layer) (all v./v.). Silver nitrate[12] and *p*-anisidine phosphate[13] spray reagents were used to detect the sugars and uronic acids.

Isolation and Purification of the Mucilage.—Tukhmalanga seeds (50 g.) were steeped in water (4 l.) at room temperature for 5 hours with continuous stirring. The solution was separated by filtration through muslin and finally through a Büchner funnel. A solution of copper sulfate (5%) was added to the filtrate and the copper salt of the mucilage precipitated almost immediately. The copper salt was collected and acidified with sulfuric acid (2 *N*) (until acidic to congo red paper) and after suspension in a limited amount of water, the mucilage was finally precipitated by pouring the solution into ethanol with vigorous stirring. The white fibrous precipitate thus obtained was washed a number of times with ethanol, filtered, pressed and dried *in vacuo* over phosphorus pentoxide (yield 8%). The mucilage was purified by reprecipitations by dissolving it in sulfuric acid (2 *N*) and pouring in ethanol and stirring. The final purification was done by passing the acidified impure solution of the mucilage through a column of strong cation-exchange resin, Amberlite IR-120, concentrating and finally pouring into alcohol (sulfated ash 0.83%).

The raw and purified mucilages are soluble in water, do not reduce Fehling solution and are free from nitrogen, sulfur and halogens.

Hydrolysis of the Mucilage—Characterization of L-Rhamnose, L-Arabinose, D-Galactose and Isolation of Aldobiouronic Acid.—A solution of the mucilage (45 g.) in sulfuric acid (1 l., 0.1 *N*) was heated on a boiling water-bath for 90 hours, the course of hydrolysis being followed by iodometric titrations.[14] The solution was neutralized (barium carbonate), filtered and evaporated. The residue was extracted with boiling methanol and the filtered extract concentrated to a sirup (a). The solid amorphous residue left after methanol extraction was the barium salt of the degraded polysaccharide. Paper chromatography of the sirup (a) using the solvent (A) and silver nitrate spray reagent for development revealed the presence of rhamnose, arabinose and galactose.

Resolution of the sirup (a) into its components on a cellulose column, using butanol half-saturated with water as the solvent, yielded (a) L-rhamnose, identified as α-L-rhamnose hydrate, m.p. and mixed m.p. 92.5°, $[\alpha]^{30}D$ +7.8° (equilibrium value in water, *c* 2.0) (after recrystallization from aqueous methanol), methylphenylhydrazone m.p. and mixed m.p. 124°; (b) L-arabinose, m.p. and mixed m.p. 156° (after purification[15]), $[\alpha]^{30}D$ +101° (equilibrium value in water, *c* 1), diphenylhydrazone m.p. and mixed m.p. 195°; (c) D-galactose, m.p. and mixed m.p. 165°, $[\alpha]^{30}D$ +81.8° (*c* 1, equilibrium value in water), methylphenylhydrazone m.p. and mixed m.p. 182–183°.

The methanol-insoluble barium salt (b) was found to be of an aldobiouronic acid. *Anal.* Calcd. for the barium salt of an aldobiouronic acid $(C_{12}H_{19}O_{11})_2Ba$: Ba, 16.9. Found: Ba, 17.0; $[\alpha]^{32}D$ +73.4° (equilibrium value in water, *c* 1).

Quantitative Hydrolysis of the Mucilage.—The purified mucilage (1.00 g.) was heated in a sealed tube with 1.5 *N* sulfuric acid (75 ml.) for 35 hours in a water-bath. The hydrolyzate was filtered to remove the undissolved residue and the filtrate made up to 100 ml. Dry D-ribose (45 mg.) was taken in a conical flask and the sealed tube hydrolyzate (25 ml.) was added to it. The resulting solution was neutralized (barium carbonate) 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[16] and titration of the formic acid liberated indicated that galactose, arabinose and rhamnose are present in the percentage of 0.74, 0.85 and 62.8%, respectively.

Hydrolysis of Aldobiouronic Acid and Characterization of L-Rhamnose and D-Galacturonic Acid.—The barium salt of the aldobiouronic acid (0.95 g.) (Ba, 17.0%) was dissolved in sulfuric acid (100 ml., 1.0 *N*) and was heated on a boiling

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water-bath for 35 hours, the course of hydrolysis being followed by iodometric titrations. The solution was neutralized (barium carbonate), filtered and evaporated. The residue was extracted exhaustively with boiling methanol and filtered extract evaporated to a sirup (c) which on cooling gave crystalline L-rhamnose hydrate which was identified as already described. The methanol-insoluble barium salt (d) was identified to be that of hexuronic acid. *Anal.* Calcd. for the barium salt of an hexuronic acid $(C_6H_9O_7)^1/_2$-Ba: Ba, 26.2. Found: Ba, 27.4. The uronic acid gave a positive basic lead acetate test[17] for galacturonic acid and was further confirmed by oxidation to mucic acid, m.p. and mixed m.p. 213°.

The aldobiouronic acid was found to be chromatographically pure, when developed with *p*-anisidine phosphate spray reagent using solvent (B).

Reduction of Aldobiouronic Acid with Potassium Borohydride.[18]—The aldobiouronic acid (1 g.) (barium salt) was set aside overnight in methanolic 4% hydrogen chloride and then refluxed for 7 hours. The solution was filtered to remove barium chloride, neutralized with silver carbonate, filtered and concentrated to a thick sirup. The dry residue then was dissolved in water (75 ml.) and the solution was added slowly to a solution of potassium borohydride (2 g.) in water (75 ml.) with stirring. After 24 hours, the excess borohydride was destroyed by the addition of dilute acetic acid, and the solution was deionized by passage through columns of Duolite C-25 and A-7 resins. After being taken to dryness, the residue was hydrolyzed by *N* sulfuric acid at 100° for 20 hours. The solution was neutralized and worked up in the usual way and on chromatographic examination showed the presence of rhamnose and galactose (corresponding to galacturonic acid of the aldobiouronic acid).

The sugars were separated on a cellulose column using butanol half-saturated with water as eluent when rhamnose and galactose were obtained. They were identified as already described.

Methylation of Aldobiouronic Acid.—To a solution of the barium aldobiouronate (10 g.) in water (50 ml.) methyl sulfate (75 ml.) was added followed by sodium hydroxide (225 ml., 30%). The sodium hydroxide was 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 (450 ml., 40%) and adding methyl sulfate (225 ml.) in the previous manner. The reaction mixture was acidified with sulfuric acid (congo red), filtered and the filtrate extracted with chloroform (in a liquid–liquid extractor).

The sirup (5.2 g.) obtained on evaporation of the chloroform extract was methylated eight times with Purdie reagent to give a sirup (5.1 g.) which was extracted exhaustively with light petroleum ether (b.p. 40–60°) and concentrated to give a sirup (4.5 g.). The sirup on fractional distillation gave: fraction I (1.87 g.), b.p. 165–170° (0.2 mm.) (bath temp.), methyl 2-*O*-(methyl 2,3,4-tri-*O*-methyl-D-galactopyranosyluronate)-3,4-di-*O*-methyl-L-rhamnopyranoside (Found: OMe, 47.7. Calcd. for $C_{19}H_{34}O_{11}$: OMe, 49.5).

Hydrolysis of Methyl 2-*O*-(Methyl 2,3,4-Tri-*O*-methyl-D-galactopyranosyluronate)-3,4-di-*O*-methyl-L-rhamnopyranoside and Identification of 3,4-Di-*O*-methylrhamnose and 2,3,4-Tri-*O*-methylgalacturonic Acid.—The fully methylated aldobiouronic acid (1.3 g.) was hydrolyzed with 2 *N* hydrochloric acid (50 ml.) for 18 hours. The cooled solution was neutralized with silver carbonate and filtered, silver ions removed by hydrogen sulfide, the solution aerated to remove hydrogen sulfide, evaporated to a small volume, neutralized with barium carbonate and filtered and the filtrate evaporated to dryness, giving a product (A) which was exhaustively extracted with ether. Concentration of the extracts gave a sirup (0.56 g.) (Found: OMe, 33.7. Calcd. for $C_8H_{16}O_5$: OMe, 32.2).

The sirup (0.56 g.) was dissolved in water (10 ml.) and oxidized with bromine (0.5 ml.) until the solution was non-reducing (48 hours). Bromine was removed by aeration, neutralized with $BaCO_3$, and worked up in the usual manner. The product (0.48 g.) crystallized almost completely and on recrystallization from ether–light petroleum needle-shaped crystals of 3,4-di-*O*-methyl-L-rhamnonolactone were obtained, m.p. and mixed m.p. 76–77°.

The residual barium salts from A were dissolved in water and oxidized with bromine water until non-reducing (2 days at 2°). The excess of bromine was removed by aeration, the solution neutralized with barium carbonate, filtered and evaporated to dryness and the residue boiled with 3% methyl alcoholic hydrogen chloride (50 ml.) for 20 hours. Hydrogen chloride was removed with silver carbonate and the filtered solution evaporated to a solid, which was exhaustively extracted with ether. Concentration of the extracts gave a sirup (0.48 g.) which was distilled, b.p. 140–150° (0.08 mm.). The distillate (0.2 g.) set solid and on recrystallization from ether–light petroleum and ether gave methyl 2,3,4-tri-*O*-methylmucate, m.p. 101° (Calcd. for $C_{11}H_{20}O_8$: OMe, 55.4. Found: OMe, 56.0).

Reduction and Hydrolysis of Fully Methylated Aldobiouronic Acid.—The fully methylated acid (250 mg.) was dissolved in dry ethyl ether (25 ml.) and the solution was added dropwise over a period of one hour to a stirred suspension of lithium aluminum hydride (1 g.) in ether (75 ml.). The reaction mixture was heated under gentle reflux for 2 hours, cooled and the excess of lithium aluminum hydride was decomposed by cautious successive additions of ethyl acetate and water. After filtration, concentration and deionization with Duolite C-25 and A-7, methyl 2-*O*-(2,3,4-tri-*O*-methyl-D-galactopyranosyl)-3,4-di-*O*-methyl-L-rhamnopyranoside was obtained as a yellow sirup (225 mg.).

Hydrolysis of Methyl 2-*O*-(2,3,4-Tri-*O*-methyl-D-galactopyranosyl)-3,4 di-*O*-methyl-L-rhamnopyranoside.—A solution of the methylated disaccharide (225 mg.) in 8% methanolic hydrogen chloride (50 ml.) was refluxed for 12 hours. After removal of the methanol, hydrolysis was effected by heating at 100° with hydrochloric acid (0.5 *N*, 25 ml.) for 15 hours. The acid was removed with silver carbonate and the solution deionized with Duolite C-25 and A-7. Chromatographic examination in solvent E showed two sugars corresponding to 2,3,4-tri-*O*-methyl-D-galactose and 3,4-di-*O*-methyl L-rhamnose.

Attempts were made to separate the two components of the methylated sugars on filter paper, using solvents C, D and E, respectively, but because of their similarity in R_f values, separation could not be effected satisfactorily.

Identification of 2,3,4-Tri-*O*-methyl-D-galactose.—The mixture of methylated rhamnose and galactose (113 mg.) was taken in absolute alcohol (5 ml.), treated with aniline (0.2 ml.) and refluxed for 4 hours. Alcohol was subsequently removed by distillation. On keeping for a few days crystals of 2,3,4-tri-*O*-methylgalactose anilide separated, which on further crystallization from methanol had m.p. 169–170° (literature m.p. of 2,3,4 tri-*O*-methylgalactose anilide is 165–170°).

Acknowledgments.—The authors record their thanks to Professor S. N. Gundu Rao, Director, National Sugar Institute, Kanpur (India), for his kind interest.

KANPUR, INDIA

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