341. *The Structure of Almond-tree Gum. Part I. The Constitution of the Aldobionic Acid derived from the Gum.*

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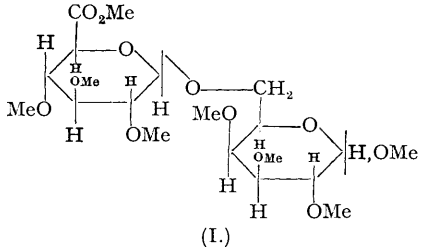
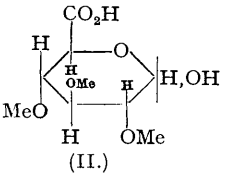
Almond-tree gum on hydrolysis yields L-arabinose (4 parts), D-xylose (2 parts), D-galactose (3 parts), and D-glucuronic acid (1 part). On graded hydrolysis, the polysaccharide yields an aldobionic acid identified as 6-(D-glucuronosido)-D-galactose, since on methylation, followed by hydrolysis of the methylated derivative, 2 : 3 : 4-trimethyl D-glucuronic acid and 2 : 3 : 4-trimethyl D-galactose are produced. The structure of this aldobionic acid is identical with that of the aldobionic acids found in gum arabic and egg-plum gum.

During the summer months, the almond tree exudes a gum which is, at first, colourless or pale yellow and very viscid, but gradually hardens, ultimately becoming insoluble in water. In physical properties and appearance it closely resembles those gums which are exuded by other members of the *Rosaceæ* family, *e.g.*, the damson, the cherry, and the egg-plum. Almond-tree gum is the neutral salt of an acidic polysaccharide which can be obtained free from ash on precipitation from acidified aqueous solution by alcohol. When an aqueous solution of the ash-free acidic gum (equiv., 1470) is boiled, hydrolysis occurs with formation of L-arabinose, D-xylose, and a degraded polysaccharide which, on further hydrolysis with 0·1N-sulphuric acid, yields D-galactose and an aldobionic acid. The yield of arabinose (40% of the original weight of the gum) was determined by the paper chromatogram method (Flood, Hirst, and Jones, *Nature*, 1947, **160**, 86). Precipitation of the arabinose as the benzoylhydrazone (Hirst, Jones, and Woods, *J.*, 1947, 1049) gave a result (36%) which, in view of the possible experimental error in the latter method, is in good agreement. D-Xylose (20%) was also detected and determined on the paper chromatogram. Hydrolysis of the degraded polysaccharide yielded D-galactose (23%, calculated on the original weight of the gum) and an aldobionic acid which on further hydrolysis gave D-galactose and D-glucuronic acid in equimolecular proportions.

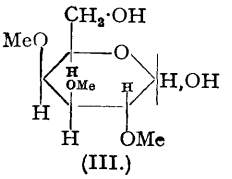
On methylation, the aldobionic acid yielded an octamethyl derivative (I) which gave, on hydrolysis, 2 : 3 : 4-trimethyl D-glucuronic acid (II) and 2 : 3 : 4-trimethyl D-galactose (III). The uronic acid fraction was identified after oxidation and esterification in the form of the crystalline methyl ester of 2 : 3 : 4-trimethyl D-saccharopyranolactone. 2 : 3 : 4-Trimethyl D-galactose was identified as the crystalline sugar and as its crystalline anilide.



(I.)



(II.)



(III.)

The ease of hydrolysis of the arabinose residues indicated that they were combined in the gum in the furanose form. Some evidence was also obtained for the production of an oligosaccharide during the autohydrolysis of the gum, since on boiling with N-sulphuric acid the sugars obtained from the autohydrolysis, the specific rotation of the solution rose from + 31° to + 66°.

It may be significant that the degraded polysaccharide resembled the degraded gums from gum arabic, egg-plum gum, cherry gum, and damson gum, in that it was built up of a repeating unit of one molecule of aldobionic acid combined with two molecules of D-galactose. The aldobionic acid was identical in structure with that isolated from gum arabic and egg-plum gum. Since the yields of aldobionic acid were good it is considered unlikely that any other differently constituted aldobionic acid may be present in the gum.

Experimental.

The gum was collected in Bristol in the summer months of 1939 and 1943 from the branches and trunks of almond trees. It was dissolved in water and filtered, and the filtrate acidified with dilute hydrochloric acid. The solution was poured into methylated spirit and the precipitated material washed with alcohol. Further purification was effected by dissolving the product in cold water and filtering the solution into alcohol. The precipitate was filtered off, washed with alcohol and with ether, and then dried under reduced pressure. The product, a white powder, was easily soluble in water with the formation of an acidic non-reducing solution. The gum did not give an insoluble copper salt [Found: Equiv., 1470 (mean value) by titration with 0·1N-sodium hydroxide; ash 1·0%: $[\alpha]_D^{20°}$ − 44° (*c*, 1·3 as sodium salt in water); galactose residues calc. as $C_6H_{10}O_5$, 33% (from the yield of mucic acid obtained on oxidation of the polysaccharide with nitric acid)].

Autohydrolysis.—A solution of the gum (5·42 g.) in distilled water (100 c.c.) was kept at 98° and the reaction followed polarimetrically: $[\alpha]_D^{10°}$ − 44° (initial value); + 17·7° (23·5 hours); + 23·6° (26·5 hours); + 25·8° (28 hours); + 28·0° (30·5 hours). (A much slower hydrolysis continued beyond this stage.) After 31 hours, barium carbonate was added and the neutral solution filtered. The insoluble barium salts were washed with hot water and the combined filtrate and washings were evaporated under reduced pressure to a syrup which was exhaustively extracted with dry methanol. The soluble fraction (A) (3·23 g.) showed $[\alpha]_D$ + 30·6° (in water). The residue (B) (2·62 g.) was the barium salt of a tetrasaccharide (Found: Ba, 8·7. Calc. for a tetrasaccharide consisting of 3 residues of galactose and 1 residue of glucuronic acid, Ba, 9·1%).

Examination of Soluble Fraction (A).—The syrup contained some oligosaccharide since in boiling N-sulphuric acid, its specific rotation increased from $[\alpha]_D$ + 31° to + 66° (constant value) in 30 minutes. The syrup isolated after this further hydrolysis was examined by the paper chromatogram method (Partridge, *Nature*, 1946, **158**, 270) and was found to contain only L-arabinose and D-xylose (by comparison with the results obtained with a synthetic mixture containing these sugars). L-Arabinose was also detected by the formation of L-arabinose benzoylhydrazone [m. p. 186° (decomp.)] and D-xylose by the isolation of its dibenzylidene dimethyl acetal derivative [m. p. 211°; $[\alpha]_D$ − 9° (in chloroform); see Breddy and Jones, *J.*, 1945, 739].

Quantitative determination of L-arabinose by the method of Hirst, Jones, and Woods (*loc. cit.*) indicated the presence of 36% of this sugar in the gum. A determination of arabinose and xylose after separation on the paper chromatogram was carried out by extracting the separated sugar zones with water followed by determination of the sugars with Somogyi's copper reagent (Flood, Hirst, and Jones, *loc. cit.*). The arabinose and xylose were in the molecular ratio 2 : 1 which is in good agreement with the observed equilibrium rotation (+ 66°) of the isolated syrup. A polysaccharide of equiv. 1470 containing 4 residues of arabinose, 2 residues of xylose, 3 residues of galactose, and 1 residue of glucuronic acid should give 42% of arabinose and 21% of xylose on hydrolysis.

Examination of Insoluble Fraction (B).—A portion (0·25 g.) was dissolved in 0·1N-sulphuric acid (25 c.c.), the precipitate of barium sulphate removed by filtration, and the filtrate boiled until the rotation became constant: $[\alpha]_D$ + 19·3° (initial value); + 33·5° (1 hour); + 30·5° (2 hours); + 30·6° (5 hours, constant value). From the final figure it follows that the aldobionic acid had a negative rotation ($[\alpha]_D$ *ca.* − 20°). It is therefore a β-D-glucuronosido-derivative. Barium carbonate was added to neutralise the acid, and the sugars were isolated in the usual way. From a portion (0·24 g.) of the resultant syrup, D-galactose (0·11 g., m. p. 167°) was obtained on extraction with methanol. This material gave D-galactose phenylmethylhydrazone (m. p. 179° alone or admixed with an authentic specimen) on treatment with phenylmethylhydrazine. In a quantitative experiment, 0·89 g. of the isolated sugar yielded 1·33 g. of the phenylmethylhydrazone. No sugar other than D-galactose was present. The material (C) (0·13 g.) insoluble in methanol contained 16·4% of Ba (Calc. for $C_{12}H_{19}O_{12}Ba_{\frac{1}{2}}$, Ba, 16·2%).

Methyl Ester of 6-(2 : 3 : 4-*Trimethyl* D-*glucuronosido*) 2 : 3 : 4-*Trimethyl Methyl*-D-*galactoside.*—The barium salt of the aldobionic acid (0·95 g.) (see above) was methylated by dissolving it in water (10 c.c.). and adding methyl sulphate (10 c.c.) and then 30% sodium hydroxide solution (20 c.c.) dropwise in 4 hours with vigorous stirring. After standing overnight the mixture did not reduce Fehling's solution. Solid sodium hydroxide (9 g.) was added followed by methyl sulphate (20 c.c.) dropwise (during 7 hours), and the stirring continued throughout the addition. When all the methyl sulphate had been added, the mixture was heated on the boiling water-bath for 30 minutes. The solution was cooled, acidified with cold concentrated sulphuric acid, and then extracted 5 times with chloroform. Evaporation of the chloroform left a syrup which was again methylated with sodium hydroxide (20 c.c.) and methyl sulphate (10 c.c.). The product was isolated by extracting the acidified solution 5 times with chloroform (see above). The syrup (0·41 g.) obtained on evaporation of the dried chloroform solution was methylated once with silver oxide and methyl iodide. The product (0·41 g.) was then distilled under diminished pressure. No low-boiling fractions were obtained, the absence of monosaccharides being thus indicated,

and the main bulk (0·37 g.) distilled at 170°/0·1 mm. (bath temp.); $n_D^{20°}$ 1·4675 (Found: OMe, 50·4. Calc. for $C_{20}H_{36}O_{12}$: OMe, 52·8%).

Hydrolysis. The methyl heptamethyl aldobionate (0·30 g.) was hydrolysed by boiling it in N-hydrochloric acid (4¼ hours). The rotation could not be observed initially, owing to the opalescence of the solution, but after 2¼ hours $[\alpha]_D$ was + 52°. Thereafter the solution became too dark to allow of polarimetric observation. The acid was neutralised with silver carbonate and the solution was filtered before and after the passage of hydrogen sulphide. The solution was neutralised with barium carbonate after being aerated to remove hydrogen sulphide. The insoluble barium salts were removed by filtration, and the filtrate evaporated to a syrup (0·26 g.); this was exhaustively extracted with ether. The ethereal extract on concentration gave a syrup (E) (0·11 g.; $n_D^{22·5°}$ 1·4680). The insoluble residue (D) (0·15 g.) consisted of the barium salt of the uronic acid fragment (see below). The syrup (E) crystallised on trituration with a small quantity of ether. It was proved to be 2 : 3 : 4-trimethyl D-galactose by its m. p. (77°) and by its conversion into a crystalline anilide (m. p. 168°, after recrystallisation from absolute ethanol; not depressed on admixture with an authentic specimen of 2 : 3 : 4-trimethyl D-galactose anilide). In cold methanolic hydrogen chloride (1%) the specific rotation rose from + 55° to + 90° in 4 hours and then remained constant. This indicated the absence of a hydroxyl group on C_4 of the galactose derivative.

The uronic acid portion (D) was identified by dissolving the barium salt (0·15 g.) in water and removing the barium as sulphate. After filtration, bromine was added to the clear solution and the oxidation was continued until the solution was non-reducing. The excess of bromine was removed by aeration, and the solution neutralised with silver carbonate. After filtration, hydrogen sulphide was passed into the solution which was again filtered. The filtrate was evaporated under reduced pressure and the residual acid boiled with 1% methanolic hydrogen chloride for 3 hours. The ester was distilled under diminished pressure, giving the methyl ester of 2 : 3 : 4-trimethyl D-saccharopyranolactone, m. p. 110°, not depressed on admixture with an authentic sample.

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