31. *The Structure of Egg-plum Gum. Part II. The Hydrolysis Products obtained from the Methylated Degraded Gum.*

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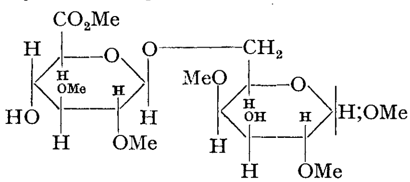
Methylated degraded egg-plum gum has been hydrolysed and the following sugars identified amongst the products of hydrolysis: (*a*) 2 : 3 : 4 : 6-tetramethyl *d*-galactose, (*b*) 2 : 4 : 6-trimethyl *d*-galactose, (*c*) 2 : 3 : 4-trimethyl *d*-galactose, (*d*) 2 : 4-dimethyl *d*-galactose and 2 : 3-dimethyl *d*-glucuronic acid. A partly methylated aldobionic acid, 6-(2 : 3-dimethyl *d*-glucuronosido) 2 : 4-dimethyl *d*-galactose was also present amongst the products of hydrolysis.

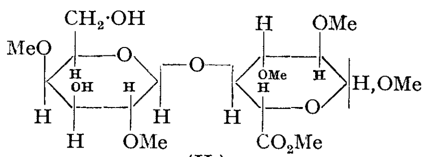
The sugar residues present in egg-plum gum have been identified after hydrolysis with dilute aqueous acid as *l*-arabinose, *d*-xylose, *d*-galactose, and *d*-glucuronic acid, which occur respectively in the approximate proportions 3 : 1 : 3 : 1 (Part I, *J.*, 1947, 1064). No evidence as to the mode of linkage of these residues has been available with the important exception that a part at least of the glucuronic acid is known to be combined with galactose and appears after hydrolysis as the aldobionic acid 6-*d*-glucuronosido-*d*-galactose identical with the aldobionic acid obtainable

from gum arabic. The arabinose residues are extremely susceptible to acid hydrolysis and can be detached readily from the gum molecule. The xylose comes away less easily but can be removed almost completely from the gum without dislocation of its main chain structure. Further insight into the structure of the stable portion of the gum molecule is derivable from a study of the hydrolysis products obtained from the methylated derivative of the degraded gum. After separation of the products by fractional distillation, the following sugars were identified; (*a*) 2 : 3 : 4 : 6-tetramethyl *d*-galactose, recognised as its crystalline anilide and as the crystalline phenylhydrazide of 2 : 3 : 4 : 6-tetramethyl *d*-galactonic acid, (*b*) 2 : 4 : 6-trimethyl *d*-galactose, recognised as its crystalline anilide, (*c*) 2 : 3 : 4-trimethyl *d*-galactose, identified as its crystalline anilide and as the crystalline phenylhydrazide of 2 : 3 : 4-trimethyl *d*-galactonic acid, and (*d*) 2 : 4-dimethyl *d*-galactose, recognised as its crystalline anilide and as the phenylhydrazide of 2 : 4-dimethyl *d*-galactonic acid. The uronic acid portion consisted mainly of 2 : 3-dimethyl *d*-glucuronic acid, identified as the methyl ester of 2 : 3-dimethyl *d*-saccharolactone. No 2 : 3 : 4-trimethyl *d*-glucuronic acid could be detected. The degraded polysaccharide is therefore not identical structurally with the degraded polysaccharide obtained from gum arabic, although on hydrolysis both these degraded gums give glucuronic acid (1 part) and galactose (3 parts) (see Smith, *J.*, 1939, 1724).

Some of the partly methylated glucuronic acid was found to be present as a disaccharide (I) which on hydrolysis gave a partly methylated derivative of *d*-glucuronic acid and a partly methylated derivative of *d*-galactose. These products, identified as 2 : 3-dimethyl *d*-glucuronic acid (III) and 2 : 4-dimethyl *d*-galactose (IV) may have been combined with one another either through the reducing group of the galactose molecule (formula II) or through the reducing group of the glucuronic acid molecule (formula I). The latter possibility is considered to be the more likely since the part of the gum molecule more resistant to hydrolysis is an aldobionic acid, 6-*d*-glucuronosido-*d*-galactose, which may be isolated from the products obtained after prolonged hydrolysis of the gum.



(I.)



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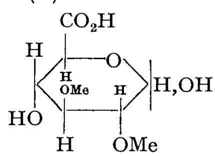
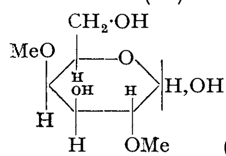


(III.)



(IV.)

If the methylated disaccharide is a derivative of this aldobionic acid it follows that in the degraded gum there are *d*-galactose units linked through their reducing groups to C_4 of the glucuronic acid residues and also *d*-galactose units linked glycosidically to C_3 of those *d*-galactose residues which occur in the aldobionic acid. At this stage, the presence of small quantities of other differently constituted aldobionic acids in the gum molecule cannot be ruled out.

Indications of the presence of small amounts of 2 : 3 : 4-trimethyl *d*-xylose in the 2 : 3 : 4 : 6-tetramethyl *d*-galactose fraction were obtained from an examination of the change in optical rotation of the sugar glycosides on hydrolysis. It is assumed that this material arises from the small amounts of *d*-xylose which remained in the degraded gum molecule, during the removal of the *l*-arabinose residues, owing to the greater resistance of *d*-xylopyranose to hydrolysis.

A satisfactory quantitative estimation of the sugars present in the methylated degraded gum molecule could not be made from an examination of the yields of sugars and of their anilides owing to the complex nature of the mixture obtained on hydrolysis, but it is evident that the degraded polysaccharide is composed of the following residues linked through the carbon atoms indicated.

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                                              :                               :
                                              3                               3,
Ga,1, . . .  . . . 6,Ga,1, . . .  . . . 3,Ga,1, . . .  . . . 6,Ga,1, . . .  . . . 4,Glu,1, . . .  . . . 4,Glu,1,6,Ga,1, . . .
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Where Ga = *d*-galactopyranose residue and Glu = *d*-glucuronic residue.

It will be observed that *d*-galactose is the major component of the degraded gum and that the -1 : 3- and -1 : 6-linkages are again encountered as in the main chain of gum arabic, damson gum, and cherry gum. The galactose residues all occur in the pyranose form and therefore cannot be the direct precursors of the *l*-arabofuranose residues present in the undegraded gum molecule.

Experimental.

The degraded arabinose-free polysaccharide (15·6 g.) was dissolved in thallous hydroxide (300 c.c.; N) and evaporated under reduced pressure at 40° to a solid which was ground to a powder (fume cupboard—use rubber gloves). The solid was boiled under reflux with methyl iodide until the solid reacted neutral to litmus (24 hours). Excess of methyl iodide was distilled off and the residue extracted with methyl alcohol and then with aqueous alcohol. The combined extracts were evaporated to dryness (40°, 12 mm.) and the residue (14·5 g.) was dissolved in aqueous alcohol (50 c.c.), and thallous ethoxide in benzene (150 c.c.; N) added. The solution was evaporated to dryness and the residual solid powdered (observing the usual precautions) and boiled with methyl iodide. After 24 hours, excess of methyl iodide was boiled off and the residual solid exhaustively extracted with acetone. Concentration of the extracts gave the crude methylated polysaccharide (14·5 g.; OMe, 43·7%) which was purified by dissolving in methyl iodide and boiling with silver oxide. The methylated polysaccharide (13·5 g.; OMe, 43·9%) was isolated as a crisp cream-coloured solid. It was fractionated by dissolving in acetone (50 c.c.) followed by the gradual addition of ether. In this way, three fractions were isolated. Fraction I (2·3 g.), $[\alpha]_D^{20°}$ + 11° (*c*, 0·6 in methyl alcohol) (Found: OMe, 38·0%). Fraction II (9·7 g.), $[\alpha]_D^{20°}$ + 9° (c, 1·7 in methyl alcohol) (Found: OMe, 44·5%). Fraction III (1·5 g.), a sticky solid, not further examined. Fraction II was used in the experiments detailed below.

Hydrolysis of the Methylated Polysaccharide.—Fraction II (8·5 g.) was dissolved in sulphuric acid (50 c.c.; N) and heated at 90° for 48 hours. The substance, initially insoluble, gradually went into solution; it was not possible to observe any change of optical rotation owing to darkening of the solution. The cooled solution was neutralised with barium carbonate, filtered, and evaporated to dryness. The residue (a brown solid) was extracted with acetone; this removed sugars and the barium salts of some oligosaccharide since the acetone extract (A) gave a positive test for barium ions. The residual barium salts were dissolved in water, filtered, and concentrated to a dry solid (B) (2·0 g.) (Found: Ba, 20·3%).

The sugars (A) were boiled with 2·5% methyl-alcoholic hydrogen chloride (50 c.c.) for 25 hours. The mixture was then cooled, neutralised with silver carbonate, and the solution worked up in the usual manner. The resultant syrupy glycosides (7·0 g.) were boiled for 5 hours with excess of barium hydroxide, cooled, and the solution neutralised (phenolphthalein) with dilute sulphuric acid. The solution was concentrated in a vacuum at 40° to a syrup which was dissolved in water (30 c.c.) and extracted exhaustively with light petroleum (b. p. 40—60°). Concentration of the extract gave a syrup (C) (0·73 g.). The aqueous solution was then extracted exhaustively with ether which on concentration gave a further quantity of syrup (2·54 g.). The aqueous solution was then evaporated to dryness under reduced pressure at 40° and the solid extracted exhaustively with ether. Concentration of this extract gave a product (1·4 g.) which was combined with the fraction, 2·54 g. above, giving a fraction (D) (3·94 g.).

The residual barium salts from the ether extraction (3·2 g.) were combined with the fraction (B) and the solid (5·1 g.) was dissolved in 5% methyl-alcoholic hydrogen chloride (100 c.c.) and kept at 20° for 30 days. The solution was then neutralised with diazomethane and concentrated immediately under reduced pressure at 40°. The residual syrup (E) was isolated from the barium chloride by extraction with acetone–ether (1 : 1). The low yield (3·1 g.) probably indicates that some incompletely hydrolysed material had not been extracted from the barium chloride by the solvent used.

Examination of the Sugar Fractions (C) and (D).—Fraction (C) was distilled, and gave: Fraction (i) (0·28 g.), b. p. 100—105° (bath temp.)/0·01 mm., $n_D^{23°}$ 1·4492 (Found: OMe, 58·3%). Fraction (ii) (0·21 g.), b. p. 100—105° (bath temp.)/0·01 mm., $n_D^{23°}$ 1·4532 (Found: OMe, 53·4%). The residue (0·20 g.) was combined with Fraction (D) and distillation was continued. Fraction (iii) (0·58 g.), b. p. 110—120° (bath temp.)/0·01 mm., $n_D^{20°}$ 1·4570 (Found: OMe, 53·4%). Fraction (iv) (0·24 g.), b. p. 120—160° (bath temp.)/0·01 mm., $n_D^{20°}$ 1·4580 (Found: OMe, 52·5%). Fraction (v) (0·97 g.), b. p. 160° (bath temp.)/0·01 mm., $n_D^{20°}$ 1·4620 (Found: OMe, 49·8%). Fraction (vi) (0·72 g.), b. p. 160—170° (bath temp.)/0·01 mm., $n_D^{18°}$ 1·4710 (Found: OMe, 42·4%). Fraction (vii) (0·36 g.), b. p. 170—220° (bath temp.)/0·01 mm., $n_D^{18°}$ 1·4740 (Found: OMe, 40·7%). Residue (1·0 g.). Loss during distillation, 0·4 g. Fractions (i), (ii), and (iii) were combined (1·05 g.) and hydrolysed with N-hydrochloric acid (25 c.c.); $[\alpha]_D^{20°}$ + 76° (initial value), + 85° (1¼ hours), + 69° (constant value). The low equilbrium value indicated that galactose was not the only sugar present. The cooled solution was neutralised with silver carbonate, filtered, and concentrated under reduced pressure at 40°. The residual syrup (1·0 g.), $n_D^{22°}$ 1·4678, was fractionally distilled giving Fraction (viii) (0·47 g.), b. p. 160° (bath temp.)/0·01 mm., $n_D^{25°}$ 1·4678 (Found: OMe, 48·5%). Fraction (ix) (0·31 g.), b. p. 170° (bath temp.)/0·01 mm., $n_D^{24°}$ 1·4738 (Found: OMe, 43·0%). Residue (0·14 g.). Loss, 0·06 g. Fraction (viii), $[\alpha]_D^{20°}$ + 46° (*c*, 2·8 in ethyl alcohol) (230 mg.), was boiled with ethyl-alcoholic aniline for 2 hours, when it gave a mixture of 2 : 3 : 4 : 6-tetramethyl *d*-galactose anilide, m. p. 194° not depressed on admixture with an authentic specimen, and 2 : 4 : 6-trimethyl *d*-galactose anilide, m. p. and mixed m. p. with an authentic specimen 179°. The two materials were separated by fractional crystallisation from absolute alcohol and were present in the proportion of approximately 2 : 1 (80 mg. of tetra- and 40 mg. of tri-methyl galactose anilides were isolated). A portion of the sugar (0·2 g.) was oxidised with bromine water. The isolated lactones did not crystallise, and no crystalline amide could be prepared. A small quantity of a crystalline phenylhydrazide was isolated, however, m. p. 137°, not depressed on admixture with an authentic specimen of the phenylhydrazide of 2 : 3 : 4 : 6-tetramethyl *d*-galactonic acid. Fraction (ix), $[\alpha]_D^{20°}$ + 40° (*c* 1·8 in ethyl alcohol) (100 mg.), was boiled with ethyl-alcoholic aniline for 2 hours, when it

gave a mixture of 2 : 4 : 6-trimethyl *d*-galactose anilide, m. p. 179°, and 2 : 3 : 4-trimethyl *d*-galactose anilide, m. p. 169°, not depressed on admixture with the corresponding authentic anilides. A sample of the sugars, on oxidation with bromine water, gave a lactone which with alcoholic phenylhydrazine gave the phenylhydrazide of 2 : 3 : 4-trimethyl *d*-galactonic acid, m. p. 174°, not depressed on admixture with an authentic specimen. Fractions (iv) and (v) (1·17 g.) were hydrolysed with boiling N-hydrochloric acid (25 c.c.); $[\alpha]_D^{20°}$ + 90° ⟶ + 85° (constant value, 5 hours). The reducing sugar (1·07 g.) was isolated after neutralisation of the hydrolysis solution with silver carbonate; $[\alpha]_D^{20°}$ + 87° (*c*, 7·1 in water) (Found : OMe, 41·0%). The syrup (0·6 g.) on refluxing with alcoholic aniline gave a mixture of anilides from which 2 : 3 : 4-trimethyl *d*-galactose anilide (0·4 g.), m. p. 169°, and 2 : 4 : 6-trimethyl *d*-galactose anilide (0·1 g.), m. p. 179°, were isolated. The sugar (0·45 g.) on oxidation with bromine water gave a syrupy mixture of acid and lactone, $n_D^{18°}$ 1·4700, $[\alpha]_D^{20°}$ + 89° (*c*, 1·8 in water, initial value) falling to + 26° (constant value, 24 hours) (Found : Equiv., 248). A portion of the lactone on being heated with alcoholic phenylhydrazine gave the phenylhydrazide of 2 : 3 : 4-trimethyl *d*-galactonic acid, m. p. and mixed m. p. 175°, after recrystallisation from alcohol. Fraction (vi) (0·72 g.) was hydrolysed with boiling N-hydrochloric acid (25 c.c.); $[\alpha]_D^{20°}$ + 92° ⟶ + 84° (constant value, 7 hours). The sugar (0·66 g.) was isolated. No crystalline anilide could be obtained on refluxing a portion of the sugar with alcoholic aniline. On oxidation with bromine water the sugar gave a mixture of lactone and acid, $[\alpha]_D^{20°}$ + 45° (*c*, 1·04 in water, initial value) falling to + 25° (constant value, 24 hours) (Found : Equiv., 209). A portion of the lactone (300 mg.) on being heated with alcoholic phenylhydrazine gave the phenylhydrazide of 2 : 4-dimethyl *d*-galactonic acid (90 mg.), m. p. and mixed m. p. 183°. Fraction (vii) (0·36 g.) was hydrolysed with boiling N-hydrochloric acid (25 c.c.); $[\alpha]_D^{20°}$ + 87° (7 hours, constant value). The sugar (0·26 g.) on being boiled with alcoholic aniline gave the anilide of 2 : 4-dimethyl *d*-galactose (0·10 g.), m. p. 206°, not depressed on admixture with an authentic specimen.

Examination of the Uronic Acid Fraction.—A portion (2·38 g.) of the syrup (E) isolated from the barium salts (B) was distilled, giving Fraction (x) (0·64 g.), b. p. 130°/0·01 mm., $n_D^{15°}$ 1·4538, $[\alpha]_D^{20°}$ + 55° (*c*, 0·4 in water) (Found : Equiv., 244; OMe, 50·1%). Fraction (xi) (0·43 g.), b. p. 165°/0·01 mm., $n_D^{15°}$ 1·4788 (Found : OMe, 39·0%. The *methyl* ester of 6-[2·3 dimethyl-*d*-glucuronosido] 2 : 4-dimethyl-*d*-galactoside requires OMe, 40·2%). Fraction (xii) (0·80 g.), $n_D^{20°}$ 1·4808 (Found : OMe, 41·0%). The residue (0·36 g.) was not further examined. Fraction (x) (0·54 g.) was hydrolysed with boiling N-hydrochloric acid (25 c.c.); $[\alpha]_D^{20°}$ + 35° (initial value) ⟶ + 50° (constant value, 4 hours). The sugar was oxidised with bromine water after isolation. The resulting 2 : 3-dimethyl *d*-saccharic acid was esterified with boiling methyl-alcoholic hydrogen chloride, the solution filtered before and after neutralisation with silver carbonate, and the isolated product distilled in a vacuum giving the methyl ester of 2 : 3-dimethyl 1 : 4-*d*-saccharolactone (0·3 g.), m. p. 101°, not depressed on admixture with an authentic specimen. Fraction (xi) appeared to consist mainly of a disaccharide; accordingly the syrup (0·32 g.) was hydrolysed with boiling N-hydrochloric acid (25 c.c.) for 7 hours; $[\alpha]_D^{20°}$ + 52° (initial value) ⟶ + 44° (constant value, 7 hours). The solution was neutralised with silver carbonate and filtered, silver ions were removed with hydrogen sulphide, and the filtered solution was concentrated under reduced pressure at 40°. The concentrated solution was neutralised by titration with barium hydroxide and the neutral solution evaporated to dryness. Exhaustive extraction with acetone gave an acetone-insoluble fraction, (xi*a*) (0·2 g.), mainly the barium salt of 2 : 3-dimethyl *d*-glucuronic acid, an acetone-soluble (xi*b*) (0·1 g.), mainly 2 : 4-dimethyl *d*-galactose since this fraction on being boiled with alcoholic aniline gave 2 : 4-dimethyl *d*-galactose anilide, m. p. and mixed m. p. 208°. Fraction (xi*a*) was dissolved in water and oxidised with bromine water. The isolated product was esterified with methyl-alcoholic hydrogen chloride and the product (0·16 g.) distilled at 130°/0·01 mm. The distillate crystallised and had m. p. 100° after recrystallisation from ether, not depressed on admixture with an authentic specimen of methyl 2 : 3-dimethyl *d*-saccharolactone ester. Fraction (xii) had the constants of a disaccharide. Attempts to convert it into a crystalline *p*-nitrobenzoyl derivative were unsuccessful. Accordingly, the material (0·40 g.) was hydrolysed with N-hydrochloric acid (25 c.c.) for 7 hours($[\alpha]_D$ not observable owing to the colour of the solution). The cooled solution was then neutralised with silver carbonate and filtered before and after the passage of hydrogen sulphide. The filtrate was concentrated (50 c.c.) and neutralised with barium carbonate, filtered, the filtrate evaporated to dryness in a vacuum, and the residue exhaustively extracted with acetone (J). The residual barium salts were oxidised with aqueous bromine and the resulting 2 : 3-dimethyl *d*-saccharic acid converted into the crystalline methyl 2 : 3-dimethyl *d*-saccharolactone ester (0·09 g., m. p. 101°) by the method described above. The acetone solution (J) on concentration gave a syrup (0·1 g.) from which no crystalline derivative could be isolated.

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