## 144. *Some Observations on the Constitution of Gum Myrrh.*

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The isolation and characterisation of D-galactose, L-arabinose, and 4-methyl D-glucuronic acid as components of gum myrrh are described.

Gum myrrh, like frankincense, gum asafœtida, and gum gamboge, is a typical oleo-gum-resin, and is composed mainly of carbohydrate, protein, and terpenoid resins (Hirst and Jones, *Research*, 1951, **4**, 411). The substance has been used as incense for many centuries. In more recent times, the gum has found medical usage as an antiseptic, the tincture being applied to inflammatory and ulcerated conditions of the throat and mouth (cf. Trease, "A Textbook of Pharmacognosy," 5th Edn., Bailliere, Tindall, and Cox, p. 356). The gum-resin is exuded from cracks and fissures in the bark of *Commiphora myrrha* Holmes (fam. Burseraceae) found in N.E. Africa and Arabia, forming nodules composed of a red oily resin and white streaks of gum. On extraction with 90% aqueous alcohol, the resins are largely removed and the crude polysaccharide thus obtained (*ca.* 40% yield) may be further purified by precipitation from acidified alcohol. A crude acidic polysaccharide prepared in this manner had an equivalent weight of 547, and contained 6·1% of methoxyl. The product gave positive tests for amino-acids and from the nitrogen content was estimated

to contain approximately 18% of protein. After its hydrolysis with acid, at least fifteen amino-acids were detected on the paper chromatogram.

Addition of copper sulphate to an aqueous solution of the alcohol-insoluble material precipitated most of the polysaccharide as an insoluble copper complex. The remainder of the polysaccharide was then precipitated from solution by the addition of alcohol. The two fractions, after removal of the copper, were obtained as cream-coloured, amorphous materials, designated the copper-insoluble polysaccharide *A* (yield, 70%) and the soluble polysaccharide *B* (yield, 19%). An examination of their hydrolysis products on the paper chromatogram indicated that each of these fractions contained galactose, arabinose, and a monomethyl uronic acid. After removal of the uronic acid component, quantitative determination by the periodate oxidation procedure of Hirst and Jones (*J.*, 1949, 1659) showed the presence of approximately 5% of arabinose and 20% of galactose (calculated as $C_5H_8O_4$ and $C_6H_{10}O_5$ respectively) in each fraction. The fractions contained protein, approximately 15% in *A* and 20% in *B*, as estimated from their nitrogen contents, and possibly adherent resin. Determination of the equivalent weights by alkaline titration gave a figure of 507 for the copper-insoluble polysaccharide *A* and 678 for the soluble polysaccharide *B*; calculation from the methoxyl contents, on the assumption that the acidic component was wholly monomethyl hexuronic acid, gave equivalent weights of 492 and 705 respectively, which are in fair agreement with those obtained by titration. Since these results show that there may be some difference in the carbohydrate moiety of these fractions, it is clear that further investigation of the homogeneity of gum is essential before the inception of structural studies.

In order to separate and hence finally to identify the two neutral sugars and the partly methylated uronic acid, a large-scale hydrolysis of the acidic polysaccharide, followed by a separation of the liberated neutral sugars from the uronic acid component, was performed, the acid being absorbed on the anion-exchange resin, Amberlite IR-4B. Crystalline specimens of D-galactose and L-arabinose were obtained by counter-current partition of the mixture of neutral reducing sugars between *n*-butanol and water in a cellulose-packed column (Hough, Jones, and Wadman, *J.*, 1949, 2511).

The partly methylated uronic acid was recovered from the ion-exchange resin by displacement with mineral acid. Examination of this fraction on the sheet-paper chromatogram indicated the presence of a substance which gave a characteristic brilliant-red colour on treatment with the *p*-anisidine spray reagent and moved at the rate of a monomethyl uronic acid.

Since the known methylated derivatives of the uronic acids are few, whereas in comparison the methylated derivatives of the corresponding neutral aldose derivatives are well characterised, the uronic acid was converted into its methyl ester methylglycuronoside, the carbomethoxy-group of which was then reduced with lithium aluminium hydride (Lythgoe and Trippett, *J.*, 1950, 1983) to the corresponding primary alcohol. The resultant neutral monomethyl methylglycoside was then converted by hydrolysis into the monomethyl hexose. The latter was a glucose derivative since demethylation with 48% hydrobromic acid furnished this sugar, identified by its rate of movement and colour reactions on the paper chromatogram. This was confirmed by methylation of the methylglycoside, followed by hydrolysis of the product, which sequence of operations afforded crystalline 2 : 3 : 4 : 6-tetramethyl D-glucose. It is clear then that the methoxy-group of the monomethyl glucose must be situated on $C_{(2)}$, $C_{(3)}$, or $C_{(4)}$, and an examination of the sugar on the paper chromatogram showed its rate of movement and colour reaction to be indistinguishable from those of 4-methyl D-glucose but different from those of 2- and 3-methyl D-glucose. Confirmation was provided by the formation of 4-methyl D-glucosazone, identical with an authentic specimen kindly supplied by Professor F. Smith of the University of Minnesota. It follows, therefore, that the original uronic acid present in gum myrrh must be 4-methyl D-glucuronic acid (CHO = 1).

A consideration of the above results for the crude acidic polysaccharide isolated from gum myrrh suggests that it contains approximately 64% of carbohydrate, and 18% of protein, the remainder (18%) being unidentified. The carbohydrate moiety contains the following monosaccharides in approximately the proportions indicated: D-galactose

(4 parts), L-arabinose (1 part), and 4-methyl D-glucuronic acid (3 parts). The occurrence of D-galactose and L-arabinose in gum myrrh is not unusual since these sugars are often encountered in close association in a wide variety of plant materials. On the other hand, the occurrence of 4-methyl D-glucuronic acid in a complex polysaccharide is of great interest, since the acidic component of these materials is usually found to be galacturonic, glucuronic, or mannuronic acid (Jones and Smith, *Adv. Carbohydrate Chem.*, 1949, **4**, 243) and hitherto in one instance only has a methylated uronic acid been obtained from a natural product—mesquite gum, from which White (*J. Amer. Chem. Soc.*, 1948, **70**, 367 and Smith (*J.*, 1951, 2646) isolated the same component, 4-methyl D-glucuronic acid. Monomethyl uronic acids are reported to occur in the hemicellulose of jute fibre (Sarker, *Nature*, 1951, **167**, 357) and of American White Oak (O'Dwyer, *Biochem. J.*, 1923, **17**, 503) but as yet remain unidentified. It may well be that methylated sugars and uronic acids are of more common occurrence in natural products than is usually supposed. Recent work (unpublished) has indicated that several gums contain partly methylated monosaccharides, *e.g.*, grapefruit gum, lemon gum, Chile plum gum, and golden apple gum. The methylated neutral sugars hitherto encountered in Nature are of the 3-methyl type (cf. Hirst, Hough, and Jones, *J.*, 1951, 323).

## Experimental

*Isolation of the Polysaccharide.*—The natural gum resin (430 g.) was extracted with 90% aqueous alcohol for removal of resins, and the residual crude polysaccharide, after dissolution in 20% alcohol, was slightly acidified with dilute hydrochloric acid and filtered into a large volume of alcohol. The insoluble acidic polysaccharide thus obtained was repeatedly reprecipitated in this manner from 20% aqueous alcohol until chloride-free, and the resultant product was dried at 60° under reduced pressure to give a light brown, friable powder (161 g.), $[\alpha]_D$ + 7° (*c*, 3·6 in water; neutral sodium salt) [Found: Sulphated ash, 1·6; N, 2·9; S, trace; OMe, 6·1%; equiv. (by alkaline titration), 547].

*Fractionation by Formation of a Copper Complex.*—The acidic polysaccharide (3·6 g.) was suspended in water (250 c.c.), and ammonia (*ca.* 2N) added with vigorous shaking until the solution was neutral; a little undissolved material was then removed on the centrifuge. A solution of copper sulphate (10%; 100 c.c.) was added to the supernatant liquid, and the resultant insoluble copper complex was removed on the filter. The filtrate was evaporated to a small volume (*ca.* 25 c.c.), whereafter the addition of methanol (250 c.c.) resulted in the precipitation of the water-soluble material, which was then isolated on the filter. The copper-insoluble and the soluble fraction were freed from copper by repeated washings on the filter with cold methanolic hydrogen chloride (5%) until the filtrate was copper-free. The mineral acid was removed from the polysaccharide by repeated washing with methanol until the washings were chloride-free. The products were dried at 60° under reduced pressure. The copper-insoluble fraction *A* (2·5 g.) had $[\alpha]_D$ +18° (*c*, 0·77 in water) [Found: OMe, 6·3; N, 2·5; sulphated ash, 2·1%; equiv. (by titration with standard alkali), 507]. The yield of soluble fraction *B* (was 0·7 g.) [Found: OMe, 4·4; N, 4·2; sulphated ash, 2·2%; equiv. (by titration with standard alkali), 678]. Each fraction gave positive tests for protein.

A qualitative examination of the hydrolysis products of these two fractions on the paper chromatogram (for methods see Hough, Jones, and Wadman, *J.*, 1950, 1702) suggested the presence of galactose, arabinose, and a monomethyl uronic acid. A quantitative estimate of the non-acidic reducing sugars was obtained in the following manner. The copper-insoluble polysaccharide *A* (186 mg.) was hydrolysed in a sealed tube with N-sulphuric acid (5 c.c.) for 16 hours at 100°. The reaction mixture was then washed into a flask containing rhamnose hydrate (67 mg.). Amberlite resin IR-4B was then added portion-wise, with shaking, until the solution was neutral, whereupon the resin was removed by filtration and the filtrate evaporated to a small volume (*ca.* 1 c.c.). A portion of the sugars was separated on the chromatogram and the position of the sugars located as described for quantitative analysis by Hirst and Jones (*loc. cit.*). The paper strips holding the galactose, arabinose, and rhamnose were then extracted with water and the quantities of sugars determined by periodate oxidation (*idem, ibid.*) (Found: 5·09, 1·09, and 8·37 mg. of these sugars respectively). From these figures, the recovery of rhamnose being assumed to be quantitative, the copper-insoluble fraction contains 21·9% of galactose and 4·7% of arabinose (calc. as $C_6H_{10}O_5$ and $C_5H_8O_4$ respectively). In a similar manner, the soluble fraction *B* (94·5 mg.) was hydrolysed, rhamnose hydrate (45·2 mg.) added, and the mixture of sugars analysed (Found: galactose, 1·46 mg.; arabinose, 0·41 mg.; rhamnose, 3·73 mg.).

Thus this fraction contains 19·6% of galactose and 5·2% of arabinose (calc. as $C_6H_{10}O_5$ and $C_5H_8O_4$ respectively).

*Large-scale Hydrolysis of the Polysaccharide.*—The purified polysaccharide (40 g.) was dissolved in 0·5N-sulphuric acid (1·6 l.) and heated under reflux on a boiling water-bath, the hydrolysis being followed polarimetrically: $[\alpha]_D$ +7° (initial value), +42° (3·5 hours), +54° (6 hours), +56° (8 hours), +65° (11 hours). After 18 hours, the dark-brown solution was cooled and Amberlite Resin IR-4B added portion-wise, with stirring, until the solution was neutral. This mixture of resin and solution was slowly introduced into a column (2 × 24 in.) containing at the base a small bed (2 in.) of fresh Amberlite Resin IR-4B to remove any residual acidity. The solution was allowed to percolate down the column, followed by water until the effluent was non-reducing. The effluents were concentrated, treated with charcoal, filtered, and evaporated to a syrup *C* (31 g.).

The uronic acids were recovered by stirring the resin with dilute sulphuric acid portion-wise until a permanent excess of sulphuric acid was present. The resin was then washed with water until the washings were both non-reducing and sulphate-free. To the filtrate barium acetate solution was added with stirring from a burette until the solution was just sulphate-free, the insoluble barium sulphate was removed on the filter, and the filtrate concentrated under reduced pressure to a syrup *D* (15 g.).

*Examination of the non-acidic reducing sugars* (C). A preliminary examination of the syrup on the paper chromatogram using a variety of solvents indicated the presence of only two sugars, which were provisionally identified by their rates of movement and colour reactions as galactose and arabinose; uronic acids were not detected. Confirmation was provided by the separation of a portion of this syrup (1·27 g.) by partition chromatography on a column of cellulose powder with butanol–water as the mobile phase (Hough, Jones, and Wadman, *loc. cit.*). A complete separation was obtained; the first fraction gave crystalline L-arabinose (0·63 g.), m. p. and mixed m. p. 158°, $[\alpha]_D$ +96° (*c*, 0·48 in water; equilibrium value); the other fraction gave crystalline D-galactose (0·56 g.), m. p. 161°, $[\alpha]_D$ +82° (*c*, 1·4 in water; equilibrium value), which yielded the characteristic *N*-methyl-*N*-phenylhydrazone (m. p. 185°).

*Examination of the acidic sugars* (D). The syrup, $[\alpha]_D$ 45° (*c*, 5·8 in water) (Found: OMe, 13·9. Calc. for monomethyl hexuronic acid, OMe, 14·2%), was examined on the paper chromatogram; *n*-butanol saturated with water, to which formic acid had been added to a concentration of 5%, was used as the mobile phase; after separation and evaporation of the solvents the chromatogram was sprayed with a solution of *p*-anisidine hydrochloride in *n*-butanol (*idem, ibid.*) and heated. This reagent gave a bright red spot ($R_F$ 0·28) corresponding to a monomethyl hexuronic acid, and showed faint spots corresponding to galactose and arabinose; no unsubstituted hexuronic acid could be detected.

*Methyl Ester Methyl Hexuronoside.*—The syrupy monomethyl hexuronic acid was converted into the methyl ester methyl hexuronoside by heating the syrup (6 g.) in 4% methanolic hydrogen chloride at 100° for 8 hours. After cooling, the solution was neutralised by the addition of silver carbonate, the insoluble silver salts were removed on the filter, and the filtrate was concentrated under reduced pressure to a syrup (6·85 g.) (Found: OMe, 38·7. Calc. for the methyl ester of a monomethyl methyl hexuronoside: OMe, 39·4%).

*Reduction with lithium aluminium hydride.* The methyl glycuronoside methyl ester (1 g.) in tetrahydrofuran was reduced with lithium aluminium hydride, under the conditions specified by Lythgoe and Trippett (*loc. cit.*). The resultant complex was decomposed by pouring the mixture into ice-cold dilute sulphuric acid, after which the solution was neutralised by the addition of barium carbonate, filtered, and evaporated under reduced pressure; the solid residue was then extracted exhaustively with hot methanol. Evaporation of the methanol extract afforded syrupy monomethyl methylhexoside (0·62 g.) (Found: OMe, 28·5. Calc. for $C_8H_{16}O_6$: OMe, 29·8%).

*Methylation of the monomethyl methylhexoside.* A portion (99 mg.) of the foregoing syrup was repeatedly methylated with Purdie's reagents, and after five treatments yielded tetramethyl methylhexoside (104 mg.), $n_D^{18}$ 1·4470, which on hydrolysis yielded 2 : 3 : 4 : 6-tetramethyl D-glucose, m. p. and mixed m. p. 83°, $[\alpha]_D$ +83° (c, 1·2 in water) (61 mg.). The *X*-ray powder photograph of the crystals was identical with that of an authentic specimen.

*Hydrolysis of the monomethyl methylhexoside.* Hydrolysis of a portion (0·51 g.) in 0·5N-sulphuric acid (25 c.c.) at 100° for 3 hours gave, after neutralisation with barium carbonate, a syrupy monomethyl hexose (0·44 g.), $[\alpha]_D$ +63° (*c*, 2·0 in water) (Found: OMe, 17·5. Calc. for a monomethyl hexose: OMe, 16·0%).

*Demethylation of the monomethyl hexose.* A portion (*ca.* 5 mg.) of this sugar was demethylated

by heating a solution in hydrobromic acid (48% w/v; 0·5 c.c.) in a sealed tube at 100° for 6 minutes, and the products were isolated in the usual manner (Hough, Jones, and Wadman, *loc. cit.*). Examination of this material on the paper chromatogram showed it to contain the original monomethyl hexose and a sugar indistinguishable from glucose.

Examination of the monomethyl sugar on paper chromatograms, using a variety of solvents, showed one discrete spot in all cases corresponding exactly in position and colour reactions with those shown by authentic 4-methyl glucose and clearly distinguishable from 2-, 3-, and 6-methyl glucose. Thus, for example, in butanol–pyridine–water (3 : 1 : 1 v/v) 2-, 3-, 4-, and 6-monomethyl glucoses had $R_F$ values of 0·235, 0·260, 0·285, and 0·275 respectively (obtained by comparison with rhamnose, the $R_F$ of which was assumed to be 0·300).

*Formation of* 4-*methyl* D-*glucosazone.* A portion (54 mg.) of the monomethyl hexose was dissolved in water (2 c.c.) containing phenylhydrazine (0·2 c.c.) and glacial acetic acid (0·2 c.c.). The mixture was heated at 60—70° for 3 hours. The osazone was isolated, washed with a little benzene and recrystallised from ethyl alcohol–water and then from benzene; it (51 mg.) had m. p. 157° and $[\alpha]_D$ +27° (initial value)→ +3° (20 hours; constant value) and was identical in crystal form with a genuine specimen of 4-methyl D-glucosazone, m. p. 158—159°, $[\alpha]_D$ +32° → 0°, and the *X*-ray powder photographs were indistinguishable (Found: OMe, 8·2. Calc. for monomethyl hexosazone, OMe, 8·3%).

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