*The Constitution of Gum Myrrh. Part II.**

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Purified gum myrrh has an equivalent weight of *ca.* 460 and contains D-galactose residues (8), L-arabinose residues (2), and 4-*O*-methyl-D-glucuronic acid residues (7 per repeating unit). On hydrolysis a mixture of reducing sugars and acidic oligosaccharides is produced. Fractionation of the oligosaccharides has yielded two aldobiuronic acids identified as 4-*O*-(4-*O*-methyl-α-D-glucuronosyl)- and 6-*O*-(4-*O*-methyl-β-D-glucuronosyl)-D-galactose in the approximate ratio of 6 : 1. The significance of these results is discussed.

In Part I * the identification of the component sugars of gum myrrh was detailed. The purpose of this communication is to describe attempts to isolate a protein-free polysaccharide and to determine the composition of the acidic disaccharides which are produced when the gum is hydrolysed with dilute acids.

* Part I, Hough, Jones, and Wadman, *J.*, 1952, 796.

Hough, Jones, and Wadman (*loc. cit.*) isolated from gum myrrh a polysaccharide containing approximately 18% of protein. Addition of copper sulphate to an aqueous solution of the alcohol-insoluble gum gave an alcohol-insoluble copper salt which contained protein. We have removed the majority of the protein from the crude polysaccharide by the usual procedures (see Experimental section) and then fractionated it through its copper salt. The results indicate that the fractionation described in the earlier work was probably a separation into carbohydrate-rich and a carbohydrate-poor component and that no real separation of the gum into different polysaccharides had occurred. It was not found possible to eliminate the last traces of protein material and it maybe that it was combined with the polysaccharide.

Hydrolysis of the purified polysaccharide with N-sulphuric acid gave high yields of a mixture of neutral sugars and acidic oligosaccharides. The latter were isolated as their barium salts, which were converted into the ash-free acids and then fractionated on cellulose columns. Two aldobiuronic acids were isolated in the approximate ratio of 1 : 6 and were identified as 6-*O*-(4-*O*-methyl-β-D-glucuronosyl)- and 4-*O*-(4-*O*-methyl-α-D-glucuronosyl)-D-galactose by the standard procedures.

The isolation of these two aldobiuronic acids shows that the polysaccharide component of gum myrrh resembles lemon gum (Connell, Hainsworth, Hirst, and Jones, *J.*, 1950, 1696) and mesquite gum (White, *J. Amer. Chem. Soc.*, 1948, **70**, 367; Smith, *J.*, 1951, 2646) in the main features of its composition. It is becoming increasingly evident that aldobiuronic acids composed of D-glucuronic acid joined to $C_{(4)}$ of a D-galactose residue *via* an α-glycosidic linkage and D-glucuronic acid united through $C_{(6)}$ of a D-galactose residue *via* a β-glycosidic linkage are common components of gums (cf. Charlson, Nunn, and Stephen, *J.*, 1955, 1428). It will be of interest to determine why these two structures are so favoured.

Experimental

Unless otherwise stated, concentration of solutions was carried out at 40°/20 mm., and specific rotations were measured in H_2O. Paper chromatograms were run in (*a*) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4) (Jones, *J.*, 1953, 1672) or (*b*) butanol–pyridine–water (9 : 2 : 2).

Isolation and Purification of the Polysaccharide.—The gum myrrh was collected by Major P. E. Glover in Central N.E. Africa in January, 1947, and given to us by Sir John L. Simonsen, F.R.S., of the Imperial Institute, London. The gum-resin (550 g.) in the form of yellow or dull red nodules, containing much debris was roughly crushed, and then thoroughly extracted with several changes of hot ethanol, which removed the bulk of the resin. The residue was filtered off, washed with hot ethanol, finely ground, and again extracted with hot ethanol. The residue from this extraction was dissolved in hot water, cooled, and poured into ethanol acidified with hydrochloric acid. The precipitate was filtered off and washed with acetone to give a pale brown powder (189 g.) (Found : sulphated ash, 2·3; N, 3·0%).

To this crude gum acid (18 g.) in water (500 c.c.), which was neutralised with sodium hydroxide, was added a slurry of cadmium hydroxide (cf. Laidlaw and Reid, *J. Sci. Food Agric.*, 1952, **3**, 19) [from cadmium sulphate (10·8 g.) and N-sodium hydroxide (86·5 g.)] at about 60° with very rapid stirring. The precipitate was centrifuged off and from the supernatant liquid more protein was removed by the formation of a water-insoluble chloroform–protein gel (cf. Sevag, Lackman, and Smolens, *J. Biol. Chem.*, 1938, **124**, 425). The method consisted of adding 0·25 vol. of chloroform and 0·1 vol. of butanol (foam prevention) to the carbohydrate–protein solution. The mixture was shaken for 60 min. and then centrifuged; two layers were formed, the lower consisting of a fairly stable chloroform–protein gel. The upper clear, aqueous layer was decanted and the process repeated. After ten such operations a negligible amount of emulsion was obtained, indicating that no more protein was being removed. The clear aqueous solution from the last treatment was passed in turn through columns of IR-120 and IRA-400 resin, and the effluent (pH 2) was poured into ethanol. The precipitate was collected and washed with alcohol and acetone in a centrifuge, to give a white powder (8 g.), $[\alpha]_D^{18}$ +32° ± 1° (*c*, 1·2) [Found, on material dried at 60°/0·2 mm. : equiv. (by titration), 500; corr. for ash, protein, and moisture, 460; sulphated ash, 0·4; N, 1·2; OMe, 6·1%]. Chromatography of the hydrolysate of this material revealed the presence of galactose, arabinose, and 4-*O*-methyl-glucuronic acid.

Quantitative estimation of the non-acidic reducing sugars in this material (cf. Hough, Jones, and Wadman, *loc. cit.*) indicated that galactose and arabinose were present in the approximate molar ratio of 4 : 1.

Attempted Fractionation of the Polysaccharide.—(a) *Action of copper sulphate in neutral solution.* Gum acid (1 g.) (N, 1·2%) was dissolved in water and adjusted to pH 7·0 with aqueous sodium hydroxide. 10% Copper sulphate solution (50 c.c.) was added with rapid stirring, and the precipitate centrifuged. The pale green complex was dissolved in 5% hydrochloric acid (5 c.c.) and poured into methanol. The precipitate was redissolved in water and neutralised with sodium hydroxide. Copper sulphate was added to precipitate the complex, and the pH adjusted to 7·0 with aqueous sodium hydroxide. The polysaccharide was recovered from the copper complex as described above, and washed with methanolic hydrogen chloride, ethanol, and acetone, to give a white powder (fraction A) (0·7 g.). The solution from the first precipitation was acidified and poured into methanol; the resulting polysaccharide was washed (fraction B) (0·1 g.).

Both these fractions were purified by dissolution in water and treatment with IR-120 and IRA-400 resins in turn. They were then precipitated (methanol), washed, and dried (60°/0·1 mm.) as before, and had: fraction A, $[\alpha]_D^{20}$ +31° ± 1°, equiv. 507; fraction B, $[\alpha]_D^{20}$ +17° ± 2°, equiv. 590.

(b) *Action of copper acetate in neutral solution.* Gum acid (1 g.) (N, 1·2%) in water (50 c.c.) was neutralised with aqueous sodium hydroxide. 5% Copper acetate solution was added dropwise with stirring. A precipitate appeared immediately which redissolved, and it required 4 c.c. of copper acetate solution before a permanent precipitate appeared, and a further 6 c.c. for complete precipitation. The precipitate was centrifuged, dissolved in 5% hydrochloric acid, and precipitated in ethanol. The resulting polysaccharide was taken up in water, neutralised with sodium hydroxide to pH 7—8, and again treated with copper acetate solution (5 c.c.). The precipitate was centrifuged and washed, dissolved in 5% hydrochloric acid, and passed through a column of IR-120 resin. The eluate was poured into ethanol, and the precipitated polysaccharide centrifuged, and washed with ethanol and acetone (0·76 g.) (Found: equiv., 460).

The solution from the first precipitation gave a faint positive Molisch test, but when it was poured into methanol only a very small precipitate was recovered.

(c) *Attempted fractionation by alcohol precipitation.* The gum acid (1·0 g.) (N, 1·2%) was dissolved in water (200 c.c.), and alcohol added in increasing quantities, the precipitates being removed by centrifuging. Up to 75% v/v ethanol there was no precipitate. At 80% 0·518 g. (equiv., 515) was precipitated; at 85%, 0·262 g. (equiv., 520); and at 90% a further trace. The material lost (*ca.* 22%) probably remained in solution.

Hydrolysis of the Polysaccharide.—When the crude gum acid was heated in N-sulphuric acid on a water-bath, aldobiuronic acid, R_{Ga} 0·67 (R_{Ga} is relative to galactose) (solvent *a*), appeared after 2 hr. This spot reached maximum intensity at about 12 hr. For the preparation of aldobiuronic acid, the crude gum acid (100 g.), containing about 18% of protein (N × 6·25), in N-sulphuric acid (1 l.) was heated on a water-bath for about 11 hr. The neutralised (barium carbonate) solution was concentrated to *ca.* 100 c.c. and poured into ethanol. The precipitated barium salts were redissolved and reprecipitated. These salts were dissolved in water and, after removal of barium (IR-120 resin), the solution was poured into ethanol. The precipitated material of high molecular weight was removed (centrifuge) and the filtrate evaporated to give a pale yellow amorphous solid (34 g.). Chromatography of this revealed the presence of traces of 4-*O*-methylglucuronic acid, arabinose, and galactose and a high concentration of aldobiuronic acid, together with higher oligosaccharides. On extended irrigation (72 hr.) (solvent *a*) the aldobiuronic acid spot was resolved into two spots, R_{Ga} 0·69 (aldobiuronic acid No. 1) and R_{Ga} 0·59 (aldobiuronic acid No. 2). This mixture (34 g.) was separated on a cellulose column (450 × 55 mm.) (Hough, Jones, and Wadman, *J.*, 1949, 2511) with butanol–formic acid–water (45 : 1 : 4) to give acid I (11·4 g.), a mixture of the acids (1·2 g.), and acid II (2·0 g.) (all weights as barium salt). A specimen of acid II showed signs of crystallisation after 9 months and had formed large colourless needles after 12 months. Attempted recrystallisation failed, because of its high solubility.

Aldobiuronic Acid I.—The barium salt, after purification by precipitation of an aqueous solution in ethanol, was dried at 60°/0·01 mm. and had $[\alpha]_D^{16}$ +87° ± 4° (*c*, 0·98) (Found: Ba, 15·75; OMe, 6·6, 6·7. Calc. for $C_{26}H_{42}O_{24}Ba$: Ba, 15·7; 2OMe, 7·1%). It (2·7 g.) was methylated with methyl sulphate (15 c.c.) and 40% aqueous sodium hydroxide (45 c.c.). This was followed by a second methylation using methyl sulphate (20 c.c.) and sodium hydroxide (25 g.). Acidification with sulphuric acid and extraction with chloroform yielded a pale yellow

syrup, which was then treated with Purdie's reagents, giving a viscous syrup (2·1 g.). It was distilled (b. p. 120—125° (bath)/0·1 mm., $[\alpha]_D^{24}$ +98° ± 7° (*c*, 0·83 in MeOH) (Found : C, 51·3; H, 7·9; OMe, 50·9. Calc. for $C_{20}H_{36}O_{12}$: C, 51·3; H, 7·7; OMe, 53·0%). This material was hydrolysed by 2N-sulphuric acid at 100° in a sealed tube for 25 hr. The neutralised hydrolysate ($BaCO_3$) was extracted with chloroform in a continuous extractor, to yield a pale yellow syrup, $[\alpha]_D^{18}$ +93° ± 3° (*c*, 0·75); $[\alpha]_D$ +15° ⟶ −22° in 15 hr. (*c*, 1·5 in 5% methanolic hydrogen chloride), R_{MG} 0·87 (solvent *b*), 0·91 (solvent *a*) (R_{MG} relative to tetra-*O*-methylgalactose). The syrup was oxidised with bromine water (4 days) and, after removal of bromine by aeration, the solution was neutralised with silver carbonate and filtered, and silver was precipitated with hydrogen sulphide. The filtrate was evaporated to dryness in a vacuum, and the residue recrystallised from dry ether as large colourless needles, m. p. and mixed m. p. 98—99° with 2 : 3 : 6-tri-*O*-methyl-D-galactofuranolactone. *X*-Ray powder diagrams of this and the authentic specimen were identical.

Isolation of 4-O-*Methyl*-D-*glucuronic Acid.*—Barium aldobiuronate I (1 g.) was hydrolysed with 2N-sulphuric acid in a sealed tube at 100° for 12 hr. The neutralised ($BaCO_3$) and filtered solution was poured into ethanol. The precipitated barium salt was centrifuged out, dissolved in water, and then shaken with IR-120 resin until all the barium had been removed. The resin was filtered off, and the solution evaporated, leaving a syrup which was fractionated on a cellulose column with butanol–formic acid–water (45 : 1 : 4) as solvent. Evaporation of the fractions containing uronic acid gave a syrup (0·23 g.), which was refluxed with 1% methanolic hydrogen chloride (10 c.c.) for 6 hr. and then neutralised with silver carbonate. Filtration and evaporation left a syrup which was treated with concentrated ammonia solution (5 c.c.) and left at 0° overnight. Evaporation of this afforded a syrup which rapidly crystallised. Four recrystallisations from absolute ethanol gave large, colourless plates of the amide of methyl 4-*O*-methyl-α-D-glucuronoside, m. p. and mixed m. p. 232°, $[\alpha]_D^{22}$ +143° (*c*, 0·67) (Found : OMe, 28·0. Calc. for $C_8H_{15}O_6N$: OMe, 28·05%).

Aldobiuronic Acid II.—The barium salt, after purification as described for acid I, had $[\alpha]_D^{16}$ +6° ± 4° (*c*, 0·9) (Found : Ba, 15·2; OMe, 6·9, 7·1. Calc. for $C_{26}H_{42}O_{24}Ba$: Ba, 15·7; 2OMe, 7·1%). This salt (0·90 g.), on methylation as above, yielded a pale yellow syrup (0·67 g.), which, on trituration with light petroleum (b. p. 40—50°) and storage at −10°, crystallised in colourless nodules. Recrystallisation from light petroleum gave needles, $[\alpha]_D^{25}$ −44° ± 10° (*c*, 0·6 in $CHCl_3$), m. p. and mixed m. p. 86—88° with the methyl ester of methyl 6-(hexa-*O*-methyl-β-D-glucuronosyl)-β-D-galactoside (Charlson, Nunn, and Stephen, *J.*, 1955, 269). The amide of methyl 4-*O*-methyl-D-glucuronoside was obtained from aldobiuronic acid II in the same manner as for acid I.

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