318. *Gum Tragacanth. Part I. Fractionation of the Gum and the Structure of Tragacanthic Acid.*

By G. O. Aspinall and J. Baillie.

Fractionation of the water-soluble part of gum tragacanth affords (i) tragacanthic acid, which contains residues of D-galacturonic acid (43%), D-xylose (40%), L-fucose (10%), and D-galactose (4%), and (ii) an arabinogalactan, which contains residues of L-arabinose (75%), D-galactose (12%), D-galacturonic acid (3%), and L-rhamnose (traces). The homogeneity of these polysaccharide preparations has been assessed by chromatography on diethylaminoethylcellulose. Controlled stepwise degradation of tragacanthic acid with acid and enzymes leads to the isolation of various oligosaccharides, including 2-*O*-α-L-fucopyranosyl-D-xylose, 2-*O*-β-D-galactopyranosyl-D-xylose, the pseudo-aldobiouronic acid, 3-*O*-β-D-xylopyranosyl-D-galacturonic acid, and oligomers of D-galacturonic acid. The main structural features of tragacanthic acid are discussed in the light of these results and of a re-examination of the cleavage products from the methylated polysaccharide.

Previous structural studies [1,2] on gum tragacanth, the exudate from various species of *Astralgus*, have shown that the gum is grossly heterogeneous and that it contains at least

[1] James and Smith, *J.*, 1945, 739.
[2] James and Smith, *J.*, 1945, 749.

two polysaccharide components, tragacanthic acid, which is composed of residues of D-galacturonic acid, D-xylose, and L-fucose,[1] and an arabinogalactan.[2] Because of difficulties in handling the gum in aqueous solution, in which only a part of it is soluble, James and Smith [1,2] carried out their structural studies on the methylated gum. They found that the methylated gum could be readily fractionated to give derivatives of tragacanthic acid, the arabinogalactan, and a glycoside of unknown structure. Some general features of the highly branched tragacanthic acid were recognised by the characterisation as methanolysis products from the methylated polysaccharide of methyl glycosides of 2,3,4-tri-*O*-methyl-L-fucose, 2,3,4-tri- and 3,4-di-*O*-methyl-D-xylose, and 2,3-di- and a mono-*O*-methyl-D-galacturonic acid; the presence of a methyl di-*O*-methyl-6-deoxy-hexoside as a further cleavage product was also suspected. In this paper we report the fractionation of the water-soluble portion of the gum and further structural studies on tragacanthic acid.

The crude gum was separated into fractions soluble and insoluble in water. Fractional precipitation of the water-soluble portion of the gum (either directly or after regeneration from the insoluble complex with Cetavlon) by addition of ethanol led to polysaccharide fractions which differed markedly in optical rotation, equivalent weight, and approximate sugar composition. Some of the fractions appeared to consist largely of either tragacanthic acid or arabinogalactan (see Table in Experimental section), but these were not sufficiently homogeneous for detailed study. The water-insoluble portion of the gum dissolved partly in dilute aqueous sodium hydroxide. The alkali-soluble material remained in solution on acidification, and graded precipitation gave fractions which were similar to those isolated from the water-soluble portion of the gum. The alkali-insoluble fraction appeared to consist largely of a glucan contaminated with adhering tragacanthic acid and arabinogalactan. It is probable that the glucan is cellulosic in nature since it remained largely insoluble after being heated with 2N-sulphuric acid but was hydrolysed by 72% sulphuric acid. The presence of cellulose microfibrils suspended in an amorphous ground substance of acidic polysaccharide has been indicated by electron microscopy for gum tragacanth and various seed mucilages.[3]

The following procedure was developed for the isolation of the two main polysaccharides in sufficiently homogeneous form for detailed studies. The crude gum was extracted with boiling ethanol to remove glycosidic components and then with cold ethanol–water (7 : 3) to afford the arabinogalactan component. The crude tragacanthic acid was most conveniently prepared as its 2-hydroxyethyl ester which was obtained by fractional precipitation of the solution resulting from treatment of the water-soluble portion of the gum with ethylene oxide.[4] Tragacanthic acid was regenerated as required from the ester by saponification with cold dilute sodium hydroxide.

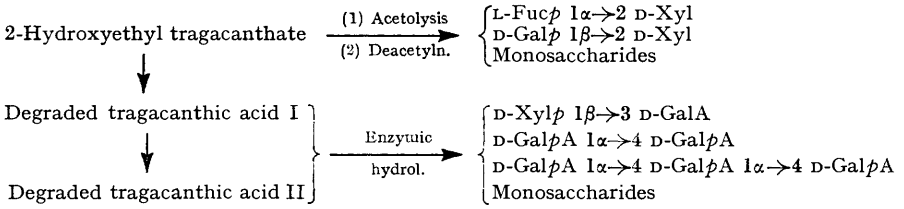
The homogeneity of the two polysaccharide preparations was assessed by glass-fibre paper ionophoresis in 2N-potassium hydroxide,[5] and by chromatography on diethylaminoethylcellulose.[6] The two polysaccharides were readily distinguished by paper ionophoresis and the individual preparations were substantially homogeneous by this criterion. Samples of each polysaccharide were then recovered by extraction of the glass paper. Paper chromatography of the hydrolysate from tragacanthic acid indicated galacturonic acid and xylose > fucose > galactose > arabinose. Subsequent experiments showed that galactose and probably also arabinose were minor constituents of tragacanthic acid and that these sugars did not arise from contaminating arabinogalactan. Likewise, chromatography of the hydrolysate from the arabinogalactan indicated arabinose > galactose > galacturonic acid > rhamnose. Rhamnose could only be detected as a constituent of the arabinogalactan. Since this sugar had not been recognised previously

[3] Mühlethaler, *Exp. Cell Res.*, 1950, **1**, 341.
[4] Deuel, *Helv. Chim. Acta*, 1947, **30**, 1523.
[5] Lewis and Smith, *J. Amer. Chem. Soc.*, 1957, **79**, 3929.
[6] Neukom, Deuel, Heri, and Kundig, *Helv. Chim. Acta*, 1960, **43**, 67.

as a constituent of gum tragacanth, a sample of the crude gum was hydrolysed and, after partial separation of the sugars formed, L-rhamnose was characterised as the toluene-*p*-sulphonylhydrazone.

When the tragacanthic acid preparation was chromatographed on diethylaminoethylcellulose the main component accounted for more than 90% of the carbohydrate material. The minor components gave arabinose, xylose, and glucose on hydrolysis and probably consisted largely of glycosidic material which had not been completely removed. The tragacanthic acid, which was recovered from the column had $[\alpha]_D$ +105° (in H_2O) and uronic anhydride content of 43%, and hydrolysis gave xylose (40%), fucose (10%), galactose (4%), and arabinose (trace). When this sample was chromatographed again on diethylaminoethylcellulose a single component was obtained.

The arabinogalactan preparation had $[\alpha]_D$ −78° (in H_2O) and uronic anhydride content of *ca.* 3%, and hydrolysis gave arabinose (75%), galactose (12%), and rhamnose (trace). When this sample was chromatographed on diethylaminoethylcellulose the main component accounted for at least 88% of the carbohydrate material; the minor components gave the same sugars in similar proportions as hydrolysis products. The arabinogalactan, which was recovered from the main peak from the column gave a single component when re-chromatographed.



Scheme showing graded hydrolysis.

2-Hydroxyethyl tragacanthate was degraded in a stepwise manner (see flow sheet). Hydrolysis of the polysaccharide ester with 0·05N-sulphuric acid on the boiling-water bath for 20 hours resulted in the release of most of the fucose residues, but only of a small proportion of the xylose and traces of the galacturonic acid residues, and degraded tragacanthic acid I was isolated. This degraded polysaccharide contained xylose and galacturonic acid residues in approximately equimolecular proportions and only traces of fucose, galactose, and arabinose residues. Under more drastic conditions (heating the polysaccharide with 0·5N-sulphuric acid on the boiling-water bath for 6 hours) further xylose residues were released and degraded tragacanthic acid II was isolated. This degraded polysaccharide had a high optical rotation ($[\alpha]_D$ +228°), approximating to those of polysaccharides of the pectic acid group.

During the early stages of the hydrolysis resulting in the formation of degraded tragacanthic acid I chromatographic examination of the products of low molecular weight indicated that neutral oligosaccharides had been released. These oligosaccharides, however, were produced more satisfactorily by acetolysis of tragacanthic acid followed by deacetylation. Two disaccharides formed in this way were isolated in pure form after chromatography on charcoal–Celite and partition chromatography on filter sheets. The first disaccharide, which was obtained crystalline, gave fucose and xylose on hydrolysis. Hydrolysis of the derived glycitol (from borohydride reduction) gave fucose and xylitol. Since the disaccharide gave no colour reaction with triphenyltetrazolium hydroxide,[7] these results pointed to the presence of a 2-*O*-fucosylxylose. The structure of the disaccharide as 2-*O*-α-L-fucopyranosyl-D-xylose was established by the isolation of 2,3,4-tri-*O*-methyl-L-fucose and 3,4-di-*O*-methyl-D-xylose on hydrolysis of the methylated disaccharide. The anomeric configuration at the glycosidic linkage may be tentatively

[7] Feingold, Avigad, and Hestrin, *Biochem. J.*, 1956, **64**, 351; Bailey, Barker, Bourne, Grant, and Stacey, *J.*, 1958, 1895.

assigned on the basis of the optical rotation of the disaccharide. In a similar manner the second disaccharide was shown to be a 2-*O*-galactosylxylose, and its structure was confirmed by the isolation of 2,3,4,6-tetra-*O*-methyl-D-galactose and 3,4-di-*O*-methyl-D-xylose on hydrolysis of the methylated derivative. The optical rotation ($[\alpha]_D$ +40°) of the disaccharide indicated the presence of a β-D-galactopyranosyl linkage. Since this work was completed Kooiman [8] has reported the isolation of 2-*O*-β-D-galactopyranosyl-D-xylose ($[\alpha]_D$ +30°) as a partial hydrolysis product of a polysaccharide from *Tamarindus indica* seeds. This disaccharide was hydrolysed by β-galactosidase.

Two commercial enzyme preparations had little action on tragacanthic acid, and only small amounts of galactose and arabinose were liberated. In contrast, degraded tragacanthic acids I and II were extensively depolymerised with the formation of xylose, galacturonic acid, and similar series of acidic oligosaccharides. The main acidic oligosaccharide from degraded tragacanthic acid was later shown to be 3-*O*-β-D-xylopyranosyl-D-galacturonic acid, and only traces of oligomers of galacturonic acid were produced in this degradation. On the other hand, degraded tragacanthic acid II gave relatively larger proportions of di- and tri-galacturonic acids. The mixtures of acidic oligosaccharides from the two enzymic degradations were separated by paper chromatography on filter sheets, and fractions of the same chromatographic mobilities were combined. The acidic oligosaccharide with the highest mobility gave xylose and galacturonic acid on hydrolysis, whereas the derived glycitol (from borohydride reduction) furnished xylose and galactonic acid, showing the compound to be a xylosylgalacturonic acid. When the glycitol was oxidised with periodate approximately 1 mol. of formaldehyde was released, indicating that the galacturonic acid residue was 3- or 4-*O*-substituted. The acidic disaccharide was methylated, the methylated derivative was reduced with lithium aluminium hydride, and the product was hydrolysed, to give 2,3,4-tri-*O*-methyl-D-xylose and 2,4-di-*O*-methyl-D-galactose. On the basis of the optical rotation ($[\alpha]_D$ +20°) of the disaccharide a β-glycosidic linkage is indicated and the structure 3-*O*-β-D-xylopyranosyl-D-galacturonic acid may be assigned. The pseudo-aldobiouronic acid, 2-*O*-α-D-glucopyranosyl-D-glucuronic acid, has been synthesised by enzymic transglucosylation,[9] but as far as we are aware the only previously recorded isolation of a pseudo-aldobiouronic acid from the degradation of a polysaccharide is that of 4-*O*-β-D-glucosaminyl-D-glucuronic acid from hyaluronic acid.[10] The structure of the latter disaccharide has not been established directly, but has been inferred from other evidence. In contrast to aldobiouronic acids [*O*-(glycosyluronic acid)glycoses], which are very resistant to acid hydrolysis, our pseudo-aldobiouronic acid was readily cleaved by dilute acid.

The di- and tri-galacturonic acids from the enzymic hydrolysis of the degraded tragacanthic acids were isolated as calcium salts whose optical rotations ($[\alpha]_D$ +122° and +151°) were indicative of α-glycosidic linkages and were in reasonable agreement with the values quoted for the di- and tri-saccharides formed on enzymic breakdown of apple pectic acid.[11] Furthermore, the infrared spectra of the calcium salts were identical with those of the corresponding compounds isolated from lucerne pectic acid,[12] but this evidence of identity cannot be regarded as final since the spectra of the calcium salts of the di- and tri-saccharides were indistinguishable. Neverthless, despite the absence of formal proof for the linkage between galacturonic acid residues in these compounds 1,4-linkages may be inferred since 2,3-di- and 2-*O*-methyl-D-galactose were isolated on hydrolysis of reduced methylated tragacanthic acid; the latter sugar presumably arises from the reduction of 3-*O*-β-D-xylopyranosyl-D-galacturonic acid residues.

Tragacanthic acid proved to be resistant to methylation, but samples of the polysaccharides were partly etherified by using methyl sulphate and sodium hydroxide or

[8] Kooiman, *Rec. Trav. chim.*, 1961, **80**, 849.
[9] Barker, Gomez-Sanchez, and Stacey, *J.*, 1959, 3264.
[10] Linker, Hoffman, and Meyer, *J. Biol. Chem.*, 1960, **235**, 924.
[11] Jones and Reid, *J.*, 1954, 1361.
[12] Aspinall and Fanshawe, *J.*, 1961 4215.

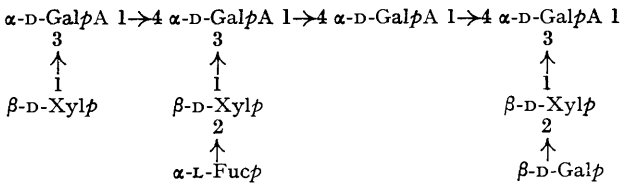
thallous hydroxide and methyl iodide, and further methylation was effected with methyl iodide and silver oxide. Methylated tragacanthic acid was reduced with lithium aluminium hydride, the reduced methylated polysaccharide was hydrolysed, and the resulting methylated sugars were fractionated by partition on cellulose. The following sugars were characterised by the formation of crystalline derivatives: 2,3,4-tri-*O*-methyl-L-fucose, 2,3,4-tri-, 3,4-di-, and 4-*O*-methyl-D-xylose, and 2,3,4,6-tetra-, 2,3,4-tri-, 2,3- and 2,4-di-, and 2-*O*-methyl-D-galactose, and some unsubstituted D-xylose and D-galactose. In addition, the following sugars, which were present only in small amounts, were provisionally characterised by one or more of the criteria, optical rotation, chromatography and paper ionophoresis of the sugars, chromatography of the products of demethylation and of periodate oxidation, and gas chromatography of the derived methyl glycosides:[13,14] di- and mono-*O*-methyl-L-fucose, di- and 3-*O*-methyl-D-xylose, 2,4,6-tri-*O*-methyl-D-galactose, and 3,5-di-*O*-methyl-L-arabinose.

With the exception of tetra- and the small proportion of 2,4,6-tri-*O*-methyl-D-galactose, the methyl ethers of D-galactose could have arisen either from D-galactose residues originally present in the polysaccharides or from D-galacturonic acid residues after reduction with lithium aluminium hydride. In view of the high proportion of D-galacturonic acid and of the low proportion of D-galactose residues in the polysaccharide, the latter alternative seemed more probable and was confirmed by the following experiments. The hydrolysis products from a sample of methylated tragacanthic acid were separated into neutral and acidic fractions. These fractions were each converted into methyl glycosides, and a portion of the acidic methyl glycosides was reduced with lithium aluminium hydride and re-treated with methanolic hydrogen chloride to ensure complete cleavage of glycosidic linkages to monosaccharide derivatives. The three mixtures of methyl glycosides from neutral sugars, acidic sugars, and reduced acidic sugars were separately examined by gas chromatography; the glycosides from the reduction of acidic sugars were also hydrolysed and the resulting sugars were examined by paper chromatography. The results showed that 2,3,4-tri-, 2,3(and probably 2,4)-di-, and 2-*O*-methyl-D-galactose were derived largely, if not exclusively, from the reduction of D-galacturonic acid residues.

The characterisation of the major neutral cleavage products from the methylated polysaccharide confirms and extends the earlier work of James and Smith[1] on tragacanthic acid. D-Xylopyranose residues occur mainly as end groups and as 2-*O*-substituted units, and L-fucopyranose residues are present largely as end groups. Since D-galactopyranose residues are present mainly as end groups it is clear that they are integral constituents of the acidic polysaccharides and that they do not arise from contaminating arabinogalactan where D-galactose residues are present in the interior chains.[2,15] Likewise, the very small proportion of L-arabinose residues in the tragacanthic acid preparation, which gives rise to 3,5-di-*O*-methyl-L-arabinose, represents a genuine minor structural feature since contamination of the polysaccharide with arabinogalactan would produce the 2,3,5-trimethyl ether as the main derivative of L-arabinose. In view of the difficulty in methylating tragacanthic acid it is possible that some of the minor cleavage products from the methylated polysaccharide are artifacts resulting from incomplete etherification. In particular, 2,4,6-tri-*O*-methyl-D-galactose, D-xylose, 4-*O*-methyl-D-xylose, and the unidentified di-*O*-methyl-L-fucose could either be products of incomplete methylation or represent genuine structural units in the interior chains of the polysaccharide. Experiments to obtain additional evidence on this and other points of fine structure are in progress.

On the basis of the results the annexed partial structure summarises the main structural features of tragacanthic acid. The polysaccharide is clearly based on essentially linear chains of 1,4-linked α-D-galacturonic acid residues. The majority of D-galacturonic acid

[13] Bishop and Cooper, *Canad. J. Chem.*, 1960, **38**, 388.
[14] Aspinall, *J.*, 1963, 1676.
[15] Aspinall and Baillie, following paper.



residues carry xylose-containing side-chains through C-3. Three types of side-chain have been recognised, namely, single β-D-xylopyranose residues, and disaccharide units of 2-*O*-α-L-fucopyranosyl-D-xylopyranose and 2-*O*-β-D-galactopyranosyl-D-xylopyranose, and these must account for the majority of the sugar residues in the outer chains. On the present evidence it is not possible to exclude the presence, in small proportions, of other units, *e.g.*, xylobiose units or branched side-chains. The location of the small proportion of 2-*O*-substituted L-arabinofuranose residues in the polysaccharide structure is not known.

Experimental

Paper chromatography was carried out on Whatman Nos. 1, 3MM, and 31ET papers with the following solvent systems (v/v): (A) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (B) benzene–ethanol–water (169 : 47 : 15, upper layer); (C) butan-2-one, half saturated with water; (D) ethyl acetate–pyridine–water (10 : 4 : 3); (E) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (F) ethyl acetate–acetic acid–water (10 : 5 : 6); (G) ethyl acetate–acetic acid–water (9 : 2 : 2). R_G values of methylated sugars refer to the rates of movement relative to 2,3,4,6-tetra-*O*-methyl-D-glucose in solvent A. Demethylations of methylated sugars were performed with hydriodic acid [16] or with boron trichloride.[17] Chromatography of the periodate oxidation products of methylated sugars was carried out by Lemieux and Bauer's method.[18] Unless otherwise stated, optical rotations were observed for water solutions at *ca.* 18°.

Gas–liquid partition chromatography of methylated and partially methylated methyl glycosides was carried out in a Pye argon chromatograph according to the procedure of Bishop and Cooper [13] (see also accompanying paper [14]). Separations were made on the following columns (120 × 0·5 cm.) at gas flow rates of 80—100 ml./min.: (*a*) 15% by weight of butane-1,4-diol succinate polyester [13] on acid-washed Celite (80—100 mesh) at 150°; (*b*) column *a* at 175°; (*c*) 10% by weight of polyphenyl ether [*m*-di-(*m*-phenoxyphenoxy)benzene] on acid-washed Celite at 200°. Retention times (*T*) are quoted relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside as an internal standard.

Fractionation of Gum Tragacanth.—(i) *Fractional precipitation from aqueous solution.* Crude gum (10 g.) was dispersed in water (1 l.) for 24 hr., and the resulting suspension was diluted by the addition of water (500 ml.), stirred for 12 hr., and allowed to stand for 24 hr. at 0°. Small particles of dirt were removed by filtration through muslin, and the insoluble portion of the gum was removed by repeated centrifugation. The water-soluble portion of the gum (fraction A, 4·09 g.) was isolated by concentrating the aqueous solution and precipitating the polysaccharide by addition of ethanol containing 2% of aqueous 2N-hydrochloric acid (7 vol.). The insoluble portion of the gum (fraction B, 5·85 g.) was washed with water and dried by solvent exchange with ethanol and ether.

Fraction A (4·09 g.) was dissolved in water (500 ml.) and ethanol was added slowly with stirring. Polysaccharide fractions A1—4, which separated when the solution contained 50%, 60%, 70%, and 80% of ethanol respectively, were separated at the centrifuge. The mother-liquors after precipitation of fraction A4 were concentrated to a brown residue, which was dissolved in water and partitioned between water and butan-1-ol. The butan-1-ol layer was concentrated to a white amorphous solid, hydrolysis of which resulted in the separation of an insoluble oil and the formation of glucose, arabinose, and xylose.

Fraction B (5·85 g.) was stirred under nitrogen with N-sodium hydroxide (600 ml.) for 15 hr., and the insoluble residue (B1) was separated at the centrifuge. The alkaline solution

[16] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[17] Bonner, Bourne, and McNally, *J.*, 1960, 2929.
[18] Lemieux and Bauer, *Canad. J. Chem.*, 1953, **31**, 814.

was neutralised with acetic acid, and ethanol was added slowly with stirring, fractions B3 and B4 separating when the solution contained 50% and 70% of ethanol.

(ii) *Fractional precipitation after regeneration of gum fractions from Cetavlon complexes.* A saturated aqueous solution of cetyltrimethylammonium bromide ("Cetavlon") was added to a solution of fraction A (10 g.) in water (1 l.) until no further precipitate formed. The polysaccharide complex was removed at the centrifuge and washed with water. The complex was decomposed by stirring it with a 10% aqueous sodium chloride solution, and addition of ethanol gave precipitates (A5 and A6) which separated at concentrations of 40% and 70% of ethanol. The combined mother-liquor and washings from the complex were concentrated and poured into ethanol (4 vol.), to give fraction A7.

A solution of the alkali-soluble, but water-insoluble, portion of the gum (fraction B2, 10 g.) was passed through a column of Amberlite resin IR-120(H) to remove cations and treated with "Cetavlon" as above. Fraction B5 was precipitated with ethanol after regeneration of polysaccharide from the insoluble complex, and fraction B6 was precipitated with ethanol from the mother-liquors from the complex.

(iii) *Fractional precipitation of 2-hydroxyethyl esters.* Fraction A (10 g.) in water (1 l.) was deionised with Amberlite resins IR-120(H) and IR-4B(OH), and ethylene oxide (125 ml.) was added to the resulting acid solution. After 12 days the neutral solution was filtered to remove precipitate A8, which had separated, and addition of acetone gave precipitates A9 and A10 at concentrations of 20% and 50% of acetone. Fraction A9 had uronic anhydride, 41·3% (corresponding to 43·5% in the acid polysaccharide) and was used in subsequent studies on tragacanthic acid (Found: OMe, 1%; glycol ester, 5·4%).

(iv) *Extraction of the gum with ethanol–water.* Powdered gum (100 g.) was extracted with boiling ethanol for 24 hr. Concentration of the extract gave a yellow solid (3 g.) which contained unidentified glycosides and traces of glucose and arabinose. A portion of this material was chromatographed on cellulose with butan-1-ol, saturated with water, to remove monosaccharides, and hydrolysis of the resulting glycosides gave glucose, arabinose, and xylose. The ethanol-extracted gum was shaken with ethanol–water (7 : 3) for two periods of 30 hr., the mixture was centrifuged, and polysaccharide was precipitated by the addition of further ethanol. Arabinogalactan (fraction A14, 3—5 g.) was isolated after reprecipitation from aqueous solution with ethanol and had uronic anhydride, 5·4%.

(v) *Fractionation of gum fractions on diethylaminoethylcellulose.* Arabinogalactan (fraction 14, 335 mg.) was dissolved in 0·005M-sodium dihydrogen phosphate buffer (pH 6; 6 ml.) and poured on to a column (32 × 3 cm.) of diethylaminoethylcellulose (phosphate form) as described by Neukom *et al.*[6] The column was eluted with 0·025M- (500 ml.), 0·05M- (500 ml.), 0·1M- (500 ml.), and 0·25M-sodium dihydrogen phosphate (pH 6) (500 ml.), and a gradient of sodium hydroxide (0·01—0·5M; 2 l.). Fractions (*ca.* 20 ml.) were collected and analysed for sugars by the anthrone method [6] and for uronic anhydride by the carbazole method. Two minor fractions (A15 and A16) were eluted at low phosphate concentrations and were isolated after dialysis, deionisation, and concentration. The concentrated solutions were hydrolysed directly and the hydrolysates were examined by paper chromatography. The main fraction (A17, 296 mg.), which was eluted with 0·25M-phosphate, was treated in the same way and the polysaccharide was isolated by precipitation with acetone [Found: uronic anhydride, 3·2 (by decarboxylation), 5·0% (carbazole method)]. When a sample of this fraction was rechromatographed on diethylaminoethylcellulose a single peak was eluted at the same phosphate concentration.

2-Hydroxyethyl tragacanthate (fraction A9, 355 mg.) was de-esterified in 0·5M-sodium hydroxide for 3 hr., and the resulting polysaccharide was precipitated with ethanol, dissolved in 0·005M-sodium dihydrogen phosphate buffer (pH 6; 5 ml.), and chromatographed on diethylaminoethylcellulose as described previously. A number of minor fractions were eluted at low phosphate concentrations and two of these (A11 and A12) were isolated after dialysis and deionisation, and their hydrolysis products were examined by paper chromatography. The main fraction (A13, 325 mg.), which was eluted with alkali, was treated in the same way and the polysaccharide was isolated by precipitation with acetone [Found: uronic anhydride, 43·2 (by decarboxylation), 40% (carbazole method)]. When a sample of this fraction was rechromatographed on diethylaminoethylcellulose a single peak was eluted with alkali.

Examination of Fractions from Gum Tragacanth.—The results of the examination of the various fractions from gum tragacanth are given in the Table. Approximate proportions of

sugar formed on hydrolysis are indicated by +++ = strong, ++ = medium, + = weak, and tr = trace. Quantitative paper chromatography was carried out by Hirst and Jones's method.[19]

Fractions A9 (after de-esterification) and A14 were examined by ionophoresis on glass fibre paper in 2N-potassium hydroxide. The major component of A9 was immobile and only traces of other components were present. The main component was eluted from the paper and hydrolysed to give galacturonic acid, xylose, fucose, and small amounts of galactose and arabinose. The major component of A14 migrated as a discrete band and only a trace of an immobile component was present. Elution of the main component from the paper followed by hydrolysis and paper chromatography showed arabinose, galactose, and traces of galacturonic acid and rhamnose.

Examination of fractions from gum tragacanth.

Starting material	Fraction	Wt. (g.)	$[\alpha]_D$	Equiv. wt.	Sugars on hydrolysis: GalA	Gal	Ara	Xyl	Fuc	Rha	G
(i) *Fractional precipitation of the gum.*											
Crude gum	A1	2·81	+101°	405	+++	tr	+	+++	++	—	—
(10 g.)	A2	0·43	+5	—	+++	+	+	+++	+	tr	—
	A3	0·32	−35	—	+	+	++	++	—	tr	—
	A4	0·18	−50	1540	+	++	+++	+	—	tr	—
	B1 *	2·10	—	—	+	+	+	+	+	—	+
	B3	2·85	+85	575	+++	+	+	+++	+	tr	tr
	B4	0·50	−20	1100	+	++	+++	+	—	tr	—
(ii) *Fractional precipitation after regeneration of Cetavlon complex.*											
Fraction A	A5	6·75	+120°	405	+++	+	+	+++	++	tr	—
(10 g.)	A6	1·38	−40	1425	++	++	+++	+	+	tr	—
	A7	1·07	−54	1650	+	++	+++	+	—	tr	—
Fraction B2	B5	7·60	+69	727	+++	+	+	+++	++	tr	tr
(10 g.)	B6	1·53	−21	1050	+	++	+++	+	tr	tr	—
(iii) *Fractional precipitation of 2-hydroxyethyl esters.*											
Fraction A	A8 *	1·78	—	—	+	tr	+	+	+	—	+
(10 g.)	A9	7·18	+104°	383	+++	+	tr	+++	+	tr	—
	A10	0·87	−47	1790	+	++	+++	tr	—	tr	—
(iv) *Extraction of crude gum.*											
Crude gum	A14	—	−78°	3500	3%	12%	75%	—	—	tr	—
(v) *Fractionation on diethylaminoethylcellulose.*											
Fraction A9	A11	—	—	—	—	tr	++	tr	—	—	++
(0·355 g.)	A12	—	—	—	tr	tr	++	++	—	—	+
	A13	0·325	+105°	—	43%	4%	tr	40%	10%	—	—
Fraction A14	A15	—	—	—	tr	++	+++	tr	—	tr	+
(0·325 g.)	A16	—	—	—	tr	++	+++	tr	—	tr	tr
	A17	0·296	−75	—	+	++	+++	—	—	tr	—

* Fractions B1 and A8 were incompletely hydrolysed with 2N-sulphuric acid at 100° in 18 hr. When the insoluble residue was treated with cold 72% sulphuric acid for 3 days and then with dilute sulphuric acid at 100° for 12 hr., glucose was formed as the main hydrolysis product.

Characterisation of L-*Rhamnose as a Constituent of the Gum.*—Powdered gum (10 g.) was hydrolysed with N-sulphuric acid (1 l.) at 100° for 12 hr., and the cooled suspension was filtered, neutralised with barium hydroxide, filtered again, deionised, and concentrated to a syrup (7·2 g.). The syrup was partitioned on a cellulose column (66 × 3·5 cm.) with butan-1-ol–ethanol–water (3 : 1 : 3, upper layer) as eluant. Most of the rhamnose was eluted together with fucose, xylose, and arabinose, and this fraction (540 mg.) was refractionated on cellulose (57 × 2·2 cm.) with solvent A as eluant and gave L-rhamnose (95 mg.), $[\alpha]_D$ +7° (*c* 1·9), which was characterised by conversion into the toluene-*p*-sulphonylhydrazone, m. p. 240—241° and mixed m. p. 241—242°.

Preparation of Degraded Tragacanthic Acids.—2-Hydroxyethyl tragacanthate (fraction A9, 5 g.) was heated in 0·05N-sulphuric acid (500 ml.) on the boiling-water bath for 20 hr. Ethanol

[19] Hirst and Jones, *J.*, 1949, 1659.

(5 vol.) was added to the cooled solution, and degraded tragacanthic acid I (3 g.) was removed by filtration, washed free from acid, and dried. The degraded polysaccharide had $[\alpha]_D$ +175°, equiv. 326, uronic anhydride (by decarboxylation) 46·4% (corresponding to 48·5% in the acid polysaccharide), and glycol ester 4·7%. The glycol ester content of degraded tragacanthic acids I and II was estimated by saponification and oxidation of the liberated glycol with periodate, followed by determination by the chromotropic acid reagent of the formaldehyde formed.[20] Hydrolysis of degraded tragacanthic acid I gave galacturonic acid and xylose in approximately equal proportion, and traces of fucose, galactose and arabinose. Neutralisation of the mother-liquors from the precipitation, followed by chromatography, showed fucose with smaller amounts of xylose, galactose, arabinose, and neutral oligosaccharides, and traces of galacturonic acid.

2-Hydroxyethyl tragacanthate (5 g.) was heated in 0·5N-sulphuric acid (500 ml.) on the boiling-water bath for 6 hr., and afforded degraded tragacanthic acid II (1·3 g.), $[\alpha]_D$ +228°, equiv. 202, uronic anhydride 68·5%, and glycol ester 0·5%. Hydrolysis of the degraded polysaccharide gave galacturonic acid and xylose.

Enzymic Degradation of Degraded Tragacanthic Acid.—Preliminary experiments showed that degraded tragacanthic acid I was similarly degraded by both " Hemicellulase " and " Pectinase " (L. Light and Co. Ltd.), but that the enzyme preparations had little action on tragacanthic acid and that only traces of arabinose and galactose could be detected as degradation products. Degraded tragacanthic acid I (3 g.) was treated in water (1 l.) at pH 3 (acetic acid) with " Hemicellulase " (1·2 g.) for 8 hr. Polysaccharide was precipitated with acetone (1 vol.) and was treated with enzyme for three further periods of 8 hr. The combined centrifugates were concentrated, neutralised with ammonia, and taken to dryness (2·05 g.). Paper chromatography in solvent E showed, in addition to xylose and galacturonic acid, an acidic oligosaccharide having $R_{\text{galacturonic acid}}$ 0·72 together with smaller amounts of acids having $R_{\text{galacturonic acid}}$ 0·51 and 0·38.

Degraded tragacanthic acid II (3 g.) was degraded similarly and afforded a similar mixture (1·71 g.) of monosaccharides and acidic oligosaccharides. The main oligosaccharide component had $R_{\text{galacturonic acid}}$ 0·51. The products from both enzymic hydrolyses were separated on filter sheets with solvent E, and fractions of the same mobility were combined.

Examination of Acidic Oligosaccharides.—Acidic oligosaccharide I (285 mg.), $R_{\text{galacturonic acid}}$ 0·72 in solvent E, had $[\alpha]_D$ +20° (as ammonium salt) (*c* 0·57). Hydrolysis gave xylose and galacturonic acid, and hydrolysis of the derived glycitol (from borohydride reduction) gave xylose and galactonic acid. The glycitol (10 mg.) was oxidised with 0·004M-sodium metaperiodate (25 ml.), and samples (1 ml.) were withdrawn and analysed for formaldehyde by the chromotropic acid method.[20] The formaldehyde liberated corresponded to 1 mole per mole of glycitol. The sugar (200 mg.) was methylated with methyl sulphate and 30% sodium hydroxide, care being taken to avoid strongly alkaline solutions until glycosidation was complete. The reaction mixture was acidified to pH 3 and extracted with chloroform. The methylated acid (70 mg.) was reduced with lithium aluminium hydride (150 mg.) in tetrahydrofuran (15 ml.) and furnished methylated neutral disaccharide (58 mg.). Hydrolysis of the methylated disaccharide with N-sulphuric acid for 4 hr. on the boiling-water bath gave two sugars, R_G 0·41 and 0·93, which were separated on filter sheets in solvent A. The first component (23 mg.), $[\alpha]_D$ +82° (*c* 1·1), was recrystallised from acetone containing 1% of water to give 2,4-di-*O*-methyl-D-galactose monohydrate, m. p. 102° and mixed m. p. 101—102°, which was further characterised as the aniline derivative, m. p. and mixed m. p. 210°. The second component (22 mg.), $[\alpha]_D$ +20° (*c* 1·1), was recrystallised from ethanol–water, to give 2,3,4-tri-*O*-methyl-D-xylose, m. p. and mixed m. p. 90—91°.

Acidic oligosaccharide II (98 mg.), $R_{\text{galacturonic acid}}$ 0·51, was chromatographically indistinguishable from the digalacturonic acid from pectic acid. It gave a calcium salt which had $[\alpha]_D$ +122° (*c* 1·2 in N-HCl) and whose infrared spectrum was identical with that of calcium digalacturonate from pectic acid.

Acidic oligosaccharide III (10 mg.), $R_{\text{galacturonic acid}}$ 0·38, gave galacturonic acid and smaller amounts of xylose on hydrolysis.

Acidic oligosaccharide IV (12 mg.), $R_{\text{galacturonic acid}}$ 0·26, was chromatographically indistinguishable from the trigalacturonic acid from pectic acid. Hydrolysis gave galacturonic acid

[20] McFadyen, *J. Biol. Chem.*, 1945, **158**, 107.

only. It gave a calcium salt which had $[\alpha]_D$ +151° (*c* 0·6 in N-HCl) and an infrared spectrum identical with that of calcium trigalacturonate from pectic acid.

Acetolysis of Acetylated Tragacanthic Acid.—Acetylated tragacanthic acid (12·5 g.), prepared by the method of Carson and Maclay,[21] was added slowly to the acetolysis mixture (500 ml.; acetic acid, acetic anhydride, sulphuric acid, 10 : 10 : 1) at 0°, shaken at room temperature for 12 hr. until dissolution was complete, and set aside for a further 60 hr. The mixture was poured into water (4 l.) and sodium hydrogen carbonate was added gradually (to pH 4). The precipitated acetates were removed at the centrifuge, washed with water, and dissolved in chloroform, and the dried solution was concentrated to a syrup (8·0 g.). The syrup was dissolved in methanol (150 ml.) containing chloroform (10 ml.), 0·5N-barium methoxide (40 ml.) was added, and the mixture was shaken overnight. The resulting mixture, which was still alkaline, was poured into water (3 l.), a small insoluble residue was filtered off, the filtrate was concentrated, barium ions were removed by passage through Amberlite resin IR-120(H), and the solution was concentrated to a syrup (3·6 g.). The syrup was added to a column of charcoal (B.D.H., Ltd., acid-washed)–Celite (160 g.; 1 : 1) which was eluted successively with water, and water containing 2%, 5%, 10%, and 15% of ethanol. Each fraction was treated with Amberlite resin IR-45(OH) to remove acids, concentrated, and examined chromatographically. The fraction (454 mg.) eluted with water containing 5% of ethanol consisted of xylose and a trace of galactose. Chromatographically pure samples of the two disaccharides were obtained by fractionation on filter sheets with solvent E.

Disaccharide A (110 mg.), $R_{glucose}$ 1·4 and 1·0 in solvents D and E, was recrystallised from ethanol–water and had m. p. 185—190° (decomp.) and $[\alpha]_D$ −61° (equil.) (*c* 0·71). The sugar gave no colour with triphenyltetrazolium hydroxide and furnished fucose and xylose on hydrolysis. Hydrolysis of the derived glycitol gave fucose and xylitol. Methylation of the sugar (80 mg.) with methyl sulphate and 30% aqueous sodium hydroxide afforded methylated disaccharide (65 mg.), hydrolysis of which furnished two sugars, R_G 0·92 and 0·75, and only traces of other products. The sugars were separated on filter sheets with solvent A. The first component (28 mg.), $[\alpha]_D$ −118° (*c* 1·4), recrystallised from ethanol–water to give 2,3,4-tri-*O*-methyl-L-fucose, m. p. and mixed m. p. 62—63°, which was further characterised as the aniline derivative, m. p. 132—133°. The second component (30 mg.), $[\alpha]_D$ +21° (*c* 1·5), was chromatographically indistinguishable from 3,4-di-*O*-methyl-D-xylose and was characterised by conversion into 3,4-di-*O*-methyl-D-xylonolactone, m. p. and mixed m. p. 67—68°.

Disaccharide B (60 mg.), $R_{glucose}$ 0·95 and 0·6 in solvents D and E, $[\alpha]_D$ +40° (*c* 3·0), gave no colour with triphenyltetrazolium hydroxide and furnished galactose and xylose on hydrolysis. Hydrolysis of the derived glycitol (from borohydride reduction) gave galactose and xylitol. Methylation of the disaccharide (55 mg.) with methyl sulphate and 30% aqueous sodium hydroxide furnished methylated disaccharide (41 mg.). Methanolysis of the methylated disaccharide afforded a mixture of methyl glycosides, the major components of which had the retention times of the methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-galactose (*T* 1·86sh, 1·99) and 3,4-di-*O*-methyl-D-xylose (*T* 1·32, 1·63) when examined by gas chromatography on column *a*. The major portion of the methylated disaccharide was hydrolysed to give two sugars, R_G 0·88, 0·75, which were separated on filter sheets with solvent A. The first component (18 mg.), $[\alpha]_D$ +118° (*c* 0·91), was characterised as 2,3,4,6-tetra-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 188—189°. The second component (17 mg.), $[\alpha]_D$ +23° (*c* 0·85), was chromatographically indistinguishable from 3,4-di-*O*-methyl-D-xylose and was characterised by conversion into 3,4-di-*O*-methyl-D-xylonolactone, m. p. and mixed m. p. 67—68°.

Preparation and Hydrolysis of Reduced Methylated Tragacanthic Acid.—Tragacanthic acid proved very resistant to methylation, and samples of partially methylated polysaccharide (Found: OMe, *ca.* 30%) were obtained by two procedures. In the first procedure the water-soluble portion of the gum was methylated with methyl sulphate and 30% aqueous sodium hydroxide, methylated glycoside and methylated arabinogalactan were removed by extraction with chloroform, and partially methylated tragacanthic acid was isolated after dialysis and concentration of the remaining solution. This material was converted into the silver salt and treated with methyl iodide and silver oxide in methanol suspension. In the second procedure, 2-hydroxyethyl tragacanthate was converted into the thallium salt with thallous hydroxide and treated with methyl iodide in methanol suspension. The combined samples of partially

[21] Carson and Maclay, *J. Amer. Chem. Soc.*, 1946, **68**, 1015.

methylated tragacanthic acid were further methylated by several treatments with methyl iodide and silver oxide; methanol and later acetone and tetrahydrofuran were added to aid solution. Methylated tragacanthic acid had $[\alpha]_D$ +90° (*c* 0·98 in $CHCl_3$) (Found: OMe, 38·6%, not raised on further methylation).

Lithium aluminium hydride (3 g.) in tetrahydrofuran (25 ml.) was added dropwise to methylated tragacanthic acid (2·8 g.) in tetrahydrofuran (150 ml.), and the mixture was refluxed for 2 hr. Further hydride (1 g.) was added, and the mixture was refluxed for 1 hr., and set aside for 18 hr. The excess of hydride was destroyed by addition of ethyl acetate, and the solution was brought to pH 4 by addition of dilute sulphuric acid. The solution was extracted with chloroform, the extract was dried and concentrated, and reduced methylated tragacanthic acid (1·9 g.) was precipitated by light petroleum. A further quantity of methylated polysaccharide was isolated from the aqueous layer after neutralisation, concentration, and extraction of the dry residue with chloroform. Reduced methylated tragacanthic acid (2·15 g.) had $[\alpha]_D$ +78° (*c* 1·2 in $CHCl_3$) (Found: OMe, 31·8%). Hydrolysis of a sample of the methylated polysaccharide followed by paper chromatography of the hydrolysate failed to reveal methylated uronic acids.

Reduced methylated tragacanthic acid (1·9 g.) was dissolved in N-hydrochloric acid (125 ml.) and after 2 days the solution was gradually warmed to 100°, and the hydrolysis was completed by heating the mixture on the boiling-water bath for 10 hr. (constant rotation). The solution was neutralised with silver carbonate and concentrated to a syrup (1·7 g.) which was separated on cellulose (74 × 4 cm.), (i) light petroleum (b. p. 100—120°)-butan-1-ol (4 : 1, later 7 : 3, and 1 : 1), saturated with water, (ii) butan-1-ol, half saturated with water, and (iii) water being

Analysis of hydrolysate of reduced methylated tragacanthic acid.

Fraction	Wt. (mg.)	$[\alpha]_D$	Paper chromatography * R_G	Sugar	Sugars given on demethyln.	Other evidence †
1	397	+16°	0·93	2,3,4-Me_3xylose	Xylose	B, C, D
			0·88	2,3,4-Me_3fucose (*t*)		
2	131	−80	0·93	2,3,4-Me_3xylose	Xylose	B, C, D
			0·88	2,3,4-Me_3fucose	Fucose	
3	148	−12	0·88	Me_4galactose	Galactose	B, C, D
			0·81	Me_2fucose	Fucose	
			0·75	3,4-Me_2xylose	Xylose	
4	172	+14	0·93	2,3,4-Me_3xylose (*t*)		B, C, D, I
			0·87	2,3,4-Me_3fucose (*t*)	Xylose	
			0·80	Me_2fucose (*t*)	Fucose (*t*)	
			0·75	3,4-Me_2xylose		
5	32	+43	0·75	3,4-Me_2xylose (*t*)	Xylose	B, C, D, I
			0·68	Me_3galactose	Galactose	
6	19	+65	0·68	Me_3galactose	Galactose	B
			0·56	Me fucose (*t*)	Fucose (*t*)	
7	20	+16	0·68	Me_3galactose	Galactose	B, C
			0·68	Me_2xylose	Xylose	
			0·56	Me fucose (*t*)	Fucose (*t*)	
8	21	+32	0·50	Me fucose	Fucose; Galactose (*t*)	B, C, I, P
9	22	+66	0·55	Me_2galactose	Galactose	B, C, I
			0·47	Me xylose	Fucose; Xylose	
10	155	+70	0·48	2,3-Me_2galactose		B, I, P
			0·39	4-Me xylose; 3-Me xylose (*t*)	Galactose; Xylose	
11	40	+66	0·40	2,4-Me_2galactose; Me xylose (*t*)	Galactose; Xylose (*t*)	D, E, I, P
12	22	+11	0·29	Me xylose; Unknown sugar	Xylose; Galactose (*t*)	D, E, I
13	30	+70	0·28	Unknown sugar	Arabinose	D, E, I
			0·24	2-Me galactose	Galactose	
14	320	+84	0·23	2-Me galactose	Galactose	
15	150	+45	0·23	2-Me galactose		D, E
			0·17	Xylose		
			0·09	Galactose		

* *t* = trace. † B, C, D, and E = paper chromatography in solvents B, C, D, and E, respectively; I = paper ionophoresis; P = paper chromatography of the periodate-oxidised sugar.

used as eluants to give fifteen fractions. The annexed Table summarises the results of preliminary examination of the various fractions.

Fraction 1. The syrup crystallised to give 2,3,4-tri-*O*-methyl-D-xylose, m. p. and mixed m. p. 90—91°, $[\alpha]_D$ +60° ⟶ +17° (equil.) (*c* 1·2), and the sugar was further characterised by conversion into 2,3,4-tri-*O*-methyl-D-xylonolactone, m. p. and mixed m. p. 55—56°.

Fraction 2. The syrup was fractionated on filter sheets with solvent D, to give fractions 2*a* (40 mg.) and 2*b* (61 mg.). Fraction 2*a*, $[\alpha]_D$ +20° (*c* 0·8), crystallised to give 2,3,4-tri-*O*-methyl-D-xylose, m. p. and mixed m. p. 90—91°. Fraction 2*b* had $[\alpha]_D$ −118° (*c* 0·75) and gave fucose and a trace of xylose on demethylation. Crystallisation from ethanol containing 1% of water furnished 2,3,4-tri-*O*-methyl-L-fucose monohydrate, m. p. and mixed m. p. 63—64°. The sugar also afforded an aniline derivative, m. p. 132—133° (James and Smith [22] give m. p. 133—134° for 2,3,4-tri-*O*-methyl-*N*-phenyl-L-fucosylamine).

Fraction 3. The complex mixture of sugars was partly fractionated on filter sheets with solvent B, giving fractions 3*a* (44 mg.) and 3*b* (96 mg.). Fraction 3*a* had $[\alpha]_D$ +116° (*c* 0·9) and chromatography in solvents A, B, C, and D and paper ionophoresis indicated that the main component was 2,3,4,6-tetra-*O*-methylgalactose and that only traces of tri-*O*-methylfucose and tri- and di-*O*-methylxylose were present. 2,3,4,6-Tetra-*O*-methyl-D-galactose was characterised by conversion into the aniline derivative, m. p. and mixed m. p. 189—190°. Chromatography of fraction 3*b*, $[\alpha]_D$ −40° (*c* 0·96), indicated the presence of 3,4-di-*O*-methylxylose, a di-*O*-methylfucose, and a trace of tetra-*O*-methylgalactose. A further component was revealed by paper ionophoresis and the major portion was further separated by ionophoresis on filter sheets into three sub-fractions. Chromatography and ionophoresis of fraction 3*b* (i) (15 mg.) showed a main component, R_G 0·80, which gave fucose on demethylation. Gas chromatography of the methyl glycosides on column *a* showed two main components, *T* 1·08 and 1·52. Fraction 3*b* (ii) (10 mg.), $[\alpha]_D$ +22° (*c* 1·0), was characterised as 3,4-di-*O*-methyl-D-xylose by conversion into 3,4-di-*O*-methyl-D-xylonolactone, m. p. and mixed m. p. 67—68°. Fraction 3*b* (iii) (6 mg.) was chromatographically and ionophoretically indistinguishable from 3,5-di-*O*-methyl-L-arabinose, gave arabinose on demethylation, and furnished methyl glycosides having the relative retention times (*T* 1·06, 2·75) of methyl glycosides of 3,5-di-*O*-methyl-L-arabinose on column *a*.

Fraction 4. The syrup was separated by ionophoresis on filter sheets into fractions 4*a* (20 mg.) and 4*b* (62 mg.). Fraction 4*a* contained three components, probably a di-*O*-methylfucose (R_G 0·80) and 2,3- and 2,4-di-*O*-methylxylose (R_G 0·73, 0·69), and gave fucose and xylose on demethylation. Fraction 4*b*, $[\alpha]_D$ +21° (*c* 1·5), was chromatographically and ionophoretically homogeneous and was characterised as 3,4-di-*O*-methyl-D-xylose by conversion into 3,4-di-*O*-methyl-D-xylonolactone, m. p. and mixed m. p. 67—68°.

Fraction 5. The syrup was converted into methyl glycosides which were examined by gas chromatography. Components having the retention times of methyl glycosides of 2,4,6- (major) and 2,3,4-tri-*O*-methyl-D-galactose (minor) (*T* 4·16 and 4·76, and 7·5 on column *b*, and 2·10 and 2·39, and 2·66 and 2·93 on column *c*) were recognised, but the complex mixture of glycosides from other sugars (probably including di-*O*-methylxylose) could not be identified with certainty.

Fraction 6. The syrup contained 2,3,4-tri-*O*-methyl-D-galactose as the main component and the sugar was characterised as the aniline derivative, m. p. and mixed m. p. 164—165°.

Fraction 8. The syrup, $[\alpha]_D$ +32° (*c* 1·0), contained a major component, M_G 0·38, which was purified by chromatography in solvent A. Chromatography of the periodate-oxidised sugar showed a series of products similar to those formed by 3-*O*-methylrhamnose. Gas chromatography of the methyl glycosides showed a main component (*T* 1·00 on column *c*) which was distinct from the methyl glycosides formed from 2-*O*-methyl-L-fucose.

Fraction 10. Gas chromatography of the derived methyl glycosides on column *c* indicated the presence of methyl glycosides of 4-*O*-methylxylose (*T* 1·10) and 2,3-di-*O*-methylgalactose (*T* 2·48, 3·23, 3·75, 4·24). The syrup was fractionated on filter sheets with solvent E, to give pure samples of 4-*O*-methyl-D-xylose (40 mg.) and 2,3-di-*O*-methyl-D-galactose (50 mg.), together with a mixture (45 mg.) of the two sugars. 4-*O*-Methyl-D-xylose crystallised when seeded, had m. p. and mixed m. p. 102—104° and $[\alpha]_D$ +11° (equil.) (*c* 2·0), and was further identified by an *X*-ray powder photograph. 2,3-Di-*O*-methyl-D-galactose, $[\alpha]_D$ +77° (*c* 0·75),

[22] James and Smith, *J.*, 1945, 746.

was characterised by conversion into the aniline derivative which was identified by m. p. 153—164° and mixed m. p. (with sample m. p. 152—153°) 152—154°, and by *X*-ray powder photograph.

Fraction 11. The syrup crystallised to give 2,4-di-*O*-methyl-D-galactose monohydrate, m. p. and mixed m. p. 97—98°, which was further characterised by conversion into the aniline derivative, m. p. and mixed m. p. 209—210°.

Fraction 14. The sugar crystallised from ethanol–water to give 2-*O*-methyl-D-galactose, m. p. and mixed m. p. 146—148°, and $[\alpha]_D$ +52° ⟶ +84° (equil.).

Fraction 15. The major portion of the syrup was fractionated on filter sheets with solvent D, to give 2-*O*-methyl-D-galactose (21 mg.), m. p. and mixed m. p. 147°, $[\alpha]_D$ +85° (equil.) (*c* 1·0), D-xylose (80 mg.), $[\alpha]_D$ +80° ⟶ +19° (equil.) (*c* 4·0), m. p. and mixed m. p. 144—145° [di-*O*-benzylidene dimethyl acetal, m. p. and mixed m. p. 210°], and D-galactose (29 mg.), $[\alpha]_D$ +80° (equil.) (*c* 1·3), m. p. and mixed m. p. 166° [mucic acid, m. p. and mixed m. p. 210°].

Examination of Hydrolysis Products from Methylated and Reduced Methylated Tragacanthic Acid.—Samples (100 mg.) of methylated and methylated reduced tragacanthic acid were hydrolysed and the hydrolysates were separated on filter sheets with solvent D into four fractions containing (i) tri- and di-*O*-methylpentoses and 6-deoxyhexoses, tetra- and tri-*O*-methylhexoses (R_G 0·93—0·60), (ii) *O*-methylpentoses and di-*O*-methylhexoses (R_G 0·60—0·25), (iii) *O*-methylhexose and unsubstituted sugars (R_G 0·25—0·10), and (iv) methylated acids (chromatographically immobile). The various fractions were converted into methyl glycosides which were examined by gas chromatography on columns *b* and *c*. In addition, the methylated acids from methylated tragacanthic acid were treated with methanolic hydrogen chloride, reduced with lithium aluminium hydride, and hydrolysed to give neutral sugars which were examined by paper chromatography and whose methyl glycosides were examined by gas chromatography on column *c*. The results indicated that 2,3,4-tri-, 2,3(and possibly 2,4)-di-, and 2-*O*-methylgalactose were formed only after reduction of hexuronic acid units.

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DEPARTMENT OF CHEMISTRY, UNIVERSITY OF EDINBURGH. [*Received, August 30th,* 1962.]