193. *The Chemistry of Gum Tragacanth. Part I. Tragacanthic Acid.*

By Sybil P. James and F. Smith.

Gum tragacanth (an exudation of shrubs belonging to the genus *Astragulus*) has been shown to be a mixture of tragacanthic acid, A, and a neutral polysaccharide, B. There is also present in the gum a small quantity of a third component, C, which appears to be a glycoside. Methyl derivatives of A, B, and C have been obtained by exhaustive methylation of the gum. A closer investigation of the methylated tragacanthic acid, A, forms the subject of this paper.

Hydrolysis of the methyl ester of methylated tragacanthic acid with methyl alcoholic hydrogen chloride and subsequent fractionation of the resulting mixture of methylated glycosides and ester uronosides, revealed the presence of 2 : 3 : 4-*trimethyl α-methyl*-l-*fucoside* (I), 2 : 3 : 4-trimethyl methyl-*d*-xyloside (II), 3 : 4-dimethyl methylxyloside, the methyl ester of 2 : 3-dimethyl methylgalactofururonoside (IV), and the methyl ester of a monomethyl β-methylgalactopyruronoside. The mode of linking of these units in tragacanthic acid is discussed.

In continuation of our investigations of plant gums we have examined gum tragacanth. This gum is an exudation from various species of shrubs belonging to the genus *Astragulus* (of the order *Leguminosæ*) occurring

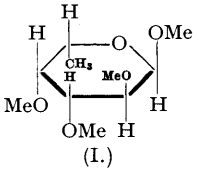
in S.W. Europe and the Middle East. The gum is produced spontaneously but its formation is artificially stimulated for commercial purposes by making vertical incisions in the trunks of the shrubs after being denuded of their leaves. The gum exudes and, when allowed to dry, forms white flakes.

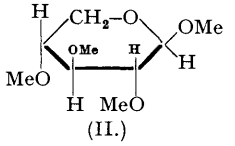
The early work of Kraut (*Ber.*, 1872, **4**, 650) who subjected the gum to fractionation indicated its great complexity. The first analytical work on the gum was by Widstoe and Tollens (*Ber.*, 1900, **33**, 132) who examined several varieties of tragacanth and identified the constituent sugars by hydrolysis with 10% sulphuric acid. From three samples of white tragacanth, arabinose was obtained and, from two samples of the brown variety, these workers isolated xylose. There was also isolated a crystalline sugar, identical with fucose obtained from sea-weed. In the same year Hilger and Dreyfuss (*Ber.*, 1900, **33**, 178) demonstrated that tragacanth contained arabinose and galactose while O'Sullivan (J., 1901, **79**, 1164) suggested that hydrolysis of gum tragacanth afforded a degraded material related to the polysaccharide acids derived in the same way from gum arabic and gedda gum.

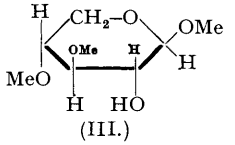
The white powder which constitutes the commercial product is slightly acid to litmus. It contains some inorganic material which may be removed by precipitation of the gum from solution in aqueous hydrochloric acid with alcohol. After further precipitation to remove mineral acid the gum has an equivalent weight of 442. It contains methoxyl groups (OMe, 3·8%) of an etheric nature since the methoxyl content was unchanged after treatment of the gum with sodium hydroxide solution. Fractionation of the gum from aqueous solution by the addition of alcohol has confirmed the view that the gum is highly complex in nature. Owing to the physical properties of the gum which made it difficult to handle it was deemed advisable to postpone fractionation until the gum had been transformed into the corresponding methyl derivative.

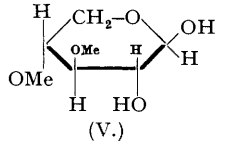
Gum tragacanth was subjected to exhaustive methylation, with methyl sulphate and sodium hydroxide, the methylation being carried out at room temperature. Since the methylated product was soluble in acid and alkaline solutions and could not be extracted with chloroform it was separated from inorganic material by dialysis. The methylated product proved to be a mixture of an acidic methylated polysaccharide *tragacanthic acid*, A, a neutral methylated polysaccharide, B, the examination of which will form the subject of a further communication, and a third small fraction, C, which is a glycoside. The latter was easily separated from the mixture of the three methylated products because of its insolubility in cold water. An initial separation of A and B was effected by reason of the fact that B was insoluble in hot water whereas the sodium salt of methylated tragacanthic acid, A, dissolved readily. Completion of the separation of A and B was achieved by conversion of A into the barium salt from which B was extracted with acetone. The methylated tragacanthic acid, A, was liberated from its barium salt by treatment with oxalic acid and its essential homogeneity demonstrated by fractionation.

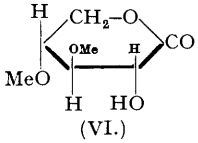
Treatment of the methylated tragacanthic acid with silver oxide gave the corresponding methyl ester. Hydrolysis of the methyl ester of methylated tragacanthic acid, A, was brought about by boiling with dry methanol containing 3% hydrogen chloride or by heating in a sealed tube with the same reagents, when there was obtained a mixture of methylated glycosides and the methyl esters of methylated uronic acids. These components were separated by conversion of the acids to their barium salts followed by extraction of the methylated glycosides with ether. The mixture of esters from the barium salts and the mixture of glycosides were each subjected to fractional distillation (Smith, J., 1939, 1724). In addition, the mixture of glycosides and esters resulting from hydrolytic cleavage was directly resolved into its components by means of fractional distillation.

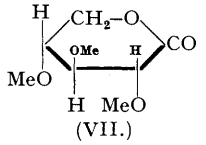

(I.)

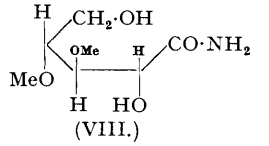

(II.)

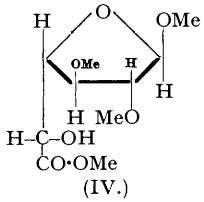

(III.)

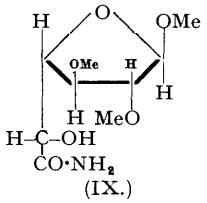

(V.)

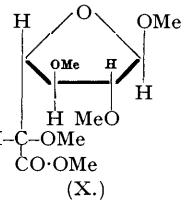

(VI.)

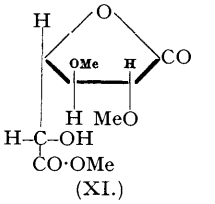

(VII.)


(VIII.)


(IV.)


(IX.)


(X.)


(XI.)

The mixture of methylated glycosides was shown to consist of 2 : 3 : 4-*trimethyl* α-*methyl*-l-*fucoside* (I), 2 : 3 : 4-trimethyl methyl-*d*-xyloside (II), 3 : 4-dimethyl methylxyloside (III) and an unidentified fragment

which may be a dimethyl methylpentoside. The presence of the methyl ester of 2 : 3-dimethyl methylgalacturonoside (IV) and the *methyl ester* of a *monomethyl methylgalacturonoside* was demonstrated.

The trimethyl methylpentoside (I), which separated from the first fractions of the distillate in the crystalline form, was proved to be 2 : 3 : 4-trimethyl α-methyl-*l*-fucoside by its identity with a specimen prepared synthetically by methylation of α-methyl-*l*-fucopyranoside (Mensaas, *Rec. Trav. Chim.*, **1932**, **51**, 475) (see Part II).

The fully methylated xyloside (II) was identified as a 2 : 3 : 4-trimethyl methylxyloside since hydrolysis gave crystalline 2 : 3 : 4-trimethyl xylose identified by m. p., rotation and comparison with an authentic specimen.

The dimethyl methylpentoside (III) was shown to be 3 : 4-dimethyl methylxyloside by the following experimental facts. On hydrolysis with 0·1N sulphuric acid a syrupy dimethyl pentose (V) was formed which was converted to the crystalline lactone (VI) by oxidation with bromine. The relatively rapid rate of mutarotation of this lactone as compared with the lactones of the xylose series suggested that this is also a δ-lactone, in which case the OH at position 4 is probably blocked by a methyl residue. This view was confirmed by methylation with methyl iodide and silver oxide when there was obtained the characteristic crystalline 2 : 3 : 4-trimethyl δ-xylonolactone (VII). It follows therefore that the two methyl groups of (III) could be present in positions 2 and 3, 3 and 4, or 2 and 4. A distinction between these possibilities was afforded by the fact that the dimethyl lactone (VI) formed an amide (VIII) which was shown to contain a free hydroxy group at C_2 since it gave the positive Weerman test for α-hydroxyamides (*Rec. Trav. Chim.*, **1917**, **36**, 16). The methyl groups of (III) must therefore occupy positions 3 and 4.

It is to be noted that a 3 : 4-dimethyl derivative of xylose has been found in the mixture of sugars obtained by hydrolysis of the methylated mucilage of *Plantago lanceolata* (Mullan and Percival, J., 1940, 1501). These authors isolated a crystalline 3 : 4-dimethyl xylonolactone, m. p. 67°, which showed a rotation of +41° changing in 6 hours to +31°. The crystalline lactone we have encountered shows m. p. 68°, $[\alpha]_D$ −56° changing in 65 hours to −27°. In comparison, it has been observed that 2 : 3 : 4-trimethyl δ-*d*-xylonolactone shows $[\alpha]_D$ −4° mutarotating, as does our lactone, in a positive direction, the equilibrium value of +21° being reached in 8 days (Haworth and Westgarth, J., 1926, 682). We are not certain yet whether the 3 : 4-dimethyl xylonolactone obtained during these investigations is a member of the *d*- or *l*-series but the problem will be settled by a synthesis, now in progress, of this compound.

The dimethyl ester (IV) proved to be the methyl ester of 2 : 3-dimethyl methylgalacturonoside since on treatment of it with methanolic ammonia there was formed the crystalline amide (IX) of 2 : 3-dimethyl β-methylgalactofururonoside previously obtained from methylated pectic acid (Luckett and Smith, J., 1940, 114). Confirmation of the structure (IV) was obtained by its giving methyl ester of 2 : 3 : 5-trimethyl β-methylgalactofururonoside (X) on methylation with methyl iodide and silver oxide (Luckett and Smith, *loc. cit.*). Furthermore, oxidation of (IV) with nitric acid followed by esterification and distillation gave the 1 : 4-lactone 6-methyl ester of 2 : 3-dimethyl mucic acid (XI).

The constitution of the monomethyl methylgalacturonoside which crystallised in the high boiling ester fractions has not been completely elucidated but it is clear that this monomethyl derivative of galacturonic acid is a derivative of β-methylgalactopyruronoside. This is deduced from the fact that methylation with methyl iodide and silver oxide of the methyl ester of monomethyl methylgalacturonoside afforded the crystalline methyl ester of 2 : 3 : 4-trimethyl β-methylgalactopyruronoside the structure of which has been established by synthesis from galactose (Luckett and Smith, J., 1940, 1506).

The complexity of the hydrolytic mixture and the difficulty experienced in the separation of the constituents of the mixture do not allow an exact estimation of the relative amounts of glycosides and esters to be made. Although the experimental facts given above do not lead to a particular structural solution they do afford for the first time proof of the nature and mode of linkage of some of the constituent units of tragacanthic acid.

Since the tragacanthic acid had been subjected to repeated methylation it follows that the free hydroxyl groups of the cleavage products must represent the mutual points of attachment of these residues. Thus 2 : 3 : 4-trimethyl methylfucoside (I) and 2 : 3 : 4-trimethyl methylxyloside (II) must arise from terminal residues in the complex molecule of methylated tragacanthic acid; such units as (I) and (II) must therefore be linked to the main structure through their reducing groups. The 3 : 4-dimethyl methylxyloside (III) is derived from units of xylose which are interposed between two other residues and moreover this 3 : 4-dimethyl derivative can only be produced from units of xylose possessing a six atom ring and joined to other residues in the complex through positions 1 and 2. Similarly, the 2 : 3-dimethyl methylgalacturonoside (IV) is produced from a uronic acid residue to which two other units are attached while the methyl ester of the galacturonoside is formed from an acid unit to which three other residues are linked. The esters are present in the hydrolytic mixture in furanose and pyranose forms but on account of the high positive rotation of the unhydrolysed polysaccharide it is believed that in the latter they all have pyranose structures and are linked to other residues through positions 1 and 4 as in the case of pectic acid (Luckett and Smith, *loc. cit.*; Beaven, Hirst, and Jones, J., 1939, 1865). Furthermore the demonstration of the presence of a relatively large proportion of xylose and fucose units, which constitute terminal residues, together with the isolation of a monomethyl methylgalacturonoside clearly indicates the branched chain character of the molecular complex as in arabic acid (Smith, J., 1939, 1724). It is also of interest that this is one of the few gums shown to contain galacturonic acid residues.

Experimental.

Properties of Gum Tragacanth.—The gum, as supplied commercially, is a white powder which shows an acid reaction to litmus. The crude material contains 3—8% OMe. A small sample of the gum was warmed with potassium hydroxide solution, the solution was acidified with acetic acid and poured into excess alcohol to precipitate the gum. The product, separated by means of the centrifuge, was washed and dried (Found: OMe, 4·6%).

A sample of the gum was freed from inorganic material by three precipitations from aqueous hydrochloric acid solution with alcohol. Two further precipitations from aqueous alcohol served to remove mineral acid and the purified gum was dried (Found: Equiv., 442).

Fractionation of Gum Tragacanth.—The gum (10 g.) was shaken with water (700 c.c.) and potassium hydroxide (30 c.c. of a 60% solution) for 20 hours. The viscous colourless solution was centrifuged to remove a small amount of insoluble material, acidified with glacial acetic acid and poured into excess alcohol. The precipitate was redissolved in water, acidified with dilute hydrochloric acid and again poured into alcohol. Purification was effected by repeated precipitation of an aqueous solution of the gum with alcohol. The material was then dissolved in water (1 l.) and alcohol was added to give a precipitate, A, which was separated on the centrifuge. The supernatant aqueous alcoholic solution was poured into alcohol and the precipitated gum (fraction B) removed and dried, 1·71 g., $[\alpha]_D^{18°}$ +30° (*c*, 0·7). Refractionation of the precipitate, A, in the same way gave a precipitate, A_1, 3·3 g., $[\alpha]_D^{18°}$ +107° (*c*, 0·6) and an aqueous alcoholic solution which yielded A_2 [0·92 g., $[\alpha]_D^{18°}$ +60·0° (*c*, 0·7)] on addition of excess alcohol. The specific rotations were observed in 0·1N NaOH and indicated that the gum is a mixture.

Methylation of Gum Tragacanth.—Commercial gum tragacanth (120 g.) was methylated in portions of 20 g. by dissolving each portion of the material in sodium hydroxide (450 c.c. of a 30% solution) followed by the gradual addition of methyl sulphate (150 c.c.) over 6 hours at room temperature with vigorous stirring. The stirring was continued for a further 4 hours after the addition of all the methyl sulphate. The solution was cooled in an ice bath, neutralised with dilute sulphuric acid, then dialysed against a continuous stream of tap-water to remove the sodium hydroxide and evaporated under reduced pressure to obtain the partially methylated compound which was subjected to a further methylation at 35°. For this second treatment with methyl sulphate the material from 30 g. of the gum was treated with sodium hydroxide (300 c.c. of 30% solution) and methyl sulphate (100 c.c.). After the second methylation part of the methylated gum separated with some sodium sulphate on acidification of the reaction mixture. The solid was filtered off and the partly methylated gum extracted with 95% alcohol while the filtrate was subjected to dialysis at 40°. The dialysed solution and alcoholic extract were combined, neutralised with sodium hydroxide solution and concentrated to a small volume under reduced pressure. Four further methylations were applied, using the method described for the second methylation. In all these methylations, acetone was added as required to keep the methylated material in solution.

Separation of methylated gum into fractions A, B, and C. The crude methylated product from gum tragacanth (120 g.) was treated with aqueous acetone (90%) to remove sodium sulphate. The latter was separated and the neutral aqueous acetone solution evaporated to dryness. Extraction of the residue with hot water left a hot water insoluble material, B. During the dialysis of the hot water extract to remove sodium sulphate there separated a light brown precipitate, C, which was removed on the centrifuge. The solution from which C had been separated was evaporated to dryness under diminished pressure to give fraction, A (41 g.; $[\alpha]_D^{18°}$ +89° in 50% aqueous acetone; equiv., 445; OMe, 33·6%). The hot water insoluble material, B, was dissolved in cold water and subjected to dialysis in the presence of dilute sulphuric acid when there separated more of the material C. When all the mineral acid had passed through the membrane the material C was removed, combined with that previously isolated and the aqueous solution evaporated to dryness under reduced pressure to give fraction, B (35 g.; $[\alpha]_D^{18°}$ −14° in 50% aqueous acetone; equiv., 1515; OMe, 37·6%).

Purification and Preliminary Fractionation of the Methylated Tragacanthic Acid, A.—The acidic substance, A, dissolved in alcohol was treated with a slight excess of barium hydroxide (tested with phenolphthalein). The mixture, after being at room temperature for several hours, was neutralised with carbon dioxide and filtered. The filtrate was concentrated to a small volume under reduced pressure and more barium hydroxide solution was then added. After keeping at room temperature for 2 hours the solution was neutralised as before and evaporated to dryness under reduced pressure at 50—60°. The product, a pale brown solid, consisting chiefly of the barium salt of the methylated tragacanthic acid, was extracted with boiling acetone. Evaporation of the extract gave a negligible amount of syrupy material which was rejected.

The free acidic compound, A, was liberated from the barium salt in the following way. The barium salt was dissolved in the minimum amount of water and a slight excess of oxalic acid (calculated from the equiv. of A) was added. The precipitated barium oxalate was filtered off and the filtrate evaporated to dryness under reduced pressure at 40°. The residue was taken up in absolute alcohol. A small amount of insoluble material (2·8 g.) was removed by means of the centrifuge. It contained barium and was probably undecomposed barium salt. The addition of ether to the alcoholic solution caused the precipitation of fraction A_1 (21·3 g.), which was removed on the centrifuge and dried. The solution was evaporated under reduced pressure and the residue dissolved in the minimum amount of alcohol. On the addition of ether, fraction A_2 (5·0 g.) was precipitated. The solution was decanted and treated with light petroleum to give A_3 (5·9 g.). The mother liquor was evaporated to dryness under reduced pressure at 50° and the residue was dissolved in water and dialysed until free from oxalate. After removal of the solvent only a negligible amount of material remained.

Fractionation of the Methylated Polysaccharide B.—The substance B was extracted with chloroform, the insoluble material constituting B_1. Fractions B_2 and B_3 were successively precipitated from the chloroform solution by the gradual addition of ligroin and fraction, B_4, was obtained by evaporation of the chloroform–ligroin mother liquors. The properties of these fractions are given in Table I.

Table I.

Fraction.	Wt., g.	OMe, %.	$[\alpha]_D^{18°}$ in H_2O.	Ash, %.	Equiv.
B_1	3·6	23·1	—	8·7	—
B_2	2·6	31·1	+19	0·5	420
B_3	21·7	31·2	−26	Nil	2390
B_4	6·5	44·4	−86	0·8	6890

Each of the fractions B_2, B_3, and B_4 was treated with barium hydroxide in the manner described for the material, A. From the barium salt of B_2 there was extracted 0·7 g. of acetone-soluble material corresponding to B. The residual barium salt was converted to the free acid by treatment with oxalic acid to give fraction, A_4, of tragacanthic acid. Treatment of B_3 with barium hydroxide gave 20·1 g. of the acetone-soluble neutral polysaccharide material, B. The small residual amount of barium salt was examined later. Fraction, B_4, was completely soluble in acetone after treatment with barium hydroxide; its high methoxyl content and syrupy nature indicated that it was partly degraded material and it was not further examined.

Finally, the barium salt from B_3 (see above) and the undecomposed barium salt from A were combined with fraction, B_1, and with 4·9 g. of material obtained from a trial methylation and fractionation. The combined materials were dissolved in water and subjected to treatment with barium hydroxide in the manner previously described. A negligible amount of material was extracted with acetone from the barium salt so formed. Decomposition of the latter with oxalic acid gave a solid which was extracted with alcohol. The addition of light petroleum to the alcoholic solution gave fraction, A_5. Evaporation of the mother liquor gave a residue which was dissolved in water freed from oxalate by dialysis. Evaporation of the solution gave fraction A_6, consisting of incompletely methylated material (see Table II), and was not examined further. The properties of the fractions of the tragacanthic acid, A, are given in Table II. The properties of the neutral methylated substance, B, are described in Part III of this series.

Fractionation of A_1.—Fraction, A_1, was extracted with 50% alcoholic acetone (200 c.c.). The insoluble material was separated and dried giving fraction, a_1. The addition of ligroin to the solution gave fraction, a_2, while evaporation of the mother liquor furnished fraction, a_3. The properties of the fractions are set out in Table II.

TABLE II.

Fraction.	Wt., g.	OMe, %.	$[\alpha]_D^{18°}$ in 50% aqueous acetone.	Equiv.
a_1	3·3	31·2	+103°	565
a_2	3·2	33·3	+101	545
a_3	9·9	33·6	+ 83	470
A_2	5·0	34·3	+ 86	420
A_3	5·9	35·7	+ 55	490
A_4	0·3	—	+ 83	430
A_5	4·9	31·3	+115	430
A_6	4·4	28·8	—	770
A_7	3·1	33·4	+ 70	495
A_8	2·8	32·0	+ 83	450

(Fractions A_7 and A_8 were obtained from a preliminary methylation of a small sample of the gum and separated in the manner described above.)

The Methyl Ester of Methylated Tragacanthic Acid.—(i) A solution of fraction A_6 (4·7 g.) in the minimum amount of methanol was boiled with methyl iodide (20 c.c.) in the presence of silver oxide (1 g.). Further additions of silver oxide (1 g.) were made at hourly intervals over six hours. The product, isolated by extraction with methanol, was given a second treatment with methyl iodide and silver oxide in the presence of a little methanol. In this way a substance completely soluble in methyl iodide was obtained. A third treatment of the substance with methyl iodide and silver oxide served to complete the methylation and there was obtained the methyl ester of methylated tragacanthic acid (3·7 g.; OMe, 38·0%). (ii) Fractions, a_3 and A_2, were combined and dissolved in dry methyl alcohol. Esterification was effected by the addition of an ethereal solution of diazomethane. The neutral solution was evaporated to dryness and the residue subjected to three methylations with methyl iodide and silver oxide. The fully methylated product (11·63 g.) had OMe, 40·0%.

Hydrolysis of the Methyl Ester of Methylated Tragacanthic Acid.—(I) In a preliminary hydrolysis the material (3·7 g.) from esterification (i) was boiled for 8 hours with methanol (115 c.c.) containing 3% dry hydrogen chloride. The solution was neutralised with silver carbonate and filtered, the residue being repeatedly washed with boiling methanol. The combined washings and filtrate were evaporated under reduced pressure and a viscous syrup remained. This was dissolved in water and heated for 1½ hours at 50° with three times the theoretical amount of barium hydroxide solution. Excess alkali was neutralised with carbon dioxide and the solution filtered after the addition of charcoal, the residue being well washed with hot water. Evaporation of the filtrate gave a yellow solid consisting of a mixture of methylated glycosides and the barium salts of the methylated galacturonosides. The former were removed by exhaustive extraction with ether; evaporation of the extract gave a colourless mobile syrup, D (0·56 g.). The residual barium salt amounted to 3·0 g. (Found: OMe, 19·8; Ba, 17·8%). Distillation of the methylated glycosides, D, gave fractions D_1 (0·22 g.), b. p. (bath temp.) 90—92°/0·01 mm., $n_D^{17°}$ 1·4440, and D_2 (0·19 g.), b. p. (bath temp.) 117—120°/0·01 mm., $n_D^{17°}$ 1·4545, OMe, 48·0%. Fraction D_1 crystallised spontaneously. The crystals of *trimethyl α-methyl-*l*-fucoside* were separated by trituration with ligroin and, after recrystallisation from the same solvent, had m. p. 85—92° (Found: OMe, 55·8. $C_{10}H_{20}O_5$ requires OMe, 56·3%).

Examination of the Barium Salt.—The yellow solid (3·0 g.) was converted to the methyl ester by boiling with dry methanol containing 1% hydrogen chloride for 6 hours. The precipitated barium chloride was separated from the cooled solution; the filtrate was neutralised with silver carbonate and filtered, the residue being repeatedly washed with hot methanol. Evaporation of the filtrate and washings gave a dark brown syrup which was dissolved in acetone. The addition of ether to the solution served to remove the inorganic impurities as a flocculent precipitate which was separated. Evaporation of the filtrate gave a clear yellow syrup which was distilled giving fractions, D_3 (0·47 g.), b. p. 150—161°/0·06 mm., $n_D^{17·5°}$ 1·4550, OMe, 48·6%, equiv., 300; D_4 (0·31 g.), b. p. 161—185°/0·06 mm., $n_D^{17·5°}$ 1·4740, OMe, 40·0%, equiv., 260; D_5 (0·47 g.), b. p. 270—300°/0·06 mm., OMe, 37·1%, equiv., 210. Fraction, D_4, crystallised slowly. The crystals of a monomethyl methylgalacturonoside were separated by trituration with ether and alcohol and, after recrystallisation from a mixture of ether, ligroin and alcohol, had m. p. 159°.

(II) Hydrolysis was effected by boiling the material (11·6 g. from esterification, ii) for 12 hours with 3% methanolic hydrogen chloride (200 c.c.). The solution was neutralised with silver carbonate, filtered and concentrated at atmospheric pressure to avoid loss of fully methylated pentosides. Distillation of the liquid gave fractions, E_1—E_4. The "still" residue was further hydrolysed by heating in a sealed tube with dry methanol (50 c.c.) containing 2% dry hydrogen chloride. The product was isolated in the manner previously described and distilled giving fractions, E_5—E_7. The undistillable residue (0·9 g.), carbonaceous material, was rejected.

Fraction.	Wt., g.	B. p. (bath temp.).	Pressure, mm.	$n_D^{18°}$.
E_1	2·65	94—96°	0·022	1·4435
E_2	1·53	126—130	0·03	1·4462
E_3	1·75	160—175	0·02	1·4533
E_4	0·43	197—205	0·03	1·4570—1·4658
E_5	2·0	132—155	0·04	1·4538—1·4570
E_6	1·56	160—225	0·04	1·4670—1·4690
E_7	0·31	225—270	0·04	—

Hydrolysis of Methylated Tragacanthic Acid at 114°.—Fractions, A_7 and A_8, were combined (5·0 g.) and heated in a sealed tube at 114° with dry methanol (50 c.c.) containing 2% dry hydrogen chloride. The products of hydrolytic cleavage isolated as described above were distilled giving fractions, F_1—F_6.

Fraction.	Wt., g.	B. p. (bath temp.) at 0·04 mm.	$n_D^{19°}$.	OMe, %.	Equiv.
F_1	1·2	90— 94°	1·4413	—	—
F_2	0·51	94—107	1·4500	—	—
F_3	0·12	107—130	—	—	—
F_4	0·88	150—160	1·4560	47·0	460
F_5	0·80	177—200	1·4620	38·3	260
F_6	0·73	Above 200	—	38·9	290

The undistillable residue (0·45 g.), charred material, was discarded.

Examination of the Methylated Glycosides.—Isolation of 2 : 3 : 4-*trimethyl* α-*methyl*-l-*fucoside* (I). The crystals formed in fractions E_1, E_2, F_1 and F_2 were separated by trituration at 0° with ligroin and recrystallised from the same solvent. This substance was 2 : 3 : 4-*trimethyl* α-*methyl*-l-*fucoside*, m. p. 85—92° alone and in admixture with a synthetic specimen (see Part II), $[\alpha]_D^{17°}$ −188° in water (*c*, 0·5) (Found: C, 54·1; H, 9·1; OMe, 55·8. $C_{10}H_{20}O_5$ requires C, 54·5; H, 9·2; OMe, 56·3%).

Fractionation of the remaining glycosides. After separation of the crystals of (I) from fractions, E_1, E_2, F_1 and F_2, removal of the solvent from the combined mother liquors afforded a liquid (3·3 g.) which upon fractional distillation gave :—

Fraction.	Wt., g.	B. p. (bath temp.) at 0·04 mm.	$n_D^{21°}$.	OMe, %.
G	1·44	90—105°	1·4405	55·9
H	0·44	105—120	1·4483	51·4
J	0·42	Above 120	1·4517	47·01
Residue	0·50			

Crystals of 2 : 3 : 4-trimethyl α-methyl-*l*-fucoside separated in fraction H and were removed by trituration. The remaining syrup was combined with fraction, G, and distilled.

Fraction.	Wt., g.	B. p. (bath temp.) at 0·04 mm.	$n_D^{18°}$.	OMe, %.
K	0·89	83— 85°	1·4413	57·0
L	0·19	100—105	1·4470	—
Residue	0·27	—	1·4503	52·4

Fraction L crystallised completely. After recrystallisation from ligroin, the trimethyl α-methyl-*l*-fucopyranoside had m. p. 85—92°.

Isolation of 2 : 3 : 4-*Trimethyl* d-*Xylose* (II).—When a solution of fraction K (0·88 g.) in 0·1N sulphuric acid (50 c.c.) was heated on the boiling water bath for 2 hours no change in rotation was observed. The concentration of mineral acid was then increased to 1N, and the hydrolysis continued. After 5 hours, when the rotation (initial value +11°) had reached a steady value (−14°), the solution was neutralised with barium carbonate, filtered and evaporated to dryness under reduced pressure. Extraction of the residue with ether gave a syrup (0·63 g.) which crystallised upon inoculation with 2 : 3 : 4-trimethyl *d*-xylose. Trituration with ligroin–ether followed by crystallisation from the same mixture gave 2 : 3 : 4-trimethyl *d*-xylose (0·1 g.), m. p. and mixed m. p. 86—87°, $[\alpha]_D^{19°}$ +17·5° (equilibrium value in water; *c*, 3·0) (Found: OMe, 47·9. Calc. for $C_8H_{16}O_5$: OMe, 48·4%).

After removal of the trimethyl xylose a small portion of the syrup from the mother liquors was treated with aniline in boiling ethyl alcohol. No crystalline anilide could be obtained.

Oxidation with bromine. The syrup (0·40 g.) was dissolved in water and treated with bromine (1 c.c.) at room temperature for 40 hours. The bromine was removed by aeration and the non-reducing solution neutralised with silver carbonate and filtered before and after passing in hydrogen sulphide. The clear solution was evaporated under reduced pressure at 40° and the syrupy residue heated at 100° under reduced pressure for 1 hour to effect lactonisation. The product (0·3 g.), purified by extraction with ether, gave, on distillation, a colourless liquid, b. p. 120° (bath temp.)/0·02 mm. The distillate crystallised on nucleation with 2 : 3 : 4-trimethyl δ-xylonolactone and after recrystallisation from ether–ligroin the crystals had m. p. 55° alone and on admixture with an authentic specimen. The remaining syrup in water had $[\alpha]_D$ −16° (initial value), −8° (after 1 hr.), −5° (2 hrs.), −5° (8 hrs.), +1·2° (66 hrs.), +2° (90 hrs.), +5° (162 hrs.); this seemed to indicate that the product was a mixture. No crystalline amide or phenylhydrazide was obtained.

Identification of 3 : 4-*Dimethyl Methylxyloside* (III).—When a solution of fraction J (0·41 g.) in 0·1N sulphuric acid (50 c.c.) was heated on the boiling water bath it had $[\alpha]_D$ +31° (initial value), +28° (after 1 hr.), +25° (2 hrs.), +22° (3·3 hrs.); +19° (4·6 hrs.), +18° (5·6 hrs.), +16° (6·6 hrs.). After a further ½ hour, the concentration of acid was increased to 1N and the hydrolysis continued. The solution then had $[\alpha]_D$ +12° (initial value), +4° (after 0·5 hr.), 0·0° (2 hrs.), −3° (2·5 hrs.) (constant value). The solution was neutralised with barium carbonate and the free dimethyl sugar (0·30 g.) isolated by the usual procedure (Found: OMe, 34·4. $C_7H_{14}O_5$ requires OMe, 34·8%).

3 : 4-*Dimethyl Xylonolactone* (VI).—The dimethyl sugar (0·30 g.) was treated in aqueous solution (15 c.c.) with bromine at room temperature for 40 hours and then no longer reduced Fehling's solution. The lactone (0·22 g.), isolated in the usual way, gave, on distillation, a colourless liquid, b. p. (bath temp.) 130°/0·008 mm., $n_D^{18°}$ 1·4670, which crystallised spontaneously. The crystals of 3 : 4-*dimethyl* δ-*xylonolactone* after recrystallisation from ether had m. p. 68°, $[\alpha]_D^{18°}$ −56° (initial value in water; *c*, 1·0), −54° (after 2 hrs.), −45° (19 hrs.), −43° (24 hrs.), −36° (43 hrs.), −27° (65 hrs.; constant value) (Found: OMe, 35·1. $C_7H_{12}O_5$ requires OMe, 35·2%).

One treatment of the crystalline lactone (10 mg.) with silver oxide (0·1 g.) and methyl iodide (2—3 c.c.) gave a crystalline product which after recrystallisation from ether–ligroin had m. p. 56° alone and on admixture with an authentic specimen of 2 : 3 : 4-trimethyl *d*-δ-xylonolactone.

The Amide of 3 : 4-*Xylonic Acid* (VIII).—3 : 4-Dimethyl xylonolactone (10 mg.) was dissolved in dry methyl alcohol and the solution, after saturation with ammonia at 0°, was treated at 0° for 24 hours. Removal of solvent by evaporation in vacuo gave the amide as a syrup. The syrupy amide was dissolved in water (0·2 c.c.) and a solution of sodium hypochlorite (0·03 c.c.; 1·5N) added. The mixture was kept at 0° for ½ hour. The excess hypochlorite was destroyed by the addition of one drop of a solution of sodium thiosulphate. Addition of sodium acetate to the solution, until the

latter became alkaline, followed by semicarbazide hydrochloride gave, within one minute, a white precipitate of hydrazodicarbonamide, m. p. and mixed m. p. 256° (decomp.); yield, based on the lactone, almost quantitative (Weerman test).

Examination of the Methyl Esters of the Methylated Galacturonosides.—Identification of 2 : 3-*dimethyl* β-*methylgalacturonoside.* The properties of fractions F_4, F_5, and F_6 (see table) indicated that they were composed of partly methylated esters and a small amount of methylated glycosides. An attempt to remove the latter was carried out as follows. The esters were digested with a slight excess of barium hydroxide solution at 50° for 1 hour and then the solution was neutralised with carbon dioxide, filtered and evaporated under reduced pressure. The residue was repeatedly extracted with boiling ether to remove the partly methylated glycosides. The ethereal extract yielded a viscous syrup (0·17 g.) on evaporation. Owing to the insolubility of the methyl glycosides in ether, this method failed and, therefore, the barium salts were reconverted into methyl esters by boiling for 10 hours with 1% methanolic hydrogen chloride. The mixture of esters and methylated glycosides, isolated in the usual way, gave on distillation :—

Fraction.	Wt., g.	B. p. (bath temp.) at 0·02 mm.	$n_D^{20°}$.	OMe, %.	Equiv.
(i)	0·72	160—163°	1·4560	47·4	410
(ii)	0·71	187—194	1·4690	41·6	250
(iii)	0·19	Above 194	1·4730	38·2	245

The equivalent weight of fraction (i) showed that the separation of methylated glycosides from the ester fraction was still incomplete.

2 : 3 : 5-*Trimethyl Methylgalactofururonoside* (X).—Fractions (i), (ii), and (iii) were combined and methylated twice with methyl iodide and distilled, giving fraction (a) (0·5 g.), b. p. 123° (bath temp.)/0·02 mm., $n_D^{17°}$ 1·4422, and fraction (b) (0·5 g.), b. p. 133—135° (bath temp.)/0·02 mm., $n_D^{17°}$ 1·4475. Fraction (b) crystallised on inoculation with the methyl ester of 2 : 3 : 5-trimethyl β-methylgalactofururonoside. The crystals were separated on porous porcelain and, after recrystallisation from ligroin had m. p. 41—42° alone and on admixture with a synthetic specimen, and $[\alpha]_D^{15°}$ −123° in methanol (*c*, 1·0).

Treatment of fraction (a) with methanolic ammonia at 0° for 24 hours followed by evaporation of the solvent gave the amide of 2 : 3 : 5-trimethyl β-methylgalactofururonoside. This was separated by trituration with a mixture of ether and ligroin and, after recrystallisation from ether–alcohol–ligroin, the amide had m. p. 105° alone and in admixture with a synthetic specimen, and $[\alpha]_D$ −144° in water (*c*, 1·0) (Found : C, 48·1; H, 7·4; N, 5·5; OMe, 49·0. Calc. for $C_{10}H_{19}O_6N$: C, 48·2; H, 7·6; N, 5·6; OMe, 49·8%).

The Amide of 2 : 3-*Dimethyl* β-*Methylgalacturonoside.*—Fractions E_5, E_6, and E_7 were combined (3·9 g.) and digested at 50° with slight excess of barium hydroxide (tested with phenolphthalein) and the barium salts formed by neutralisation of the solution with carbon dioxide followed by filtration and removal of the solvent under reduced pressure. These barium salts of the partly methylated galacturonosides were extracted with the minimum amount of dry methanol and filtered to remove the small amount of barium carbonate. A mixture of ether and ligroin was gradually added to the methanolic solution of the barium salts until no further precipitate was formed. The barium salts were separated on the centrifuge and repeatedly washed with ether containing a little alcohol in order to remove the last traces of the methylated glycosides. This operation served to remove a small quantity of methylated glycoside. The barium salts (3·9 g.) were dried and converted into the methyl esters by boiling for 6 hours with 1% methanolic hydrogen chloride. The solution was neutralised with silver carbonate, filtered and evaporated under diminished pressure to give a syrup (3·0 g.) which upon distillation gave :—

Fraction.	Wt., g.	B. p. (bath temp.) at 0·04 mm.	n_D.	OMe, %.	Equiv.
E_9	0·7	140—145°	1·4540	51·8	235
E_{10}	0·6	145—151	1·4535—1·4596	47·5	255
E_{11}	1·2	Above 160	1·4590	—	270

Treatment of fraction E_9 with methanolic ammonia (0·06 g.) gave the crystalline amide (IX) of 2 : 3-dimethyl β-methylgalactofururonoside. The crystals were separated by trituration with alcohol and ether, and after recrystallisation from ethyl acetate had m. p. and mixed m. p. 122°, $[\alpha]_D^{16°}$ −146° in water (*c*, 1·2) (Found : C, 46·5; H, 6·8; N, 6·5. Calc. for $C_9H_{17}O_6N$: C, 46·0; H, 7·3; N, 6·0%).

Two methylations of fraction E_{10} with methyl iodide and silver oxide gave the methyl ester of 2 : 3 : 5-trimethyl methylgalactofururonoside (0·31 g.), b. p. 120—125° (bath temp.)/0·03 mm., $n_D^{20°}$ 1·4430—1·4475, which crystallised on inoculation with the crystalline methyl ester of 2 : 3 : 5-trimethyl β-methylgalactofururonoside. After separation as previously described, the crystals of (X) had m. p. and mixed m. p. 42—43°, $[\alpha]_D^{18°}$ −117° in water (*c*, 2·0).

2 : 3-*Dimethyl Mucic Lactone Methyl Ester* (XI).—Fraction E_9 (0·28 g.) was heated with concentrated nitric acid (5 c.c.) at 50° for ½ hour and then at 70—80° for 2 hours. The solution was diluted with water and distilled under diminished pressure, fresh additions of water being made from time to time until most of the nitric acid had been eliminated; the last traces were removed by the simultaneous addition and distillation of methanol. The syrup thus obtained was dried and esterified by boiling for 6 hours with 1% methanolic hydrogen chloride (20 c.c.). The solution was neutralised with silver carbonate, filtered and evaporated under reduced pressure to give a syrup (0·12 g.) which distilled as a colourless liquid, b. p. (bath temp.) 150°/0·02 mm. The distillate crystallised completely on inoculation with 2 : 3-dimethyl mucic lactone methyl ester and, after recrystallisation from ether, the crystals had m. p. and mixed m. p. 91°, $[\alpha]_D^{17°}$ −56° in water (*c*, 1·0).

Isolation of the Methyl Ester of a Monomethyl Methylgalactopyruronoside.—The crystalline material formed in fractions D_4 and E_7 was separated by trituration with alcohol–ether and after recrystallisation from alcohol–ether–ligroin had m. p. 159° and $[\alpha]_D^{15°}$ −36° in water (*c*, 1·2) (Found : C, 46·0; H, 7·0; OMe, 37·6. $C_9H_{16}O_7$ requires C, 45·7; H, 6·8; OMe, 39·4%).

Methylation of the crystalline *methyl* ester of monomethyl methylgalactopyruronoside (17 mg.) gave the methyl ester of 2 : 3 : 4-trimethyl β-methylgalactopyruronoside, m. p. 96—99°, $[\alpha]_D^{14°}$ −16° in methanol (*c*, 1·5) (after one recrystallisation from ether–ligroin). It gave no depression of the m. p. when mixed with an authentic specimen of the methyl ester of 2 : 3 : 4-trimethyl β-methylgalactopyruronoside. The constituents of fraction E_3, E_4, and E_{11} remain to be identified.

The Constituent, C, of Gum Tragacanth.—This material, which separated during the dialysis of the methylated gum, was dried and dissolved in acetone. The solution was poured into ligroin and the precipitated brown amorphous mass separated. Evaporation of the mother liquor under reduced pressure gave a glassy residue (5 g.; OMe, 20·7%). To this was added 6·5 g. of a similar product from a previous methylation and the combined material was treated with a slight excess of barium hydroxide (tested with phenolphthalein) at 50° for 10 minutes. The solution was neutralised with carbon dioxide and filtered after the addition of charcoal. The filtrate was evaporated under reduced pressure

and the residue extracted with a 50% mixture of acetone and ether. Evaporation of the extract gave a brown glassy residue, $[\alpha]_D^{18°}$ −40° in aqueous acetone, OMe, 32·0%. Two treatments with Purdie's reagents gave a product having OMe, 33·7%.

The material was dissolved in acetone (15 c.c.) and ether (20 c.c.) was added followed by ligroin (400 c.c.). The liquid was decanted from the precipitated syrup (C_1) and freed from solvent giving a pale yellow glassy solid, (C_2) (3·4 g.; OMe, 33·9%).

Hydrolysis of fraction, C_2. When a solution of C_2 in 3% methanolic hydrogen chloride was boiled the rotation changed from −16° to −14° reaching a constant value in 3½ hours. The solution was neutralised with silver carbonate and filtered. Evaporation of the filtrate gave a syrup (2·9 g.) which upon distillation gave: fraction (i), a mobile liquid (1·4 g.), b. p. (bath temp.) 113°/0·03 mm., $n_D^{18°}$ 1·4445; fraction (ii), a clear viscous liquid setting to a hard glass, b. p. (bath temp.) 300°/0·03 mm., $[\alpha]_D^{18°}$ +25° in ethyl alcohol (*c*, 0·7) (Found: OMe, 7·8%). No decomposition was observed.

Examination of fraction (i). The mobile syrup (0·47 g.) was twice methylated with methyl iodide and silver oxide and distilled, b. p. (bath temp.) 93°/0·004 mm., $n_D^{15°}$ 1·4380, $[\alpha]_D^{18°}$ +36° in water (Found: OMe, 55·9. Calc. for $C_{10}H_{20}O_5$ (trimethyl methylmethylpentoside): OMe, 56·3%). A solution of this syrup (0·26 g.) in 0·1N sulphuric acid (20 c.c.) was heated on the boiling water bath and showed $[\alpha]_D$ +28° (initial value); +21° (after ½ hour); +17° (1 hr.) (constant value). No change in rotation was observed when the acid concentration was increased to 1N and the solution heated on the boiling water bath. The free sugar isolated in the usual way (0·15 g.) had $[\alpha]_D^{16°}$ +11° in alcohol (*c*, 3·0) (Found: OMe, 42·0. Calc. for a trimethyl methylpentose $C_9H_{18}O_5$: OMe, 45·1%).

Examination of fraction (ii). One treatment of a portion of fraction (ii) with methyl iodide and silver oxide gave a glassy solid (Found: OMe, 11·1%). This material (0·2 g.) was not affected by treatment with 2% methanolic hydrogen chloride at 114°. Fraction (ii) did not give a Molisch test for carbohydrates. With concentrated sulphuric acid it gave a red colour and no charring was observed. It did not react with concentrated nitric acid. The material did not decolourise a solution of bromine in carbon tetrachloride and it showed no selective absorption in the ultra-violet region of the spectrum (examined in a concentration of 14 mg. % in ethanol). It is possible that this material is of a steroid nature (Found: C, 73·2; H, 9·3%; *M*, by depression of the m. p. of camphor using $K = 50$, 680).

The authors thank Professor W. N. Haworth, F.R.S., for his interest in this work.

A. E. Hills Laboratories, The University, Edgbaston, Birmingham. [*Received, July 2nd*, 1945.]