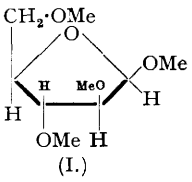
195. *The Chemistry of Gum Tragacanth. Part III.*

By Sybil P. James and F. Smith.

The neutral constituent of gum tragacanth, separated as the methyl polysaccharide (B) (see Part I of this series), undergoes smooth hydrolysis with methanolic hydrogen chloride. Separation of the resulting mixture of methylglycosides by fractional distillation demonstrated the presence of 2 : 3 : 5-trimethyl methyl-*l*-arabofuranoside, 2 : 3-dimethyl methyl-*l*-arabinoside, β-methyl-*l*-arabopyranoside and a dimethyl methylhexoside. The high negative rotation of the methylated polysaccharide together with its ease of hydrolysis lend support to the view that the arabinose units are of the furanose type. The isolation of the dimethyl hexose shows that the polysaccharide is not a simple araban (which affords tri-, di-, and mono-methyl arabinose), a view also supported by the isolation of β-methyl-*l*-arabopyranoside as one of the cleavage products.

Investigations into the chemistry of gum tragacanth reported in Part I of this series (James and Smith, this vol., p. 739) have revealed that this gum is composed of three main constituents, namely, tragacanthic acid, a neutral polysaccharide designated (B), and a third product (C) which appeared to be possibly steroid in nature. This communication deals with the chemistry of the neutral polysaccharide (B). Methylation of the gum enabled (B) to be separated in the form of its methyl derivative. Treatment of the latter with methyl iodide and silver oxide completed its methylation and fractional precipitation of the methylated polysaccharide from an acetone solution with ligroin demonstrated its essential homogeneity. The methyl derivative of the neutral polysaccharide had $[\alpha]_D$ −92° in methanol and OMe, 39·8%.

When the methylated polysaccharide was boiled with 2% methanolic hydrogen chloride simultaneous hydrolysis and glycoside formation occurred rapidly. This procedure afforded a mixture of glycosides which was separated into its components by fractional distillation. The presence of 2 : 3 : 5-trimethyl methyl-*l*-arabofuranoside (I), 2 : 3-dimethyl methyl-*l*-arabinoside (II), β-methyl-*l*-arabopyranoside (III), and a dimethyl methylgalactoside in the mixture of glycosides has been established.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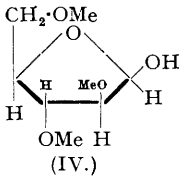
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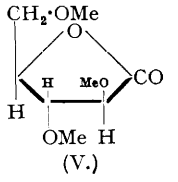
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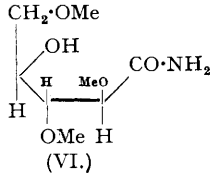
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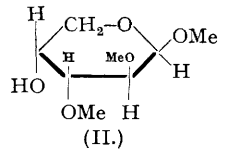
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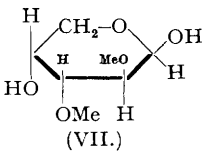
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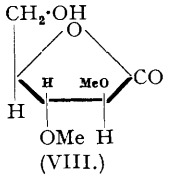
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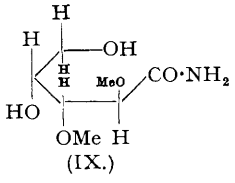
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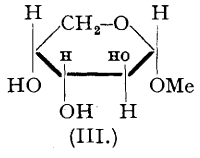
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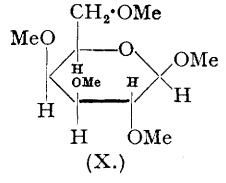
(X.)

The following reactions served to demonstrate the presence of 2 : 3 : 5-trimethyl methyl-*l*-arabofuranoside (I). Hydrolysis of (I) with dilute acid gave the corresponding free sugar (IV) which on treatment with bromine was converted to 2 : 3 : 5-trimethyl-γ-arabonolactone (V) recognised by its properties and by its transformation into the crystalline amide (VI) with methanolic ammonia. This amide was identical with an authentic specimen of 2 : 3 : 5-trimethyl-*l*-arabonamide.

The 2 : 3-dimethyl-*l*-arabonamide (II) was identified by a similar series of reactions. Hydrolysis of (II) with dilute acid led to the formation of the free sugar (VII) which on treatment with bromine gave 2 : 3-dimethyl arabonolactone (VIII). This in turn furnished crystalline 2 : 3-dimethyl-*l*-arabonamide (IX) which proved to be identical with an authentic specimen (Hirst and Jones, J., 1938, 504; Smith, J., 1939, 753).

The β-methyl-*l*-arabopyranoside (III) which crystallised from the higher boiling fractions obtained on distillation of the mixture of glycosides was recognised by its rotation, m. p., and by comparison with an authentic specimen prepared by the method of Purdie and Rose (J., 1906, 1207).

A dimethyl methylgalactoside was shown to be present inasmuch as a fraction of the hydrolysate, which had a methoxyl content corresponding to that of a dimethyl methylhexoside, yielded on methylation with methyl iodide and silver oxide 2 : 3 : 4 : 6-tetramethyl methylgalactoside (X). The latter was recognised by the fact that upon hydrolysis it afforded 2 : 3 : 4 : 6-tetramethyl galactose which gave on treatment with aniline in alcohol the known 2 : 3 : 4 : 6-tetramethyl galactose anilide. In view of the methoxyl content of the mixture of glycosides remaining after the removal of β-methyl-*l*-arabopyranoside it is unlikely that any monomethyl methylpentoside is present. Nevertheless, careful search for such a product will made in future investigations.

From these results it is evident that the methylated polysaccharide examined consists of arabinose and galactose units. The isolation of 2 : 3 : 5-trimethyl methyl-*l*-arabofuranoside (I) as a product of hydrolytic cleavage of the methylated polysaccharide with methyl alcoholic hydrogen chloride proves that in this polysaccharide there are present units of the furanose type which constitute terminal residues and which are linked to the main structure only through their reducing groups. From the proof of the existence of 2 : 3-

dimethyl methyl-*l*-arabinoside (II) as a product of hydrolysis it is possible that this derivative arises from either furanose or pyranose residues. If the dimethyl derivative is derived from furanose units these must be joined to the main structure through positions 1 and 5 whereas if they arise from pyranose residues then the latter are joined to other groups in the complex through positions 1 and 4. In view of the relatively rapid rate at which hydrolysis of the methylated polysaccharide is effected with acid methanol accompanied by liberation of almost all the arabinoses units it is believed that the arabinose units which afford 2 : 3-dimethyl arabinose are also of the furanose ring type. The identification of crystalline β-methyl-*l*-arabopyranoside (III) as a product of hydrolysis of the methylated polysaccharide is interesting from a structural point of view because it means that the arabinose units must be joined through all available hydroxyl groups and must therefore be mutually joined to four other sugar residues (cf. Beaven, Hirst, and Jones, J., 1939, 1865). There is no evidence at this stage which indicates the type of ring structure possessed by these units of arabinose which are isolated after hydrolysis in the form of crystalline β-methyl-*l*-arabopyranoside. It would appear, however, that this polysaccharide present in gum tragacanth is not of the simple araban variety, a view also supported by the identification of galactose in the form of a dimethyl derivative as a cleavage fragment. When opportunity arises it is our intention to return to a study of this polysaccharide in order to elucidate further the significance of the formation of the various cleavage fragments encountered in this preliminary investigation.

Experimental.

The Neutral Methylated Polysaccharide from Gum Tragacanth. Completion of the Methylation with Methyl Iodide and Silver Oxide.—The methylated polysaccharide (B) (20 g.; OMe, 37·3%) obtained during the methylation of crude gum tragacanth with methyl sulphate and sodium hydroxide (see Part I) was subjected to methylation with methyl iodide and silver oxide in the usual way. After each methylation the product was isolated by means of acetone and the methoxyl content determined. The methoxyl value was constant after the second methylation (Found : OMe, 38·9%).

Fractionation of the Methylated Polysaccharide.—The fully methylated material (13·1 g.) was dissolved in acetone and fractions A_1, A_2, and A_3 were precipitated from the solution by graded addition of ligroin. The supernatant liquid was decanted from each fraction precipitated and the latter was washed by decantation with a mixture of ether and ligroin and dried under reduced pressure at 40° (bath temp.). Evaporation of the mother liquor gave a syrup (A_4) which probably consisted of degraded material and was not further examined at this stage. Fraction A_1 (6·1 g.; $[\alpha]_D$ −71° in 50% aqueous acetone) which contained inorganic impurities was refractionated by dissolving in acetone and adding ligroin until a small precipitate formed. This precipitate which was dark coloured and contained inorganic impurity was neglected. Evaporation of the mother liquor gave a purer specimen (5·4 g.; $[\alpha]_D$ −72° in 50% aqueous acetone) which, however, still contained inorganic impurities. These were eliminated by dissolving the material in acetone and adding ligroin. Evaporation of the mother liquor then gave an inorganic-free specimen of A_1 (3·0 g.). The properties of the fractions are tabulated below.

Fraction.	Wt., g.	OMe, %.	$[\alpha]_D$ (in 50% aqueous acetone).	Fraction.	Wt., g.	OMe, %.	$[\alpha]_D$ (in 50% aqueous acetone).
A_1	3·0	39·3	−84°	A_3	2·1	40·9	−84°
A_2	2·7	40·2	−85	A_4	2·1	—	−62

Hydrolysis of the Methylated Polysaccharide.—When a solution of the methylated derivative (0·5 g., A_4) was boiled with 4% methanolic hydrogen chloride (50 c.c.) it showed $[\alpha]_D$ −73° (initial value), +9° (after 1½ hrs.), +7° (2·75 hrs.), constant value. After 4 hours the solution was neutralised with silver carbonate, filtered and evaporated under slightly diminished pressure to give a mobile syrup (0·45 g.).

The main bulk of the fractions A_1, A_2, A_3 (7·6 g.) was then boiled for 6 hours with 2% methanolic hydrogen chloride (150 c.c.). The solution was neutralised with silver carbonate, filtered, and evaporated to dryness under slightly reduced pressure to give a non-reducing syrup (7·4 g.). In order to remove a small amount of colloidal silver the syrup (7·4 g.) was dissolved in acetone and to the solution was added an excess of ether and a small amount of charcoal. The solution was filtered and freed from solvent. The syrup was transferred to a Widmer flask and fractionally distilled giving :

Fraction.	Wt., g.	B. p. (bath temp.).	Pressure, mm.	n_D.	OMe, %.
I	2·5	132—145°	10·00	1·4350	58·9
II	1·1	120—130	0·05	1·4445	55·7
III	0·7	130—140	0·04	1·4600	46·5
IV	0·6	140—160	0·02	1·4670	43·6
V	0·7	160—185	0·02	1·4670	38·9
VI	0·4	185—190	0·04	1·4783	33·4
VII	0·3	Above 190	0·04	1·4873	40·3

The residue (0·95 g.) was boiled with methanol (20 c.c.) containing hydrogen chloride (4%) for 20 hours and the product, isolated by the method previously described, distilled giving fraction VIII (0·3 g.), b. p. (bath temp.) 123—160°/0·03 mm., n_D 1·4565—1·4683, and fraction IX (0·5 g.), b. p. (bath temp.) above 160°/0·03 mm., n_D 1·4775. Fraction I was pure 2 : 3 : 5-trimethyl methyl-*l*-arabofuranoside and since no variation of the refractive index was observed during the first fractional distillation it was not redistilled.

Refractionation of Methylated Glycosides : Fractions II, III, and IV.—Fraction II was redistilled very slowly and when the refractive index of the distillate began to change, the distillation was stopped; fraction III was then added to the still residue, the receiver changed and the distillation continued. Similarly when the refractive index of the distillate had reached that of the first drop collected in fraction IV the latter was added to the still residue and the fractionation was continued. In this manner fractions X to XIII were obtained; fraction XIV consists of the still residue which could not be distilled from the Widmer flask. The properties of the fractions are tabulated :

Fraction.	Wt., g.	B. p. (bath temp.)/0·05 mm.	$n_D^{18°}$.	OMe, %.
X	0·42	70— 80°	1·4380	59·6
XI	0·68	100—125	1·4515	49·9
XII	0·25	125—130	1·4550	46·6
XIII	0·45	135—137	1·4620	44·4
XIV	0·36	Still residue	1·4675	39·0

Examination of Fractions of Methylated Glycosides.—Identification of 2 : 3 : 5-trimethyl methyl-l-arabinoside (I): *2 : 3 : 5-trimethyl l-arabinose* (IV). When a solution of fraction (1) (1·0 g.) (Found: OMe, 58·9. Calc. for $C_9H_{18}O_5$: OMe, 60·2%) in 0·1N sulphuric acid (100 c.c.) was heated on a boiling water bath it showed $[\alpha]_D$ −82° (initial value), −78° (after 1 hr.), −54° (3·75 hrs.), −36° (7·75 hrs., constant value). The solution was neutralised with barium carbonate, filtered and evaporated to a syrup under slightly reduced pressure. The free sugar, 2 : 3 : 5-trimethyl *l*-arabinose (0·9 g.) extracted from the residue with ether had $[\alpha]_D^{18°}$ −37° (*c*, 0·94) (Found: OMe, 47·0. Calc. for $C_8H_{16}O_5$: OMe, 48·4%).

2 : 3 : 5-*Trimethyl* l-*Arabonolactone* (V).—A solution of the free sugar (0·87 g.) in water was treated with bromine (0·4 c.c.) at room temperature for 48 hours after which time the solution did not reduce Fehling's solution. The solution was freed from the excess of the bromine by aeration, neutralised with silver carbonate, filtered, treated with hydrogen sulphide, filtered and evaporated to dryness under reduced pressure at 40°. The syrup so obtained was heated for one hour at 100° under reduced pressure in order to effect lactonisation. The 2 : 3 : 5-trimethyl *l*-arabonolactone (0·58 g.) purified by extraction with ether followed by distillation had b. p. (bath temp.) 120°/0·02 mm., $n_D^{18°}$ 1·4450 (Found: OMe, 49·6. Calc. for $C_8H_{14}O_5$: OMe, 48·9%), $[\alpha]_D^{18°}$ −40° in water (*c*, 0·7) (initial value).

2 : 3 : 5-*Trimethyl* l-*Arabonamide* (VI).—Treatment of the lactone (V) with methanolic ammonia (solution saturated with ammonia at 0°) for 4 days at 0° gave in good yield the 2 : 3 : 5-trimethyl *l*-arabonamide, m. p. and mixed m. p. 138°, $[\alpha]_D^{19°}$ +23° in water (*c*, 1·6) (after recrystallisation from ethyl acetate).

Identification of 2 : 3-*Dimethyl* l-*Arabinose* (VII).—Fractions XI and XII were combined (0·83 g.) and hydrolysed by heating on a boiling water bath with 0·5N-hydrochloric acid (50 c.c.). The change in rotation of the solution was observed: $[\alpha]_D$ −11° (initial value), +57° (after ½ hr.) (constant after a further 2·25 hrs.). The solution was neutralised with barium carbonate, filtered, and evaporated to dryness. The residue of the reducing dimethyl sugar and barium chloride was extracted with boiling ether and alcohol. Concentration of the extract gave a syrup (0·66 g.), $[\alpha]_D^{16°}$ +44° in water (*c*, 0·7) (Found: OMe, 29·5. Calc. for $C_7H_{14}O_5$: OMe, 34·8%).

2 : 3-*Dimethyl* l-*Arabonolactone* (VIII).—The dimethyl sugar (0·65 g.) was treated with bromine (2·5 mols.) in aqueous solution and the resulting lactone isolated as in the case of the trimethyl γ-arabonolactone. The syrup (0·52 g.) was distilled giving: fraction (i) (0·24 g.), b. p. (bath temp.) 117—135°/0·04 mm., $n_D^{15°}$ 1·4595 (Found: OMe, 42·5%); fraction (ii), b. p. (bath temp.) 135°/0·04 mm., $n_D^{16°}$ 1·4605 (Found: OMe, 33·9. Calc. for $C_7H_{12}O_5$: OMe, 35·4%). Treatment of fraction (i) with methanolic ammonia by the usual method gave crystalline 2 : 3-dimethyl *l*-arabonamide, m. p. and mixed m. p. 161°, $[\alpha]_D^{18°}$ +19° in water (*c*, 1·2) (after recrystallisation from ethyl alcohol). No trimethyl arabonamide was detected in this preparation. Fraction (ii) was similarly treated with methanolic ammonia and this also gave the crystalline 2 : 3-dimethyl *l*-arabonamide, m. p. 161°, together with a syrup which did not crystallise.

Isolation of β-Methyl-l-arabopyranoside.—The fractions (V), (VI), (IX), (XIV) were partly crystalline and the crystals of β-methyl-*l*-arabopyranoside (0·14 g.), separated by trituration with acetone and alcohol followed by recrystallisation from a mixture of acetone and methanol, had m. p. and mixed m. p. 170° alone and in admixture with an authentic specimen, $[\alpha]_D^{21°}$ +224° in water (*c*, 1·1) (Found: C, 44·0; H, 7·0; OMe, 18·8. Calc. for $C_6H_{12}O_5$: C, 43·9; H, 7·3; OMe, 18·9%). Removal of the solvent from the combined mother liquors after separation of β-methyl-*l*-arabopyranoside gave a liquid (1·5 g.), $n_D^{17°}$ 1·4720 (Found: OMe, 45·2%).

Examination of fraction VII. This syrup (0·3 g.) (Found: OMe, 40·3. Calc. for $C_6H_{18}O_6$: OMe, 41·9%) was methylated twice with the Purdie reagents, a small amount of acetone (1 c.c.) being necessary for the first methylation as the syrup was insoluble in methyl iodide. The syrup (0·26 g.) isolated by means of ether had b. p. (bath temp.) 100—110°/0·02 mm., $n_D^{19°}$ 1·4470. When a solution of the syrupy distillate (0·14 g.) in N sulphuric acid (10 c.c.) was heated on a boiling water bath it showed $[\alpha]_D$ +100° (initial value), +107° (after 1 hr.), +105° (2 hrs.), +97° (3 hrs.), +86° (9 hrs.) (constant value). The solution was neutralised with barium carbonate and the free tetramethyl sugar isolated by the method previously described. The colourless syrup (0·1 g.) thus produced was boiled with an ethanolic solution of aniline (1 mol.) for 3 hours. When the solvent was removed by evaporation under reduced pressure the residual syrup crystallised completely. After recrystallisation from ethanol the crystals had m. p. 193° alone or in admixture with an authentic specimen of 2 : 3 : 4 : 6-tetramethyl galactose anilide.

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