## 476. *The Structure of Karaya Gum* (Cochlospermum gossypium).

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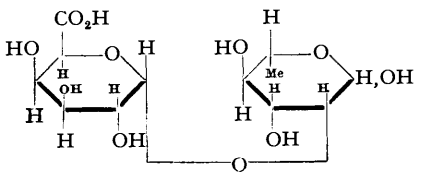
Karaya gum, from *Cochlospermum gossypium*, is a partly acetylated acidic polysaccharide, which gives on hydrolysis equimolecular proportions of L-rhamnose, D-galactose, and D-galacturonic acid, together with traces of a ketohexose. On partial hydrolysis, it gives rhamnose, galactose, galacturonic acid, 2-D-galacturonosyl-L-rhamnose, and 4-D-galacturonosyl-D-galactose, together with unidentified oligosaccharides of high uronic acid content.

Methylated karaya gum gives on hydrolysis 2 : 3 : 4-trimethyl (1 part), 3 : 4-dimethyl (1 part), and 3-methyl rhamnose (1 part), 2 : 3 : 4 : 6-tetramethyl (2 parts), 2 : 3 : 6-trimethyl (1 part), and 2 : 6-dimethyl galactose (*ca.* 1%), and a mixture of methylated uronic acids.

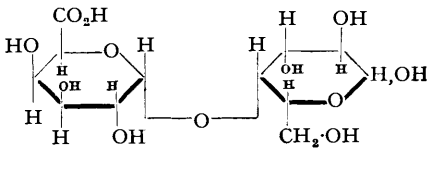
The oxidation of the deacetylated polysaccharide with periodic acid has been studied.

These investigations were carried out on a variety of karaya gum occurring as an exudate from the bark of *Cochlospermum gossypium* (family Bixineae), a small deciduous tree which grows in the forests of the North-West Himalaya. An exudate from *Sterculia urens* has also been termed karaya gum and a preliminary investigation of the gum from *S. urens* (Hough and Jones, unpublished results) has shown that the two gums are closely alike. This applies also to other gums from trees of the Sterculiaceae family, namely, *S. tormentosa* (Beauquesne, *Compt. rend.*, 1946, **222**, 1056) and *S. setigera* (Hirst, Hough, and Jones, *J.*, 1949, 3145), and is borne out by the acetyl content (18·9%), the high proportion of uronic acid residues, and the occurrence of residues of L-rhamnose, D-galactose, D-galacturonic acid, and a ketohexose. Two of these gums, namely, those from *S. setigera* and *S. urens*, have given D-tagatose on hydrolysis (see Hirst, Hough, and Jones, *loc. cit.*).

*Cochlospermum gossypium* gum, which will be referred to as karaya gum, swells in water to a bulky gel. Purification was effected by precipitation by alcohol from acidified aqueous solutions, the ash-free gum being a white, amorphous, hygroscopic powder. After hydrolysis by aqueous acid and separation of the products into neutral and acid fractions, it was found that L-rhamnose, D-galactose, and D-galacturonic acid were present in equimolecular proportions, together with a trace of a labile ketohexose. It is likely that most of this ketose is destroyed even under mild conditions of hydrolysis.



(I)



(II)

Partial hydrolysis of the gum gave a mixture of monosaccharides and acidic oligosaccharides. After methylation of the latter followed by distillation of the product, fractions were obtained which yielded on hydrolysis 3 : 4-dimethyl L-rhamnose, 2 : 3 : 6-trimethyl D-galactose, and 2 : 3 : 4-trimethyl D-galacturonic acid. Traces of lower methylated derivatives were also present. These observations indicate that the oligosaccharides obtained by this partial hydrolysis of karaya gum were mainly 2-D-galacturonosyl-L-rhamnose (I) and 4-D-galacturonosyl-D-galactose (II).

The gum was converted into its fully methylated derivative, which appeared to be substantially homogeneous. Hydrolysis with formic acid then gave a complex mixture of neutral methylated reducing sugars and methylated uronic acids. Examination of the neutral fraction by paper partition chromatography revealed six spots, corresponding to 2 : 3 : 4-trimethyl rhamnose, 2 : 3 : 4 : 6-tetramethyl galactose, 3 : 4-dimethyl rhamnose, 2 : 3 : 6-trimethyl galactose, a crystalline monomethyl rhamnose, and 2 : 6-dimethyl

galactose (see Table, p. 2336). The mixture of methylated sugars was separated into eight fractions by partition chromatography on a column of cellulose with light petroleum–butanol saturated with water as mobile phase.

The monomethyl rhamnose was chromatographically different from 4-methyl rhamnose. Its methylglycoside did not reduce potassium periodate. Although too much significance cannot be attached to the results of such oxidations when applied to methylated sugars (Greville and Northcote, *J.*, 1952, 1945), the total lack of periodate consumption suggests the location of the methyl group at $C_{(3)}$, since in the event of two contiguous hydroxyl groups, at $C_{(2)}$ and $C_{(3)}$, or $C_{(3)}$ and $C_{(4)}$, some oxidation might be expected, however incomplete.

Examination of the mixture of methylated uronic acids by paper partition chromatography revealed four spots, three cerise and one brown, on spraying with aniline oxalate. The mixture was separated into four fractions by partition chromatography on a column of celluose with butanol–glacial acetic acid saturated with water as mobile phase. The nature of these methylated uronic acids could not be ascertained with certainty but the second fraction appeared to consist of dimethyl galacturonic acids. The absence of any downward shift in optical rotation in cold methanolic hydrogen chloride indicates the absence of a free hydroxyl group on $C_{(4)}$, and suggests that this material was 2 : 4- or 3 : 4-dimethyl galacturonic acid or a mixture of the two.

On the basis of the evidence obtained it is possible to put forward the following figures as an estimate of the proportions of sugar derivatives formed on hydrolysis of methylated karaya gum: 2 : 3 : 4-trimethyl L-rhamnose (1 part), 2 : 3 : 4 : 6-tetramethyl D-galactose (2 parts), 3 : 4-dimethyl L-rhamnose (1 part), 2 : 3 : 6-trimethyl D-galactose (1 part), 3-methyl L-rhamnose (1 part), 2 : 6-dimethyl D-galactose (*ca.* 1%), and a mixture of methylated uronic acids the identity of which is still unresolved.

The deacetylated polysaccharide, on oxidation with potassium periodate, gave one mole of formic acid per 225 g. of gum, the reaction being complete in two days. This amount is in excess of that which would be liberated by the end groups known to be present in the gum and may be attributable to the oxidative degradation of uronic acid residues (Halsall, Hirst, and Jones, *J.*, 1947, 1427). On hydrolysis at this stage, the polysaccharide gave rhamnose (10·2%) and galactose (7·5%). It is not certain that this proportion of rhamnose and galactose gives an accurate representation of portions resistant to oxidation, since on oxidation for a further seven days the molecular proportion of galactose to rhamnose had decreased from 0·75 to 0·17. It is known that some methylated sugars are slowly and incompletely attacked by unbuffered periodate (Greville and Northcote, *loc. cit.*) and it is possible that the decreasing proportion of galactose as oxidation proceeds is due to a similar slow attack by the periodate.

At this stage it is not possible to formulate a unique molecular structure for the gum. The monomethyl rhamnose and monomethyl galacturonic acid, isolated from the hydrolysate of the methylated polysaccharide, must have arisen from residues which were triply-linked in the gum. It is clear, therefore, that the gum possesses a highly-branched structure. The small amount of dimethyl galactose, which may have arisen from incomplete methylation of the polysaccharide or from demethylation occurring during hydrolysis, scarcely influences the result as regards the main structural features.

Sugar residues, the presence of which has been definitely established, are as follows: Gal*p* 1 . . ., . . . 4 Gal*p* 1 . . ., Rha*p* 1 . . ., . . . 2 Rha*p* 1 . . ., . . . $\begin{matrix} 4\ \mathrm{Rha}p\ 1 \\ 2\ \ldots \end{matrix}$ (Gal*p* = D-galactopyranose, Rha*p* = L-rhamnopyranose). Evidence from the isolation of acidic disaccharides by partial hydrolysis of the gum indicates that the residues Gal*p*A 1——2 Rha*p* and Gal*p*A 1——4 Gal*p* are also structural features of the molecule. The main linkages of the D-galacturonic acid residues appear to be of the types . . . *x* Gal*p*A 1 . . . (*x* = 2 or 3) and $\begin{matrix} \cdots\ x \\ \ldots\ y \end{matrix}$ Gal*p*A 1 . . . where *y* is unknown (Gal*p*A = D-galactopyranuronic acid).

The increase in the proportion of uronic acid residues after methylation (constituting one-half of the methylated polysaccharides, compared with one-third of the unmethylated material) may be due to a weakness in the glycosidic linkage of the ketose residues, with

the result that parts of the molecule are broken off during the reaction. It is to be remembered also that the location of the acetyl groups and their function in the molecular chain are as yet unexplored.

## Experimental

B. p. recorded are bath-temperatures; optical rotations are measured in aqueous solution; solutions were evaporated under reduced pressure unless otherwise stated. Chromatography was carried out, unless otherwise stated, on Whatman No. 1 filter paper with butanol–ethanol saturated with water containing a little ammonia as mobile phase for neutral sugars, and with butanol–glacial acetic acid saturated with water in the case of uronic acids. $R_G$ values are rates relative to that of tetramethyl glucopyranose.

*Purification and Properties of Karaya Gum.*—The gum from *Cochlospermum gossypium* occurs as the partly acetylated derivative of the inorganic salt of an acidic polysaccharide (Found: OAc, 18·9%). The gum was purified in the following way. The nodules were dried *in vacuo* at 100° over silica gel and were ground to a coarse powder which was slowly stirred into hot water. The resulting gel was heated with 5% aqueous sodium hydroxide at 60° for 24 hr. The opalescent orange solution was acidified with hydrochloric acid and filtered. The filtrate was poured, with stirring, into alcohol (4 vols.), the gum being precipitated as a white fibrous mass, which was triturated with alcohol until free from chloride ions, washed with methyl alcohol, then with ether, and dried *in vacuo* at 40°, giving a white powder, $[\alpha]_D^{20}$ +64° (*c* 4·0 in N-NaOH) [Found: C, 39·6; H, 7·6; ash, 0·61%; N, nil; S, nil; halogen, nil; equiv., 470 (by titration); furfuraldehyde, liberated by boiling hydrochloric acid, equiv. to 14·8% pentosan content]. The sodium salt of the gum was non-reducing and with copper sulphate gave an insoluble copper salt. The homogeneity of the gum was established by purifying each of four nodules *via* the insoluble copper salt. The properties of the purified samples were substantially the same.

*Hydrolysis of the Acidic Polysaccharide.*—The purified polysaccharide (2·32 g.) was hydrolysed with boiling N-sulphuric acid for 18 hr. The solution was neutralised with barium carbonate, filtered, and evaporated to a brown solid, which was extracted with boiling anhydrous methyl alcohol. The extracts were evaporated to a syrup (1·31 g.), which was dissolved in water. Barium ions were removed by sulphuric acid, and the filtrate evaporated to a syrup (0·70 g.). Paper chromatographic analysis showed the presence of rhamnose and galactose on spraying with aniline oxalate, and of a trace of ketose on spraying with urea hydrochloride. Estimation by periodate oxidation showed the presence of equimolecular proportions of rhamnose and galactose.

A large-scale hydrolysis of the purified polysaccharide (10 g.) resulted in the isolation of pure crystalline samples of D-galactose and L-rhamnose. D-Galactose {m. p. 160—162°; $[\alpha]_D^{18}$ +78·4° (*c*, 4·0)} was further characterised as its methylphenylhydrazone and by conversion into mucic acid. L-Rhamnose {m. p. 90—92·5°; $[\alpha]_D^{19}$ +8·0° (*c*, 2·0)} was characterised as its benzoylhydrazone, and its identity was confirmed by an *X*-ray powder photograph. The uronic acid was identified as D-galacturonic acid by oxidation to mucic acid and also by conversion in boiling 1% methanolic hydrogen chloride into the methyl ester of α-methyl-D-galacturonoside, m. p. 140°; $[\alpha]_D$ +124·0° (*c*, 0·5).

*Methylation of Degraded Karaya Gum.*—The purified polysaccharide (27 g.) was hydrolysed with boiling N-sulphuric acid until the optical rotation reached the maximum value ($[\alpha]_D$ +80·5° after 1 hr.). The solution was neutralised with barium carbonate and evaporated to dryness, and the neutral reducing sugars were removed by extraction with boiling methyl alcohol. The residual barium salts were purified by precipitation from aqueous solution by methyl alcohol. They were methylated three times with methyl sulphate and 30% sodium hydroxide solution (yield, 7·45 g.; OMe, 36·6%) and once with Purdie's reagents, giving a yellow syrup (6·48 g.; $n_D^{19}$ 1·4667; OMe, 48·9%), which was fractionally distilled at $10^{-2}$ mm., giving fractions (i) a mobile liquid (1·07 g.), b. p. 144—170°, $n_D^{18}$ 1·4487 (OMe, 50·2%; equiv., 267), (ii) a mobile liquid (0·49 g.), b. p. 170—174°, $n_D^{18}$ 1·4546 (OMe, 50·3%; equiv., 264) (these two consisted almost entirely of methylated galacturonic acid and were discarded), (iii) a viscous syrup (0·99 g.), b. p. 225—240°, $n_D^{18}$ 1·4678, $[\alpha]_D^{15}$ +73° (*c*, 1·9 in MeOH) (OMe, 47·9%; equiv., 354), and (iv) a semi-solid (1·67 g.), b. p. *ca.* 300°, $n_D^{18}$ 1·4808, $[\alpha]_D^{15}$ +60° (*c*, 2·5 in MeOH) (OMe, 44·8%; equiv., 240). Fractions (iii) and (iv) were boiled with 7% aqueous hydrochloric acid. The products, separated into neutral sugars and the barium salts of the uronic acids, were worked up in the usual way. The barium salts were then converted into the free acids. Fraction (iii) was

shown by paper chromatography to be mainly 2 : 3 : 4-trimethyl galacturonic acid ($R_G$ 0·65), and fraction (iv) contained also dimethyl galacturonic acid ($R_G$ 0·47). Traces of lower methylated derivatives were also present. The free sugars from fractions (iii) and (iv) gave similar patterns of four spots when examined chromatographically: $R_G$ 0·82 (3 : 4-dimethyl rhamnose), 0·70 (2 : 3 : 6-trimethyl galactose), 0·55 (monomethyl rhamnose, trace), and 0·43 (2 : 6-dimethyl galactose, trace). The intensities of the spots showed that the bulk of the material consisted of 3 : 4-dimethyl rhamnose and 2 : 3 : 6-trimethyl galactose. The experiment was then repeated on a larger scale, the neutral methylated sugars being separated by partition chromatography on a cellulose column with light petroleum (b. p. 100—120°; 3 vols.) and *n*-butanol (2 vols.) as mobile phase. Two main fractions were obtained: (*a*) 3 : 4-dimethyl L-rhamnose, identified as 3 : 4-dimethyl δ-L-rhamnonolactone, m. p. 76°, $[\alpha]_D^{20}$ −152° (*c*, 0·5), and as 3 : 4-dimethyl L-rhamnonamide, m. p. and mixed m. p. 154—156°, not depressed on admixture with an authentic sample, and (*b*) 2 : 3 : 6-trimethyl D-galactose, identified as 2 : 3 : 6-trimethyl γ-D-galactonolactone, m. p. and mixed m. p. 99°. The amounts of (*a*) and (*b*) separated were in the ratio 2 : 1. The main component of the methylated uronic acid fraction was 2 : 3 : 4-trimethyl D-galacturonic acid, identified after oxidation and esterification as dimethyl 2 : 3 : 4-trimethyl mucate, m. p. and mixed m. p. 98—100°.

*Methylation of Karaya Gum.*—Methylations by methyl sulphate and sodium hydroxide were carried out in an atmosphere of nitrogen at ⩾15°. The dry powdered gum (45 g.) was slowly added with stirring to 30% aqueous sodium hydroxide (500 c.c.) at 0°. More sodium hydroxide (400 c.c.) was added, followed by methyl sulphate (560 c.c. dropwise). The mixture was stirred overnight and then dialysed for 48 hr. The solution was concentrated to small volume, and another methylation was carried out. After four methylations the partly methylated acidic polysaccharide was freed from inorganic material by dialysis. The solution was evaporated to a light brown solid (36·0 g.) (Found: OMe, 33·6; sulphated ash, 8·2%) which was dissolved in water and neutralised with silver carbonate. The silver salt was dried *in vacuo* at 60° ($P_2O_5$) and was stirred with boiling methyl iodide. After two such methylations with silver oxide and methyl iodide the methylated polysaccharide was obtained as a pale yellow solid (33·5 g.) (Found: OMe, 39·2%). This (20 g.) was separated into four portions when heated under reflux with mixtures (100 c.c. each) of light petroleum (b. p. 38—43°) and chloroform, *viz.*: Fraction I, extracted by light petroleum (90 vols.) and chloroform (10 vols.); a viscous yellow syrup (2·1 g.), $[\alpha]_D^{17}$ +38·3° (*c*, 0·533 in MeOH). Fraction II, extracted by light petroleum (85 vols.) and chloroform (15 vols); a cream-coloured powder (6·4 g.), $[\alpha]_D^{17}$ +41·2° (*c*, 0·335 in MeOH) (Found: OMe, 39·9%). Fraction III, extracted by light petroleum (80 vols.) and chloroform (20 vols.); a pale yellow powder (12·0 g.), $[\alpha]_D^{17}$ +41·2° (*c*, 0·461 in MeOH) (Found: OMe, 40·4%). Fraction IV, extracted by light petroleum (70 vols.) and chloroform (30 vols.); a yellow solid (1·5 g.), $[\alpha]_D^{17}$ +40·3° (*c*, 0·337 in MeOH) (Found: OMe, 38·5%).

The methylated polysaccharide (fraction III; 9·5 g.) was hydrolysed with 90% formic acid at 95° for 28 hr. Formic acid was removed under reduced pressure, last traces being eliminated by repeated dilution with water and evaporation. The residual solution was neutralised with barium carbonate, and the filtrate evaporated to a light brown solid which was extracted with boiling ether. The extracts were evaporated to a syrup (fraction A; 4·0 g.) containing the methylated sugars, which gave on a paper chromatogram sprayed with aniline oxalate a pattern consisting of six spots of $R_G$ 1·01 (yellow-brown), 0·88 (red-brown), and 0·82 (brown), 0·71 (red-brown), 0·55 (yellow-brown), and 0·45 (red-brown) respectively. The residue was dissolved in water and freed from barium ions by sulphuric acid, and the filtrate was evaporated to a syrup (fraction B; 4·1 g.) containing the methylated uronic acids, which gave four spots on a paper chromatogram sprayed with aniline oxalate of $R_G$ 0·82, 0·49, 0·32 (all cerise), and 0·29 (brown) respectively.

*Identification of the Neutral Sugars in Fraction A.*—The material was separated into eight fractions by partition chromatography on a column of powdered cellulose with as mobile phase 3 : 2 light petroleum (b. p. 100—120°)–*n*-butanol saturated with water. The solvent was removed from each fraction by distillation, the residue in each case taken up in water and treated with charcoal, and the filtrate evaporated to a syrup. The results are tabulated. Fraction AI gave an aniline derivative, m. p. and mixed m. p. 111—112° (Found: OMe, 34·3. Calc. for $C_{15}H_{23}O_4N$: OMe, 33·1%). In fraction AII the relative proportions of trimethyl rhamnose and tetramethyl galactose, from the optical rotation and methoxyl content of the mixture, were 15 and 85% respectively. Fraction AIII gave an aniline derivative, m. p. and mixed m. p. 192° (Found: OMe, 40·8. Calc. for $C_{16}H_{25}O_5N$: OMe, 39·9%). In

fraction AIV the relative proportions of tetramethyl galactose and dimethyl rhamnose, calculated from the optical rotation and methoxyl content of the mixture, were 55 and 45% respectively. Fraction AV was oxidised with bromine water until non-reducing. The solution was worked up in the usual way and the product converted into the amide with liquid ammonia. The product crystallised on being seeded with 3 : 4-dimethyl rhamnonamide, and had m. p. and mixed m. p. 154—156°. Fraction AVI, in 2% methanolic hydrogen chloride kept at room temperature, was observed polarimetrically: $[\alpha]_D$ +40·4° (initial); +23·3° (20 hr.); −3·8° (44 hr.); −22·0° (68 hr.); −40·4° (170 hr.). The syrup was oxidised with bromine water, giving 2 : 3 : 6-trimethyl D-galactonolactone, which crystallised from ether as needles, m. p. and mixed m. p. 98—99° (Found: equiv., 223. Calc. for $C_9H_{16}O_6$: equiv. 220). Fraction AVII (Found for 4-methyl L-rhamnose: $R_G$ 0·59) crystallised spontaneously as rosettes, m. p. 115° (after recrystallisation from ether). A portion (0·101 g.) was boiled with 1% methanolic

| Fraction | Yield (%, w/w) | $[\alpha]_D^{18}$ (*c*) | Found: OMe (%) | Sugars present | $R_G$ | Formula | Calc.: OMe (%) |
|---|---|---|---|---|---|---|---|
| AI ..... | 10·5 | 25·5° (1·1) | 44·4 | 2 : 3 : 4-Trimethyl rhamnose | 1·01 | $C_9H_{18}O_5$ | 45·1 |
| AII ... | 8·0 | 103·6° (0·734) | 51·3 | ,, ,, | 1·01 | — | — |
| | | | | 2 : 3 : 4 : 6-Tetramethyl galactose | 0·88 | — | — |
| AIII ... | 13·7 | 103·5° (1·01) [1] | 51·3 | ,, ,, ,, | 0·88 | $C_{10}H_{20}O_6$ | 52·5 |
| AIV ... | 20·4 | 68·9° (1·048) | 44·1 | ,, ,, ,, | 0·88 | — | — |
| | | | | 3 : 4-Dimethyl rhamnose | 0·84 | — | — |
| AV ..... | 1·1 | 19·7° (0·76) [2] | 31·9 | ,, ,, | 0·82 | $C_8H_{16}O_5$ | 32·3 |
| AVI ... | 16·2 | — | 41·5 | 2 : 3 : 6-Trimethyl galactose | 0·71 | $C_9H_{18}O_6$ | 41·9 |
| AVII ... | 9·5 | 39·1° (1·13) [3] | 18·4 | Monomethyl rhamnose | 0·55 | $C_7H_{14}O_5$ | 17·4 |
| AVIII... | 1·8 | — | 29·0 | 2 : 6-Dimethyl galactose | 0·45 | $C_8H_{16}O_6$ | 29·8 |

[1] Changed to 105·6° in 27 days. [2] Changed to 26·8° in 8 days. [3] Changed to 36·6° in 35 days.

hydrogen chloride (30 c.c.), $[\alpha]_D$ +15·0° (initial) changing to −51·1° (const.) in 6 hr. The methylglycoside was isolated by neutralisation of the solution with silver carbonate and evaporation of the filtrate to a syrup (0·106 g.) (Found: OMe, 32·6. Calc. for $C_8H_{16}O_5$: OMe, 32·3%). A portion (18·3 mg.) was dissolved in water (115 c.c.) and treated with 0·31M-sodium metaperiodate (2 c.c.) in the presence of potassium chloride (1 g.). After 8 days' shaking there was no uptake of oxidant. Methylation of a portion of the methylglycoside with Purdie's reagents gave (after hydrolysis to the free sugar) 2 : 3 : 4-trimethyl rhamnose, the identity of which with an authentic specimen was shown chromatographically. A portion of fraction AVII was oxidised with bromine water. The product had $[\alpha]_D$ −27·2° (initial; *c*, 0·8) ⟶ −20·1° (44 hr.) (Found: OMe, 19·6. Calc. for $C_7H_{12}O_5$: OMe, 17·6%) {cf. methyl γ-L-rhamnonolactone, $[\alpha]_D$ −28° (initial) ⟶ −21° (16 hr.) (Hough and Jones, *J.*, 1950, 1202)}. Fraction AVIII had m. p. 106—108° (after recrystallisation from ether), not depressed on admixture with 2 : 6-dimethyl galactose.

*Methylated Uronic Acids from Fraction B.*—The material was separated into four fractions by partition chromatography on a column of cellulose with 4 : 1 (vol.) butanol–acetic acid saturated with water as mobile phase. The solvent was removed from each fraction and the residues were treated in water with charcoal and the filtrates evaporated to the following pale yellow solid fractions: BI (1·325 g.), probably a dimethyl D-galacturonolactone, $R_G$ 0·82, $[\alpha]_D$ +82·8° (initial; *c*, 0·785) ⟶ +86·4° (11 days) (Found: OMe, 33·9%; equiv., 200). BII, 2 : 4(or 3 : 4)-*dimethyl* D-*galacturonic acid* (0·384 g.), $R_G$ 0·49, $[\alpha]_D$ +91·4° (initial; *c*, 0·52) ⟶ +89·4° (12 days) (Found: OMe, 27·8%; equiv., 215. $C_8H_{14}O_7$ requires OMe, 27·9%; equiv., 222). BIII (0·54 g.), $R_G$ 0·32, $[\alpha]_D$ +94·5° (initial; *c*, 0·785) ⟶ +98·1° (14 days) (Found: OMe, 18·3%; equiv., 224). BIV (0·855 g.), $R_G$ 0·29, $[\alpha]_D$ +66·9° (initial; *c*, 0·595) ⟶ +74·8° (20 days) (Found: OMe, 17·9%; equiv., 176). The OMe contents and equivalent weights of BIII and BIV indicate that they consisted mainly of monomethyl D-galacturonic acid.

A solution of a portion of BII in 1% methanolic hydrogen chloride (kept at room temperature) was observed polarimetrically: $[\alpha]_D$ +69·9° (initial; *c*, 0·8); +55·8° (41 hr.); +55·8° (4 days); +57·4 (13 days). It is improbable, therefore, that this substance contains a free hydroxyl group at $C_{(4)}$. Fractions BI, BIII, and BIV were examined with respect to (*a*) their change in optical rotation in 1% methanolic hydrogen chloride at room temperature, (*b*) the behaviour of the methyl ester of their methylglycosides on periodate oxidation, and (*c*) their conversion into esters of methylated mucic acids, but no certain identifications were achieved.

*Periodate Oxidation of Karaya Gum.*—(1) *Determination of formic acid produced.* The method of Halsall, Hirst, and Jones (*J.*, 1947, 1399, 1427) was used. The acidic deacetylated polysaccharide (104·2 mg.) was suspended in water (100 c.c.) in a 250-c.c. stoppered bottle, and

sodium metaperiodate solution (0·207M; 10 c.c.) was added. A blank was also prepared. The bottles were shaken continuously in diffuse daylight and at intervals 5-c.c. portions of the clear supernatant liquid were removed and, after the addition of ethylene glycol (1 c.c.), were titrated with 0·01N-barium hydroxide (methyl-red). A suspension of karaya gum in water was neutral to methyl-red. The following results (g. of polysaccharide/mole of $H{\cdot}CO_2H$) were obtained: 19 hr., 368; 43 hr., 260; 66 hr., 213; 115 hr., 186; 139 hr., 171; 163 hr., 164; 191 hr., 152; 257 hr., 136; 615 hr., 104.

(2) *Uptake of periodate.* In the first of the above-mentioned experiments the periodate uptake was estimated at suitable intervals. A 5-c.c. sample was diluted to 20 c.c., and saturated sodium hydrogen carbonate solution (10 c.c.) was added, followed by excess of solid potassium iodide and standard sodium arsenite solution. After 15 min. the excess of arsenite was back-titrated with standard iodine solution. The following results (g. of polysaccharide/mole of $NaIO_4$ uptake) were obtained: 19 hr., 179; 43 hr., 153; 66 hr., 134; 115 hr., 119; 139 hr., 106.

*Examination of the Hydrolysis Products from Oxidised Karaya Gum.*—The dry purified polysaccharide (0·406 g.) was oxidised with potassium periodate. The oxidation was stopped by the addition of excess of ethylene glycol after 48 hr.; 1 mole of formic acid had been liberated by 270 g. of gum. The mixture, containing the insoluble degraded polysaccharide, was dialysed and evaporated to a white solid (0·21 g.) which was hydrolysed with boiling N-sulphuric acid: $[\alpha]_D$ +17·2° (15 min.); +26·6° (35 min.); +32·4° (95 min.); +38·1° (3 hr., constant). The solution was neutralised with barium carbonate, the filtrate was evaporated to dryness, and the neutral reducing sugars were extracted by boiling methyl alcohol. Evaporation of the solvent gave a syrup (0·070 g.), which was shown to contain rhamnose and galactose in the ratio of 1 : 0·75 (mole) (quantitative chromatographic method).

Another sample of the polysaccharide (0·407 g.) was oxidised for 9 days, 1 mole of formic acid being liberated by 232 g. The oxidised polysaccharide was isolated (0·20 g.) and hydrolysed as before: $[\alpha]_D$ −25·0° (15 min.); +25·0° (2 hr., constant). Determination of the neutral reducing sugars showed the presence of rhamnose and galactose in the ratio of 1 : 0·17 (mole).

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