485. *Gum Ghatti (Indian Gum). Part IV.* Acidic Oligosaccharides from the Gum*

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The acidic oligosaccharides, which are formed on partial acid hydrolysis of gum ghatti, have been further examined. The configuration of the glycosidic linkage in 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose has been confirmed by degradation to 2-*O*-β-D-glucopyranosylglycerol. An acidic trisaccharide has been characterised as *O*-(β-D-glucopyranosyluronic acid)-(1 ⟶ 2)-*O*-D-mannopyranosyl-(1 ⟶ 2)-D-mannose.

Two aldobiouronic acids, 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose and 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, have been characterised as partial acid hydrolysis products from gum ghatti.[1] In an attempt to isolate higher acidic oligosaccharides the gum was hydrolysed under less drastic conditions, and the acidic oligosaccharides were separated from neutral sugars by adsorption on anion-exchange resin. The acids were eluted from the resin and fractionated by partition chromatography on filter sheets. In addition to the two aldobiouronic acids, only one higher oligosaccharide was obtained in sufficient quantity for detailed examination.

The configurations of the glycosidic linkages in the two aldobiouronic acids formed from gum ghatti were assigned on the basis of their optical rotations.[1] The configuration of the glycosidic linkage in 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, which is also formed from gum arabic [2] and many other gums,[3] has been established by synthesis,[4] but that of 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose, which is also formed on partial hydrolysis of several gums,[3] has not been firmly established. This aldobiouronic acid was converted into the methyl ester of the derived glycitol, which was oxidised with lead tetra-acetate and the reduced with potassium borohydride according to the procedure of Perlin and his collaborators.[5] This sequence of reactions, which resulted in degradation of the reducing sugar residue to a glycerol moiety and reduction of the glucuronic acid residue, furnished 2-*O*-β-D-glucopyranosylglycerol, thus confirming the assigned configuration.

The acidic trisaccharide was shown to be *O*-(D-glucopyranosyluronic acid)-(1 ⟶ 2)-*O*-D-mannopyranosyl-(1 ⟶ 2)-D-mannose on the basis of the following experiments. Reduction of the methyl ester methyl glycosides followed by hydrolysis gave glucose and

* Part III, G. O. Aspinall, B. J. Auret, and E. L. Hirst, *J.*, 1958, 4408.

[1] G. O. Aspinall, E. L. Hirst, and A. Wickstrøm, *J.*, 1955, 1160.
[2] S. W. Challinor, W. N. Haworth, and E. L. Hirst, *J.*, 1931, 258.
[3] F. Smith and R. Montgomery, "Chemistry of Plant Gums and Mucilages," Reinhold, New York, 1959.
[4] R. D. Hotchkiss and W. F. Goebel, *J. Biol. Chem.*, 1936, **115**, 285.
[5] A. J. Charlson, P. A. J. Gorin, and A. S. Perlin, *Canad. J. Chem.*, 1957, **35**, 365.

mannose. Partial hydrolysis of the sugar afforded the aldobiouronic acid, 2-*O*-(gluco-pyranosyluronic acid)mannose, and mannose, whilst partial hydrolysis of the glycitol (from borohydride reduction) gave the aldobiouronic acid, mannose, and mannitol. The similarity of the staining reaction with aniline oxalate to that given by 2-*O*-(β-D-gluco-pyranosyluronic acid)-D-mannose, and the absence of a colour reaction with triphenyl-tetrazolium hydroxide,[6] indicated that the reducing mannose residue was also 2-*O*-substituted. This conclusion was confirmed by partial hydrolysis of the methylated trisaccharide, which afforded crystalline 3,4,6-tri-*O*-methyl-D-mannose as the sole neutral sugar together with a methylated acidic fraction. The acidic fraction, which probably consisted largely of methylated aldobiouronic acid, could not be characterised directly by derivative formation, but reduction of the methyl ester methyl glycosides with potassium borohydride followed by hydrolysis furnished 2,3,4-tri-*O*-methylglucose and 3,4,6-tri-*O*-methylmannose, which were identified by gas chromatography of their methyl glycosides and of the methyl glycosides of their periodate-oxidation products. The characterisation of this acidic trisaccharide provides evidence for the presence of contiguous D-mannose residues in the gum. Parts of the gum molecule may contain blocks of three mannose residues linked in a similar manner, since preliminary evidence was obtained for the presence of a polymer homologous aldotetraouronic acid amongst the minor products of partial hydrolysis.

The disaccharide, 3-*O*-β-D-galactopyranosyl-L-arabinose, which is formed on partial hydrolysis of gum ghatti[7] and of *Anogeissus schimperi* gum,[8] has been assigned the β-configuration on the basis of its optical rotation. The reducing unit of the disaccharide was degraded as described by Perlin *et al.*[5] for the α-anomer and involving limited oxidation with lead tetra-acetate followed by reduction with potassium borohydride, and the crystalline 2-*O*-β-D-galactopyranosyl-L-erythritol ($[\alpha]_D$ −10°, cf. $[\alpha]_D$ +145° for the α-anomer) was isolated. A further degradation, performed in a similar way, furnished a D-galacto-pyranosylglycerol which could not be induced to crystallise but had the correct optical rotation, $[\alpha]_D$ 0°, for the β-glycoside (cf. $[\alpha]_D$ +164° for the α-anomer).

Experimental

The sample of gum was that used in previous investigations.[1] Paper chromatography was carried out on Whatman Nos. 1 and 3MM papers with the following solvent systems (v/v): (A) ethyl acetate–pyridine–water (10 : 4 : 3); (B) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (C) ethyl acetate–acetic acid–formic acid–water (18 : 8 : 3 : 9). Gas chromatography of methylated and partially methylated methyl glycosides was carried out on columns of (*a*) 15% by weight of butane-1,4-diol succinate polyester on Celite at 175°; (*b*) 10% by weight of polyphenyl ether [*m*-bis-(*m*-phenoxyphenoxy)benzene] on Celite at 200°. Retention times (*T*) are quoted relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside as an internal standard.[9] Unless otherwise stated, optical rotations were observed for water solutions at *ca.* 18°.

Partial Hydrolysis of Gum Ghatti, and Fractionation of Acidic Oligosaccharides.—Gum ghatti (60 g.) was dispersed in water (1·5 l.), a small amount of insoluble material was removed by filtration, 4N-sulphuric acid (500 ml.) was added to the filtrate, and the resulting solution was heated on a boiling water bath for 6 hr. The cooled solution was neutralised with barium hydroxide and barium carbonate, filtered, treated with Amberlite resin IR-120(H) to remove barium ions, and poured on to a column of Amberlite resin IR-45(OH). Elution of the column with water removed most of the neutral sugars, and elution of the column with 2% (later, 10%) aqueous formic acid desorbed a complex mixture (*ca.* 9 g.) of acidic oligosaccharides.

[6] D. S. Feingold, G. Avigad, and S. Hestrin, *Biochem. J.*, 1956, **64**, 351; R. W. Bailey., S A. Barker, E. J. Bourne, P. M. Grant, and M. Stacey, *J.*, 1958, 1895.
[7] G. O. Aspinall, B. J. Auret, and E. L. Hirst, *J.*, 1958, 4408.
[8] G. O. Aspinall and T. B. Christensen, *J.*, 1961, 3461.
[9] C. T. Bishop and F. P. Cooper, *Canad. J. Chem.*, 1960, **38**, 388; G. O. Aspinall, *J.*, 1963, 1676.

The acidic sugars were further fractionated by chromatography on filter sheets using solvents B and C, to give seven fractions.

Examination of Acidic Oligosaccharides.—Fraction 1. The sugar (40 mg.) was presumed to be an aldobiouronic acid in view of its greater relative chromatographic mobility in acid solvent B ($R_{galactose}$ 0·5) than in solvent A ($R_{galactose}$ 0·1). Reduction of the methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave galactose only. Since galacturonic acid has not been shown to be a constituent of the gum, the origin of galactose only in this experiment is obscure.

Fraction 2. *Degradation of* 2-O-(β-D-*glucopyranosyluronic acid*)-D-*mannose to* 2-O-β-D-*glucopyranosylglycerol.* The aldobiouronic acid (*ca.* 1 g.), $R_{galactose}$ 0·35 and 0·74 in solvents B and C, had $[\alpha]_D$ −32° (*c* 3·0) and was chromatographically indistinguishable from 2-*O*-(D-glucopyranosyluronic acid)-D-mannose from other gums. The acid (0·71 g.), as the potassium salts, was reduced with potassium borohydride (0·3 g.) in water for 20 hr. Excess of hydride was destroyed, potassium ions were removed by treatment with Amberlite resin IR-120(H), and the resulting solution was evaporated with methanol to remove boric acid as methyl borate. The resulting glycitol was refluxed with methanolic 3% hydrogen chloride for 3 hr., the solution was neutralised with Amberlite resin IR-45(OH), and the methyl ester of 2-*O*-(D-glucopyranosyluronic acid)-D-mannitol (0·53 g.) was obtained on concentration. Lead tetra-acetate (2·2 g., 3·3 mol.) was added with stirring to the methyl ester in acetic acid (50 ml.) and water (1 ml.), and the mixture was kept for 20 hr. Lead was removed from the solution by precipitation as lead oxalate followed by treatment of the filtrate with Amberlite resin IR-120(H), the solution was concentrated, and the residue reduced with potassium borohydride (0·3 g.) in water (10 ml.) for 20 hr. The reduction product was worked up as described above and a portion of the resulting mixture was chromatographed on filter sheets in solvent B, to give, as the major product, 2-*O*-β-D-glucopyranosylglycerol, $R_{galactose}$ 1·25, which after recrystallisation from methanol had m. p. and mixed m. p. (with sample kindly supplied by Dr. A. S. Perlin) 163—164°, and $[\alpha]_D$ −16° (*c* 0·5).

Fraction 3. The aldobiouronic acid (*ca.* 1 g.), $R_{galactose}$ 0·50 in solvent C, was chromatographically indistinguishable from 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose. It was recrystallised from ethanol–water and had m. p. 132—134° and $[\alpha]_D$ +14° ⟶ −14° (equil.) (*c* 0·7) (Found: C, 34·1; H, 6·0. Calc. for $C_{12}H_{20}O_{12},4H_2O$: C, 33·7; H, 6·5%) {Heidelberger and Kendall [10] give m. p. 116° (decomp. 128°), $[\alpha]_D$ −8·3° for the dihydrate}.

Fraction 4. The syrup (30 mg.), $R_{galactose}$ 0·40 in solvent C, could not be resolved into two components although it gave an elongated spot on paper chromatograms, suggesting the presence of more than one component. Partial hydrolysis furnished the two aldobiouronic acids, 2-*O*-glucuronosylmannose and 6-*O*-glucuronosylgalactose, galactose, and mannose.

Fraction 5. *Characterisation* as O-D-*glucopyranosyluronic acid*)-(1 ⟶ 2)-O-(D-*mannopyranosyl*-(1 ⟶ 2)-D-*mannose.* The sugar (420 mg.), $R_{galactose}$ 0·32 in solvent C, gave no colour reaction with triphenyltetrazolium hydroxide, but when stained with aniline oxalate gave the same characteristic orange-brown fluorescence in ultraviolet light as 2-*O*-D-glucopyranosyluronic acid)-D-mannose. Reduction of the methyl ester methyl glycosides with potassium borohydride followed by hydrolysis afforded glucose and mannose. Partial hydrolysis of the sugar yielded 2-*O*-glucuronosylmannose and mannose, and partial hydrolysis of the derived glycitol (borohydride reduction) furnished 2-*O*-glucuronosylmannose, mannose, and mannitol. The acidic oligosaccharide (390 mg.) was methylated with methyl sulphate and sodium hydroxide, and the methylated acid (200 mg.), which was isolated by extraction of the acidified solution with chloroform, was partially hydrolysed with N-sulphuric acid on a boiling water bath for 4 hr. The cooled solution was neutralised with barium carbonate, filtered, and barium ions were removed by treatment with Amberlite resin IR-120(H). Chromatography of the resulting syrup on filter sheets in solvent A furnished a neutral and an acidic fraction. The neutral fraction (50 mg.) was chromatographically and ionophoretically homogeneous, and on crystallisation from light petroleum–ether afforded 3,4,6-tri-*O*-methyl-D-mannose, m. p. and mixed m. p. 104—106°. The acidic fraction was converted into the methyl ester methyl glycosides (50 mg.), but no crystalline product could be obtained, and the ester glycosides were reduced with potassium borohydride and hydrolysed to give a mixture (32 mg.) of methylated sugars. Chromatography of the syrup showed a mixture of tri-*O*-methyl-hexoses, which could not be

[10] M. Heidelberger and E. C. Kendall, *J. Biol. Chem.*, 1929, **84**, 639.

resolved, and a trace of di-*O*-methyl-hexose. Gas chromatography of the derived methyl glycosides showed main components having the retention times of methyl glycosides of 3,4,6-tri-*O*-methyl-D-mannose (*T* 3·08 and 1·71) and 2,3,4-tri-*O*-methyl-D-glucose (*T* 2·59 and 3·70, and 1·36 and 1·82) on columns *a* and *b*. The tri-*O*-methylhexoses were separated from traces of di-*O*-methylhexose by chromatography on filter sheets in solvent B, and the resulting fraction (18 mg.) was divided into two portions. The first portion was oxidised with sodium metaperiodate, and chromatography of the product showed 2,3,5-tri-*O*-methylarabinose in addition to unchanged sugars; gas chromatography of the derived methyl glycosides showed *inter alia* components having the retention times of methyl glycosides of 2,3,5-tri-*O*-methyl-L-arabinose on columns *a* (*T* 0·55, 0·72) and *b* (*T* 0·47, 0·58). The second portion was reduced with potassium borohydride, and the resulting glycitols were oxidised with sodium metaperiodate. Chromatography of the products showed 2,3,5-tri-*O*-methylarabinose and 2,3,4-tri-*O*-methylxylose, and gas chromatography of the derived methyl glycosides showed components having the retention times of methyl glycosides of 2,3,4-tri-*O*-methyl-D-xylose (*T* 0·46, 0·58) and 2,3,5-tri-*O*-methyl-L-arabinose (*T* 0·55, 0·71) on column *a*.

Fraction 6. The syrup (40 mg.), $R_{galactose}$ 0·22 in solvent C, gave the two aldobiouronic acids, 2-*O*-glucuronosylmannose and 6-*O*-glucuronosylgalactose, mannose, and galactose on partial hydrolysis. Reduction of the methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave galactose, glucose, and mannose.

Fraction 7. The sugar (90 mg.), $R_{galactose}$ 0·12 in solvent C, gave no colour reaction with triphenyltetrazolium hydroxide, but when stained with aniline oxalate gave the same characteristic fluorescence in ultraviolet light as 2-*O*-(D-glucopyranosyluronic acid)-D-mannose. Partial hydrolysis furnished mannose, 2-*O*-glucuronosylmannose, and *O*-glucuronosyl-(1 ⟶ 2)-*O*-mannosyl-(1 ⟶ 2)-mannose.

Degradation of 3-O-β-D-*Galactopyranosyl*-L-*arabinose*.—Lead tetra-acetate (0·39 g., 1 mol.) was added with stirring to the disaccharide (0·31 g.) in water (0·8 ml.) and acetic acid (45 ml.), and the mixture was kept for 30 min. Lead was removed from the solution by precipitation as lead oxalate followed by treatment of the filtrate with Amberlite resin IR-120(H), the solution was concentrated, and the residue was reduced with potassium borohydride (0·15 g.) in water (5 ml.) for 20 hr. Excess of hydride was destroyed, and potassium ions were removed by treatment with Amberlite resin IR-120(H), and boric acid was removed by repeated evaporation with methanol. The residue had $R_{galactose}$ 0·9 in solvent B, and yielded galactose and erythritol on hydrolysis. Crystallisation from ethanol furnished 2-O-β-D-*galactopyranosyl*-L-*erythritol* (120 mg.), m. p. 160—162°, $[\alpha]_D$ −10° (*c* 0·9) (Found: C, 41·9; H, 6·7. $C_{10}H_{20}O_9$ requires C, 42·3; H, 7·1%). The glycitol (100 mg.) was oxidised with lead tetra-acetate (1 mol.), and the product was reduced with potassium borohydride as described previously. Chromatography of the residue showed the presence of unchanged starting material and a substance having $R_{galactose}$ 1·1 in solvent B. The latter compound (22 mg.) was isolated by chromatography on filter sheets, and had $[\alpha]_D$ 0° (*c* 0·9). The compound was chromatographically indistinguishable from 2-*O*-β-D-galactopyranosylglycerol (prepared from lactose [11]), and yielded galactose and glycerol on hydrolysis, but could not be induced to crystallise.

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[11] A. J. Charlson, P. A. J. Gorin, and A. S. Perlin, *Canad. J. Chem.*, 1956, **34**, 1811.