The Isolation of Oligosaccharides from Gums and Mucilages. Part III. Golden Apple Gum.*

By P. ANDREWS and J. K. N. JONES.

[Reprint Order No. 5553.]

Autohydrolysis of golden apple gum yields a mixture of mono- and oligosaccharides, from which 3-*O*-β-L-arabopyranosyl- (*A*) and 3-*O*-α-D-xylopyranosyl-L-arabinose (*B*) have been isolated and characterised. Five other oligosaccharides have been isolated from the mixture and examined.

THE Golden Apple (*Spondias cytheria*) is a tree which occurs in the West Indies and exudes gum in the dry season. Dr. W. G. C. Forsyth kindly arranged for a sample of this gum to be sent to us. Paper-chromatographic examination of the sugars produced by its hydrolysis showed that it is similar in composition to numerous other plant gums, in that it is composed of galactose, arabinose, xylose, glucuronic acid (mainly, if not entirely, in the form of a monomethyl ether), and traces of rhamnose and fucose. As with other plant gums, graded hydrolysis could be effected by the prolonged action of cold aqueous sulphuric acid or by heating a concentrated aqueous solution of the ash-free gum. The mixtures of reducing sugars obtained by each method gave similar patterns of spots on the paper chromatogram, but autohydrolysis is preferred as a preparative method for the oligosaccharides because of the ease with which the products may then be isolated and of the reduced possibility of formation of reversion products.

Autohydrolysis of golden apple gum yielded a complex mixture of reducing sugars, consisting of arabinose and traces of galactose and rhamnose, together with a number of neutral pentose-containing oligosaccharides, traces of several acidic oligosaccharides, and the degraded gum, which still contained arabinose and xylose besides the galactose and uronic acid units. The sugar mixture was fractionated on a column of charcoal–Celite (Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 677), and the mixture of oligosaccharides so obtained was further fractionated by chromatography on sheets of filter paper. In this way, seven oligosaccharides were obtained, in various amounts, in a state of purity such as to give only one spot on the paper chromatogram in three different solvent mixtures.

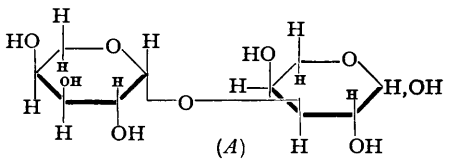
The main component of the oligosaccharide mixture was 3-*O*-β-L-arabopyranosyl-L-arabinose (*A*), which had previously been isolated from the partial hydrolysis products of larch ε-galactan (Jones, *J.*, 1953, 1672), and of peach gum and cherry gum (Andrews, Ball, and Jones, *J.*, 1953, 4090). The identity of the disaccharide (*A*) from golden apple gum with that from these other sources was established by comparing the *X*-ray photographs of their crystalline phenylosazones.

The other main component of the oligosaccharide mixture was obtained crystalline, and shown to be 3-*O*-α-D-xylopyranosyl-L-arabinose (*B*) on the following evidence. Hydrolysis yielded arabinose and xylose, but after oxidation with bromine water it yielded only xylose, demonstrating that the reducing unit was arabinose. The disaccharide gave

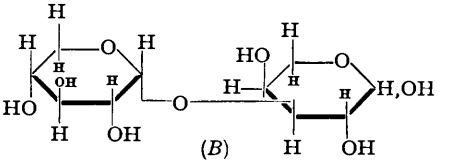
* Part II, *J.*, 1954, 1724.

a crystalline phenylosazone (which analysed as the hemihydrate), thus proving the presence of a 2-hydroxyl group in the arabinose unit. The fully methylated derivative of (*B*) was a syrup, giving on hydrolysis approximately equimolar amounts of 2 : 3 : 4-tri-*O*-methyl-D-xylose and 2 : 4-di-*O*-methyl-L-arabinose. The former crystallised and was characterised by its m. p., optical rotation, and the properties of the derived *N*-phenyl-D-xylopyranosylamine 2 : 3 : 4-trimethyl ether. The di-*O*-methyl-L-arabinose was recognised as an L-arabopyranose derivative by its high positive optical rotation, but its rate of movement on the paper chromatogram was different from that of the 2 : 3- and the 3 : 4-isomer. Its identity as the 2 : 4-isomer was confirmed by the preparation from it of crystalline *N*-phenyl-L-arabopyranosylamine 2 : 4-dimethyl ether. In addition to these two sugars, the hydrolysate of the methylated derivative of (*B*) contained a trace of 2 : 5-di-*O*-methyl-arabinose (identified chromatographically), showing that the arabinose unit in (*B*) could exist in either the (preferred) pyranose or the furanose form. Therefore, the two pentose units in (*B*) are united by a 1–3′-linkage to which is assigned the α-configuration on the basis of the high positive optical rotation of (*B*).

Very small amounts of two other disaccharides, (*C*) and (*D*), both of which yielded only arabinose on hydrolysis, were obtained from the reducing-sugar mixture. The optical rotation of (*C*) indicated that it might be composed of one L-arabopyranose and one L-arabofuranose unit, whereas (*D*), from its negative optical rotation, might be composed of two L-arabofuranose units. It is thought that the high rate of movement of (*D*) on the paper chromatogram is consistent with such a structure.



(*A*)



(*B*)

The remaining oligosaccharides (*E*, *F*, *G*) isolated from the autohydrolysate were apparently trisaccharides, as judged by their rates of movement on the paper chromatogram and their hydrolysis products. Paper chromatography showed that complete hydrolysis of (*E*) yielded only arabinose but partial hydrolysis, effected with 0·01N-sulphuric acid, gave roughly equimolecular amounts of arabinose and a disaccharide indistinguishable from (*A*) in its chromatographic behaviour. Hence (*E*) probably contained at least one non-reducing arabopyranose unit; its high positive optical rotation indicated that it contained two arabopyranose units.

Paper chromatography of their hydrolysates indicated that both (*F*) and (*G*) were composed of one xylose and two arabinose units. After oxidation with bromine water, both these trisaccharides yielded roughly equal amounts of xylose and arabinose on hydrolysis, thus the reducing unit of each of them appeared to be arabinose. The trisaccharide (*F*) reacted with phenylhydrazine to form a yellow product in very small yield.

It is considered unlikely that disaccharides (*A*) and (*B*) were formed by reversion because, if this were so, prolonged autohydrolysis should increase their yield at the expense of the yield of monosaccharides whereas the reverse effect was observed. The oligosaccharides (*C*), (*D*), (*E*), (*F*), and (*G*) which were formed in very small amounts might be adventitious. This possibility is being further investigated. However, since no free xylose was detected at this stage of the hydrolysis it is considered unlikely that those oligosaccharides, *viz.*, (*B*), (*F*), and (*G*), which contained xylose were reversion products.

The isolation of (*A*) from golden apple gum establishes the presence of L-arabopyranose units in the gum, in which respect it is similar to peach gum, cherry gum, sapote gum (White, *J. Amer. Chem. Soc.*, 1953, **75**, 257), and possibly other plant gums as well. Disaccharide (*B*), on the other hand, has not hitherto been described. Examination of the autohydrolysis products of plant gums thus provides a means of determining some of the details of their fine structure but, as in the present case, yields of most of the oligosaccharides are likely to be small.

Experimental

Chromatographic separations, on Whatman No. 1 paper, were carried out with the following solvent mixtures: (*a*) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (*b*) butan-1-ol–pyridine–water (10 : 3 : 3); and (*c*) butan-1-ol–ethanol–water (40 : 11 : 19) (all v/v). *p*-Anisidine hydrochloride (*ca.* 3% w/v in butanol, with a little stannous chloride added) was used as spray reagent to detect the sugars. The rates of movement on the paper chromatogram of oligosaccharides are expressed relative to that of galactose, *i.e.*, R_{gal} values.

Optical rotations were determined at 20° ± 2° in H_2O, and solutions were concentrated under reduced pressure.

Golden Apple Gum.—The gum, somewhat purified by precipitation from alkaline solution with acidified alcohol, was a white, water-soluble powder (Found: N, 0·3; OMe, 4·7; sulphated ash, 3·0%). On hydrolysis with N-sulphuric acid for 18 hr. at 100° it yielded rhamnose, fucose, arabinose, xylose, galactose, and uronic acid derivatives, all detected chromatographically.

Graded Hydrolyses of the Gum.—In preliminary experiments, portions of the gum were dissolved in N- and 3N-sulphuric acid, and the solutions (*ca.* 5% w/v of gum) kept at room temperature. A further portion of gum was dissolved in water, inorganic ions were removed from the solution by passing it successively down columns of Amberlite resins IR-120 and IR-4B, then the solution (*ca.* 5% w/v of gum) was heated at 90°. The progress of each hydrolysis was followed by paper chromatography, in solvents (*a*) and (*b*), of drops of the reaction mixtures. Similar chromatographic patterns were obtained in each case. Hydrolysis in N-sulphuric acid was very slow, and after a month the amounts of arabinose and oligosaccharides present in the solution were much less than in the 3N-sulphuric acid solution after the same period. The autohydrolysis gave a good yield of oligosaccharides after 12 hours' heating; after 24 hours the yield was somewhat better, but during longer periods of heating hydrolysis of the oligosaccharides was apparent and the yield of them decreased steadily. The separation of neutral and acidic sugars is conveniently carried out by two-dimensional chromatography. The sugars are first separated with the acidic solvent (*a*), the paper is then taken out of the chromatographic tank, dried, and turned through 90°, and the neutral sugars are further separated with the basic solvent (*b*).

For isolation of the oligosaccharides, autohydrolysis was carried out as follows: The crude neutral salt of golden apple gum (30 g.) was dissolved in water, and the solution passed down columns of Amberlite resins IR-120 and IR-4B. The resultant solution (*ca.* 500 c.c.) of gum acid was heated at 90° for 24 hr. The cooled solution was passed down a column of Amberlite resin IR-4B to remove acidic products of low molecular weight, then the column was washed with water, and the combined solution and washings brought to pH 7 with aqueous barium hydroxide, concentrated to 200 c.c., and poured into ethanol (400 c.c.). The precipitated barium salt (X) of degraded golden apple gum was isolated on the filter, washed with alcohol and ether, and dried under reduced pressure (yield, 18 g.). The filtrate was evaporated and yielded a syrup (9·9 g.) which partly crystallised. After trituration with methanol, crystalline L-arabinose (3·8 g.), m. p. and mixed m. p. 158—159°, $[\alpha]_D$ +104° (*c*, 0·9), was filtered off. Evaporation of the methanol gave a syrup (Y) (6·2 g.) consisting of neutral mono- and oligosaccharides.

Examination of the Salt (X).—The powder was extracted exhaustively with methanol to remove adsorbed sugars. A small portion was then hydrolysed in N-sulphuric acid for 18 hr. at 100°. Paper-chromatographic examination of the hydrolysate, after it had been neutralised with barium carbonate, filtered, and concentrated, indicated the presence of galactose, arabinose, some xylose, a trace of rhamnose, 4(?)-*O*-methylglucuronic acid, biuronic acids, and oligosaccharides.

Examination of the Syrup (Y).—Paper chromatography showed that this consisted of arabinose (mainly), a little galactose, considerable amounts of disaccharides (*A*) and (*B*), with R_{gal} 0·70 and 1·00 respectively in (*b*), and several other oligosaccharides in smaller amounts. The syrupy mixture (*ca.* 6 g.) was adsorbed on a column (27 × 3 cm.) of charcoal–Celite (1 : 1 w/w) (Whistler and Durso, *loc. cit.*), and the sugars were fractionally eluted with water and aqueous ethanol. Each solvent was applied until it eluted no more sugar. The effluent was collected in portions of *ca.* 100 c.c., and a few drops of each portion (or a concentrate when necessary) were examined on the paper chromatogram. Water sufficed to elute the monosaccharides and some (*A*) from the column; 2% ethanol eluted a little more (*A*) (this being the only fraction to be obtained which contained only one sugar); 5% ethanol eluted the rest of (*A*), all of (*B*), and much smaller amounts of three other oligosaccharides (designated

C, *D*, *E*, this being the order in which they appeared in the eluate); 20% ethanol eluted somewhat larger amounts of (*E*), together with oligosaccharides (*F*) and (*G*), and others of higher molecular weight; and absolute ethanol eluted further materials (0·2 g.) with very slow rates of movement on the paper chromatogram. After appropriate combination of the effluent portions, the solvent was removed from each fraction, the residual syrups were dissolved in methanol, small amounts of insoluble flocculent materials were filtered off, and the methanol was evaporated, giving colourless products (total weight, 5·8 g.).

The fractions consisting of monosaccharides only were not further examined. Those consisting of oligosaccharide mixtures were further fractionated by chromatography on large sheets of filter-paper, with solvent (*c*). After separation, the oligosaccharides were recovered from the paper strips by washing with cold water. The isolated oligosaccharides gave colours with *p*-anisidine hydrochloride similar to that given by arabinose, and they had the following approximate R_{gal} values :

Oligosaccharide	*A*	*B*	*C*	*D*	*E*	*F*	*G*
Solvent (*a*)	0·8	0·9	1·1	1·3	0·6	0·7	0·8
Solvent (*b*)	0·7	1·0	1·5	2·0	0·4	0·6	0·9
Solvent (*c*)	0·7	0·9	1·3	1·6	0·4	0·6	0·8

The oligosaccharides were further examined as follows (the weight given is that of the chromatographically pure sugar) :

Disaccharide (*A*). This (940 mg.) was a syrup with $[\alpha]_D$ + 214° (*c*, 1·7). It was completely hydrolysed in boiling N-sulphuric acid in 1·5 hr., and yielded only arabinose (detected chromatographically). Phenylhydrazine (0·35 c.c.), glacial acetic acid (0·4 c.c.), and a little sodium metabisulphite were added to a solution of the disaccharide (180 mg.) in water (3 c.c.). The solution was kept overnight at 50—60°, and yielded an osazone (200 mg.) which recrystallised from ethanol as small yellow needles, m. p. 235° (decomp.) (Found : C, 57·6; H, 6·2; N, 12·0. Calc. for $C_{22}H_{28}O_7N_4$: C, 57·4; H, 6·1; N, 12·2%). An *X*-ray diffraction photograph of this osazone was identical with those of the osazones of the specimens of 3-*O*-β-L-arabopyranosyl-L-arabinose from larch ε-galactan and peach and cherry gums.

Disaccharide (*B*). This (380 mg.) was initially a syrup, with $[\alpha]_D$ +173° (*c*, 3·5). A sample crystallised after about 4 months, and had m. p. 123°, but insufficient was obtained for analysis. It yielded xylose and arabinose (ratio *ca.* 1 : 1 ; sugars identified chromatographically) after hydrolysis in boiling N-sulphuric acid for 1 hr. A portion (20 mg.) was dissolved in water (3 c.c.), and a drop of bromine added. After 18 hr., excess of bromine was removed by aeration, then 2N-sulphuric acid (3 c.c.) was added, and the solution heated at 100° for 1 hr. Anions were then removed from the solution with Amberlite resin IR-4B, and the solution was concentrated. Paper-chromatographic examination indicated the presence of xylose only.

The sugar (80 mg.) was heated in water (3 c.c.) with phenylhydrazine (0·2 c.c.), glacial acetic acid (0·3 c.c.), and a little sodium metabisulphite added, at 70° for 2 hr. On cooling, a gelatinous *phenylosazone* separated. It was filtered off, washed with water, dried (yield, 40 mg.), and recrystallised from ethanol–benzene as yellow needles (33 mg.), with m. p. 226° (decomp.) (Found : C, 56·5, 56·6; H, 6·0, 6·2; N, 12·4. $C_{22}H_{28}O_7N_4,0{\cdot}5H_2O$ requires C, 56·3; H, 6·2; N, 11·9%).

The disaccharide (*B*) (180 mg.) was dissolved in water (5 c.c.), methyl sulphate (1 c.c.) was added, then sodium hydroxide solution (30% w/v; 1·5 c.c.) dropwise, with vigorous stirring, during 2 hr. The solution was then non-reducing to Fehling's solution; thereafter sodium hydroxide (30% w/v; 7·5 c.c.) was added, followed by methyl sulphate (5 c.c.) dropwise. After a further addition of the same quantity of these reagents the alkaline solution was stirred for 18 hr., then heated on the boiling-water bath for 30 min., cooled, and extracted continuously with chloroform for 18 hr. Evaporation of the chloroform gave a syrup (201 mg.) (Found : OMe, 44·8%), n_D^{20} 1·4687, which after a further methylation with Purdie's reagents was isolated (180 mg.) and distilled under reduced pressure. The main fraction (150 mg.) of the distillate was the *hexa*-O-*methyl* derivative of (*B*) with b. p. 160—170° (bath-temp.)/0·3 mm., n_D^{20} 1·4660 [Found : OMe, 49·1. $C_{10}H_{12}O_3(OMe)_6$ requires OMe, 50·8%].

The syrupy hexa-*O*-methyl derivative (142 mg.) was heated in N-hydrochloric acid (10 c.c.), at 100°, the optical rotation being $[\alpha]_D$ +127° (initial value), +76° (30 min.), +71° (1 hr.), +67° (1·5 hr.; final value). After 2 hours' heating the solution was neutralised with silver carbonate, filtered, treated with hydrogen sulphide, again filtered, and concentrated to a syrup (138 mg.) which consisted of two sugars [R_G 0·97 and 0·65 in (*c*)] together with a trace of a third (R_G 0·71). The mixture was separated into three fractions by partition chromatography

on a large sheet of filter paper, with solvent (*c*); the sugars were recovered in 3 fractions from appropriate sections of the paper by Soxhlet-extraction with acetone.

Fraction 1 (58 mg.), which consisted only of the sugar with R_G 0·97 in solvent (*c*), crystallised, and recrystallisation from ether–light petroleum (b. p. 40—60°) afforded colourless cubes of 2 : 3 : 4-tri-*O*-methyl-α-D-xylose, m. p. and mixed m. p. 87—88°, with $[\alpha]_D$ +58° (initial; *c*, 0·7) ⟶ +17° (equil.) [Found : OMe, 48·7. Calc. for $C_5H_7O_2(OMe)_3$: OMe, 48·4%]. The derived *N*-phenyl-D-xylopyranosylamine 2 : 3 : 4-trimethyl ether, prepared by heating the sugar with one equiv. of aniline in ethanol, crystallised from ether–light petroleum (b. p. 40—60°) as colourless cubes, m. p. and mixed m. p. 102—103°.

Fraction 2 (10 mg.) was a syrup, of which the main component was the compound with R_G 0·71 in (*c*). This gave a brown colour with *p*-anisidine hydrochloride, and was indistinguishable from 2 : 5-di-*O*-methylarabinose. The fraction also contained traces of the sugars in Fractions 1 and 3.

Fraction 3 (48 mg.) was a syrup with n_D^{20} 1·4700 and $[\alpha]_D$ +125° (*c*, 1·0), and consisted only of the compound with R_G 0·65 in (*c*). It gave a red colour with *p*-anisidine hydrochloride, and was indistinguishable from 2 : 4-di-*O*-methylarabinose on the paper chromatogram. The 2 : 3- and the 3 : 4-isomer also gave red colours, but had R_G 0·70 and 0·58 respectively in (*c*). When heated under reflux in alcohol with aniline the fraction yielded *N*-phenyl-L-arabopyranosylamine 2 : 4-dimethyl ether, which crystallised from ether–light petroleum (b. p. 40—60°) in colourless plates, m. p. 142—143° (lit., m. p. 145°) [Found : C, 61·5; H, 7·6; OMe, 24·7. Calc. for $C_{11}H_{13}O_2N(OMe)_2$: C, 61·7; H, 7·6; OMe, 24·5%].

Examination of the Other Oligosaccharides.—Insufficient of the other oligosaccharides was isolated for their structures to be elucidated. They were all completely hydrolysed in N-sulphuric acid at 100° in 1 hr.; the sugars so produced were identified by their behaviour on paper chromatograms. Additional information was obtained as follows :

(*C*) was a syrup (16 mg.), with $[\alpha]_D$ +42° ± 4° (*c*, 0·8), and was composed of arabinose units.

(*D*) was also a syrup (11 mg.) with $[\alpha]_D$ −34° ± 5° (*c*, 0·6), and also composed of arabinose units.

(*E*) was a solid (98 mg.) with $[\alpha]_D$ +120° (*c*, 1·0).

Complete hydrolysis of (*E*) yielded only arabinose; partial hydrolysis, effected by heating a solution of the sugar in 0·01N-sulphuric acid at 100° for 0·5—1 hr., yielded roughly equimolecular amounts of arabinose and a compound indistinguishable from 3-*O*-β-L-arabopyranosyl-L-arabinose (*A*) on the paper chromatogram in solvents (*a*), (*b*), and (*c*). The material (*E*) (50 mg.) did not yield a phenylosazone when heated with phenylhydrazine (0·1 c.c.), glacial acetic acid (0·13 c.c.), and a little sodium metabisulphite in water (2 c.c.) at 70° during 2 hr. The solution became brown in colour and a tarry deposit formed.

The material (*F*) was a crisp solid (60 mg.) with $[\alpha]_D$ +59° ± 3° (*c*, 1·2), which on hydrolysis yielded arabinose and xylose in the approximate proportion of 2 : 1. After oxidation with bromine water, followed by hydrolysis [as described for (*B*)], some diminution in the proportion of arabinose was observed. In the attempted preparation of a phenylosazone from (*F*) (40 mg.) in the manner described for (*E*), the very small amount of yellow precipitate which formed was insufficient for its isolation. The reaction mixture became yellow, indicating osazone formation, but on prolonged heating dark tar was precipitated.

The material (*G*) was a crisp solid (25 mg.) with $[\alpha]_D$ +49° ± 7° (*c*, 1·3). It yielded arabinose and xylose, in the approximate ratio 2 : 1, on hydrolysis; after oxidation with bromine water, it yielded arabinose and xylose in roughly equal amounts.

We thank Dr. W. G. C. Forsyth for a gift of golden apple gum and Dr. D. A. Brown for the *X*-ray diffraction photograph. One of us (P. A.) thanks the Directors of Monsanto Chemicals Ltd. for the award of a fellowship.

THE UNIVERSITY, BRISTOL.
[Present address (J. K. N. J.) :
QUEEN'S UNIVERSITY,
KINGSTON, ONTARIO, CANADA.]

[*Received, July 14th*, 1954.]