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The Composition of Plum Gums

By L. HOUGH

Chemistry Department, University of Bristol

AND J. B. PRIDHAM

*Chemistry Department, Royal Holloway College (University of London),
Englefield Green, Surrey*

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Gummosis, or the formation of gums, is a common phenomenon in the plant kingdom, and in temperate climates fruit trees of the Rosaceae family, e.g. peach, plum, almond, etc., are particularly susceptible. These gums may appear as exudates on the epidermal surfaces of the various plant organs or as internal pockets ('stone gum') within the tissues. They are composed predominantly of carbohydrate in the form of inorganic salts of complex acidic polysaccharides.

The biosynthetic mechanisms involved in the formation of gum polysaccharides are not understood. It has been variously suggested that gums are the products of micro-organisms (Grieg-Smith,

1904; Butler & Jones, 1949) and that they are produced from pectin (Higgins, 1919) and from hemi-cellulose (Sands & Gary, 1933). Recent histochemical studies by Ceruti & Scurti (1954) have shown that the precursor of cherry gum (*Prunus avium* L.) is probably a galactan. There seems little doubt that the gums originating from *Prunus* species are metabolic products of the plant normally found in the tissues but produced in excess when the plant is stimulated by mechanical or microbiological injury (Hirst, 1949) or by some physiological disorder. The apparent function of gum formation is to seal off surface wounds and to prevent the spread of invading micro-organisms.

This paper describes some studies on the composition of plum gums in relation to their possible function in the plant. A preliminary account of this work has been given by Pridham (1954).

MATERIALS AND METHODS

General methods. M.p. values are uncorrected. Optical rotations are given as equilibrium values in aqueous solution at 25°. Evaporations were carried out under reduced pressure. A Unicam SP. 500 spectrophotometer (1 cm. cell) was used for all u.v. measurements.

Purification of the gum polysaccharides. The gums, which were collected from trees (var. Victoria) were vigorously shaken with water at room temperature, the solutions were clarified in the centrifuge and the polysaccharides precipitated from the supernatant liquors by addition of excess of ethanol. After isolation by centrifuging, the white precipitates were washed with absolute ethanol followed by ether, and dried at 60° under reduced pressure.

Hydrolysis of the gum polysaccharides. (i) Qualitative. The gums were hydrolysed in $N-H_2SO_4$ in sealed glass tubes for 16 hr. After neutralization ($BaCO_3$) and concentration, the residues were examined on paper chromatograms. Barium ions were removed from the hydrolysates by treatment with Amberlite IR-120 (H) resin and, after concentration, the residues were again examined on paper chromatograms for glucurono- γ -lactone, which appeared as a characteristic red spot [$R_{\text{galactose}}$ 4.8 in solvent (1), see below] with the *p*-anisidine hydrochloride reagent. Further characterization of the monosaccharide constituents of the fruit-surface gum was carried out as follows. The gum (5 g.) was hydrolysed in $N-H_2SO_4$ (160 ml.) at 95–100° for 21 hr. After neutralization ($BaCO_3$) the solution was evaporated to a thick syrup which was dried by repeated evaporation in the presence of methanol. The resulting material was then extracted with boiling methanol (100 ml.; three portions) to separate the neutral monosaccharides (A) from the insoluble barium salts (B). Evaporation of the filtered methanol extracts gave a mixture of sugars (A; 2.54 g.) which was fractionated by chromatography on a column of cellulose with aq. butan-1-ol (half-saturated with water) as the mobile phase. The barium salts (B, 4.46 g.) were dissolved in water and then passed through a column of Amberlite IR-120 (H) resin, and the acidic effluent was concentrated to a small volume. After examination on paper chromatograms this solution was passed through a charcoal–Celite column, which was washed with water to elute D-glucurono- γ -lactone. Further elution of the column with aq. 50% ethanol afforded a mixture of oligosaccharides (C, 139 mg.). A portion (39 mg.) of this mixture (C) was hydrolysed ($2N-H_2SO_4$, 100°, 16 hr.) and the neutralized ($BaCO_3$) hydrolysate examined on paper chromatograms. The remainder of this mixture (C, 100 mg.) was heated under reflux with methanolic 4% hydrogen chloride until the solution was non-reducing to Fehling's solution. The neutralized (Ag_2CO_3) solution was concentrated, the resulting syrup was dissolved in tetrahydrofuran and treated with lithium aluminium hydride (Lythgoe & Trippett, 1950). Excess of hydride was decomposed with ethyl acetate, the solution was poured into ice-water and then filtered. Concentration gave a mixture of methyl glycosides which were hydrolysed ($N-H_2SO_4$, 100°, 16 hr.) and then examined on paper chromatograms.

(ii) Quantitative. (a) The fruit-surface and bark gums were hydrolysed in $N-H_2SO_4$ by heating in an autoclave (15 lb./in.², 120°) for exactly 2 hr. The hydrolysates were neutralized with Amberlite IR-4B (OH) and the sugars determined by the benzidine method (Jones & Pridham, 1954). (b) The analyses were repeated, as described above, but the period of hydrolysis was reduced to 1 hr. with $N-H_2SO_4$ at 15 lb./in.² pressure.

Paper chromatography. All separations were carried out on Whatman no. 1 paper by the descending method, unless otherwise stated, with one of the following solvent systems: (1) ethyl acetate–acetic acid–water (9:2:2, by vol.); (2) ethyl acetate–acetic acid–formic acid–water (18:3:1:4, by vol.); (3) butan-1-ol–pyridine–water (10:3:3, by vol.); (4) butan-1-ol–ethanol–water (40:11:19, by vol.); (5) phenol–water (bottom layer); (6) butan-1-ol–acetic acid–water (2:1:1, by vol.); (7) propan-1-ol–water (4:1, v/v); (8) light petroleum (b.p. 100–110°) saturated with water. Solvents (5) and (6) were used for the examination of amino acids which were detected by the use of ninhydrin.

Reducing sugars were located on the paper chromatograms with the following spray reagents: (a) ammoniacal $AgNO_3$ (Partridge, 1948); (b) *p*-anisidine hydrochloride; (c) resorcinol–HCl (Hough, Jones & Wadman, 1950).

Phenols and their derivatives were separated by using solvents (4) and (6) and they were located on the paper chromatograms with diazotized *p*-nitroaniline– Na_2CO_3 (Swain, 1953) and diazotized sulphanilic acid (Evans, Parr & Evans, 1949) spray reagents. In some cases the spots were also detected by their fluorescence under ultraviolet light with or without exposure to NH_3 .

Phosphates were examined on Whatman no. 542 acid-washed paper, irrigated with solvent (2) and subsequently detected by the procedure of Hanes & Isherwood (1949).

Alkaline nitrobenzene oxidation products were detected on paper chromatograms with 2,4-dinitrophenylhydrazine solution (0.3% in 2*N*-HCl, Stone & Blundell, 1951).

The inorganic cations present in fruit-surface gum and stone gum of plums were studied by the qualitative paper chromatographic procedures described by Pollard & McOmie (1953).

Hexuronic acid anhydride content. The method of Johansson, Lindberg & Theander (1954) was used, and each sample of gum (approx. 0.1 g.) analysed in duplicate. The results were corrected for ash and water content of the gums.

Periodate oxidations. A known weight of the gum was dissolved in water by vigorous agitation at room temperature; 0.3*M*-sodium metaperiodate solution (20 ml.) was added and the volume made to 100 ml. with water. A control solution containing 0.3*M*-sodium metaperiodate solution (20 ml.) and water (80 ml.) was prepared at the same time. The oxidation was carried out in the dark and at room temperature. At varying intervals of time, two samples (5 ml. each) were withdrawn from each flask. To one sample ethylene glycol (2 ml.) was added; it was then diluted to about 12 ml. with water and the acidity titrated potentiometrically with 0.01*N*-NaOH (Anderson, Greenwood & Hirst, 1955). The other sample was added to a mixture of 20% KI solution (5 ml.) and 2*N*- H_2SO_4 (5 ml.) and the periodate content was determined by titration of the liberated iodine with 0.1*N*-sodium thiosulphate (Schwartz, 1954). Oxidations were carried out on crude and purified gums.

Electrophoresis. The gum was dissolved in aqueous buffer solution by vigorous agitation at room temperature. After centrifuging, the solution was dialysed against buffer until the pH and conductivity of both buffered gum solution and the dialysate were approximately the same. Electrophoresis was carried out at 4° in a Hilger electrophoresis apparatus at a potential of 280–380 v and a current of 12–18 ma.

Mono- and oligo-saccharides. Crude gums were finely powdered and extracted by shaking with methanol at room temperature for 20 hr. The filtered extracts were concentrated and examined on paper chromatograms. In one instance extraction was effected with methanol containing about 1% of aq. NH_3 soln.

Lignin. Crude gum was heated with 2N- H_2SO_4 at 100° for 16 hr., care being taken to see that the samples did not contain any plant tissue. The residue was then filtered off and subjected to alkaline nitrobenzene oxidation (Freudenberg, Lautsch & Engler, 1940). The resulting products were examined on paper with solvent (8) and 2:4-dinitrophenylhydrazine as spray reagent.

Phenolic compounds. The gum was agitated with an excess of water at room temperature, the solution was clarified by centrifuging, acidified with acetic acid and then extracted continuously with ether for 10 hr. After concentration, the ethereal extract was examined on paper chromatograms. Further extraction of the aqueous gum solution was then carried out with ethyl acetate: this extract and the residual aqueous solution were concentrated and the syrups examined on paper chromatograms.

Amino acids and protein. Several specimens of gum were analysed for nitrogen. Gums were examined by paper chromatography, before and after partial hydrolysis, for ninhydrin-positive compounds. Partial hydrolysis was effected by leaving a 1% solution of gum in 3N-HCl at room temperature for 3 weeks. The acid was neutralized (Ag_2CO_3) and the solution then treated with H_2S and concentrated.

Peroxidase. Benzidine (Madeling, 1911) and pyrogallol (Willstätter & Stoll, 1918), both in the presence of traces of H_2O_2 , were used as colorimetric spot tests for peroxidase.

Peroxidase activity was measured by adding a saturated aqueous solution of benzidine (0.04%; 5 ml.) to a gum solution diluted to 25 ml. Hydrogen peroxide (20 vol.; 0.05 ml.) was then added and after 5 min. the resulting blue was measured with an EEL colorimeter (Ilford no. 608 filter, peak transmission 680 m μ) with diluted gum solution as a blank.

Determination of reducing substances in plum-fruit tissues. Fruits were selected in July and cylindrical pieces of tissue removed from gumming and non-gumming mesocarps with a cork borer (no. 4). The tissue was disintegrated with a spatula and individually extracted three times with small volumes (10 ml.) of boiling methanol. Each extract was poured through a weighed sintered-glass funnel (medium porosity) and, finally, the tissue residues were transferred quantitatively to the funnels, washed with hot methanol (20 ml.) and dried to constant weights at 100°. The methanolic extracts were concentrated to syrups, then diluted with water to 10 ml. and small portions were analysed for reducing sugars by the Somogyi (1945) and benzidine methods (Jones & Pridham, 1954). Examination of these extracts on paper chromatograms showed that the main reducing component was glucose. The extracts were then separated on paper chromatograms and the glucose was determined with benzidine.

Respiration of plum-fruit tissues. Cylinders (1 cm. $\times$ 1 cm.) of mesocarp tissue were cut from healthy plum fruit and from gumming fruit (tissues adjacent to the 'lesion') with a cork borer. Respiration measurements were by the manometric method of Dixon (1951).

Examination of plum-fruit tissues for micro-organisms. Gumming fruits were surface-sterilized with ethanol, the nodules carefully removed and pieces of tissue from beneath were plated on to a nutrient-agar medium [Lab Lemco (10 g.) (Oxo Ltd.), peptone (10 g.), NaCl (5 g.), agar (20 g.) and water (1 l.)]. The plates were examined after incubation at 25° for 48 hr.

Enzyme preparations. 'Carterzymes' ADFP 3 and C.Z. 103 were supplied by H. W. Carter and Co. Ltd., Coleford, Gloucestershire, and enzyme 19 A.P. was obtained from Rohm and Haas Co., Philadelphia, Pa., U.S.A.

RESULTS

Anatomical observations

Observation of plum trees in various orchards showed that nodules of gum were frequently present on the bark of the trunks and branches of the trees, particularly if the tissues had been damaged mechanically or if signs of disease were present as, for example, 'die-back'. Gum nodules were less common on the surface of the fruit, but these appeared much later in the season than those on the bark. Fruit with gum nodules at the distal end more often contained internal pockets of gum than those fruit with gum nodules along the ventral suture or other parts of the epicarp.

Microscopic examination of the cut surfaces of plums (picked in July) free from signs of external gummosis showed small globules of gum exuding from the severed ends of the vascular bundles rather than from the parenchymatous tissue. Initially these globules were colourless and mobile, but on standing they rapidly darkened and became highly viscous. In an atmosphere of nitrogen the globules remained colourless, and the increase in viscosity of the gum was inhibited by a water-saturated atmosphere.

Sections through the mesocarp which were stained with a mixture of Congo red and chrysoidin revealed the presence of gum in the cells adjacent to the vascular bundles and also in the phloem itself. Gum nodules were invariably produced when the fruit epicarp was punctured with a sterile needle. The time taken for the gum to appear after the skin was punctured varied from approximately 1 hr. to 2–3 days. The exudation occurred with fruit on the tree and with 'picked fruit'.

Gum in the branches and trunks of trees appeared to be associated with the bast and rarely penetrated into the wood.

An examination of gumming 'lesions' on plums in an orchard showed that various bacteria and fungi were associated with the tissues directly

beneath the external gum nodules, but the mesocarp tissues adjacent to internal pockets of gum were invariably sterile. It is probable that in the majority of cases the micro-organisms found beneath the nodules were saprophytic rather than parasitic.

Sugars of the fruit

Glucose appeared to be the main reducing substance present in the mesocarp tissues of both gumming and non-gumming fruit. Quantitative analysis (Table 1) showed that in the latter the glucose concentration was approximately 40% higher than in the former. Traces of D-xylose, which were tentatively identified by Hay & Pridham (1953) in the mesocarp, have now been confirmed by a comparison of the X-ray powder photograph of an authentic dibenzylidenedimethylacetal derivative of D-xylose (Breddy & Jones, 1945) with a similar derivative of the xylose isolated from the fruit.

In addition to glucose and xylose, sucrose, fructose and traces of raffinose were detected on paper chromatograms (cf. Bradfield & Flood, 1950).

Measurement of respiration showed that normal tissues had a significantly higher rate than gumming tissues (Table 2) and that the R.Q. values for the latter were higher than those for the normal tissue.

Table 1. *Reducing power and concentration of glucose in gumming and non-gumming fruit-mesocarp tissues*

Tissue		Reducing power (as % of glucose, based on dry wt. of tissue)		Glucose (% based on dry wt. of tissue)
		Benzidine method	Somogyi method	
Non-gumming	1	68.8	57.7	—
	2	60.8	59.3	48.2
Gumming	1	34.2	30.7	27.0
	2	35.6	31.4	28.4

Table 2. *Respiration of gumming and non-gumming fruit-mesocarp tissues*

Tissue		Gas (μ l./g. dry wt.)		R.Q.
		CO ₂	O ₂	
Non-gumming	1	386	188	2.1
	2	355	187	1.9
	3	353	191	1.9
Average value		365	189	1.9
Gumming	1	306	79	3.9
	2	381	145	2.6
	3	280	103	2.7
Average value		322	109	3.0

R.Q. values for both types of tissue were greater than unity.

Some preliminary experiments designed to test the influence of gums on the growth of micro-organisms showed that the crude materials were bacteriostatic. A pure culture of an unidentified bacillus was isolated from the cotyledons of gumming almonds. When this organism was grown on a nutrient-agar plate which contained small nodules of plum-bark gum (surface-sterilized with ethanol) embedded into the medium, zones of growth inhibition were evident around each nodule. Bacilli growing on the boundaries of these zones were pleomorphic and many spore forms were present.

Chemical constitution of Victoria-plum gums

Paper-chromatographic examination of the hydrolysates of bark, fruit and stone gums showed that glucuronic acid, galactose, mannose, arabinose, xylose and rhamnose were present in all the gums, and in similar proportions with the exception of mannose, which appeared to be more prevalent in the bark gum.

Hydrolysis of fruit-surface gum followed by separation of the component sugars afforded crystalline specimens of D-galactose ($[\alpha]_D + 79^\circ$; m.p. and mixed m.p., 156°); L-arabinose ($[\alpha]_D + 102^\circ$; m.p., 149° , mixed m.p. 148°) and L-rhamnose hydrate ($[\alpha]_D + 10.5^\circ$; m.p. and mixed m.p. 86°). D-Mannose ($[\alpha]_D + 11.9^\circ$) and D-xylose ($[\alpha]_D + 20.7^\circ$) were obtained as syrups and were characterized as the phenylhydrazones (m.p. and mixed m.p. 181 – 182°) and dibenzylidenedimethylacetal derivative (m.p. and mixed m.p. 190°) respectively.

The uronate fraction obtained from the hydrolysate was shown to contain at least three acidic oligosaccharides and two other compounds which co-chromatographed with glucurono- γ -lactone and 4-O-methylglucuronic acid respectively. The former was obtained as a crystalline compound (m.p. and mixed m.p. 173°). The mixed oligosaccharides on acid hydrolysis yielded galactose, mannose, glucuronic acid and a trace of arabinose. Examination of the hydrolysis products of the reduced oligosaccharides showed the presence of glucose, galactose, mannose, arabinose and 4-O-methylglucose.

Mild acid hydrolysis of hexuronic acid-containing polysaccharides with 100% recovery of the liberated monosaccharide components is rarely achieved, owing to the stability of the glycuronosidic bonds, and with strong mineral acids marked destruction of the monosaccharides liberated occurs. The conditions necessary for the optimum hydrolysis of Victoria-plum gum to a mixture of monosaccharides and aldobiouronic acids with N-H₂SO₄ were 4–6 hr. at 100° in a sealed tube. This was revealed by following the change in optical rotation during

Table 3. *Relative proportions of monosaccharides liberated on hydrolysis of plum gums in an autoclave*

Hydrolysis (15 lb./in.², 120°) was for 1 and 2 hr. respectively, with $N-H_2SO_4$. The values for 1 hr. hydrolysis are averages of two determinations; those for 2 hr. hydrolysis are averages of four determinations.

Gum	D-Galactose		D-Mannose		L-Arabinose		D-Xylose	
	1 hr.	2 hr.	1 hr.	2 hr.	1 hr.	2 hr.	1 hr.	2 hr.
Bark	1.0	1.0	0.18	0.71	0.66	1.51	0.27	0.24
Fruit surface	1.0	1.0	0.18	0.11	0.98	0.88	0.22	0.21

hydrolysis. In order to speed the routine analysis of the gum polysaccharides, attempts were made to shorten the hydrolysis time by carrying out the hydrolysis in an autoclave at 120° with $N-H_2SO_4$.

The change in optical rotation of solutions of various monosaccharides after varying periods of time was studied. They were extensively degraded under these conditions; after 2 hr., 33 % of L-arabinose and 22 % of D-galactose were lost (assuming that the degradation products had little or no optical activity). The technique is therefore of little use in determining absolute concentrations of monosaccharides liberated from polysaccharides, but it is useful when rapid qualitative comparative results are needed, particularly for hexuronic acid containing materials.

Aqueous solutions of plum gums readily undergo autohydrolysis because of the lability of the L-arabinose residues, which are presumably present as non-reducing end groups in the furanose form. For this reason, a comparative quantitative analysis was carried out with the crude gums to alleviate the loss of monosaccharides by hydrolysis during purification.

Analysis for uronic acid anhydride revealed that the fruit-surface gum contained less hexuronic acid (13 %) than the bark gum (18 %) when corrections were made for the ash and water content in both.

The relative proportions of D-mannose, L-arabinose and D-xylose to D-galactose were determined after acid hydrolysis of bark and fruit-surface gums under identical conditions (Table 3). After hydrolysis for 1 hr. there was little difference in the composition of the hydrolysis mixtures of the two types of gum except that a slightly higher concentration of L-arabinose had been liberated from the fruit-surface gum. However, after hydrolysis for 2 hr. it was evident that the bark gum contained a much higher concentration of L-arabinose and D-mannose than the fruit-surface gum.

When the hydrolyses (100°, sealed tube) were followed by measuring the optical rotations of the reaction mixtures, the results were similar for both types of gum. As the hydrolyses proceeded the reaction mixtures were examined on paper chroma-

Table 4. *Periodate oxidation of plum gums*

Gum	Wt. of gum consuming 1 mole of periodate (g.)	Wt. of gum liberating 1 mole of formic acid
		(g.)
Purified { Bark	158	717
{ Fruit surface	219	1225
Crude { Bark	179	726
{ Fruit surface	233	1003

tograms at varying intervals. With both gums L-arabinose was liberated a few minutes after the reaction had started, and after 30 min. L-arabinose, L-rhamnose, D-galactose, D-xylose and three acidic oligosaccharides were detectable, together with traces of D-mannose. The last-named sugar gradually increased to a maximum after about 8 hr. After 5 hr. glucuronic acid was also detectable, its appearance on chromatograms coinciding with the gradual fall in intensity of the oligosaccharide spots. There appeared to be less hexuronic acid liberated from the fruit-surface gum than from the bark gum and the relative concentrations of the acidic oligosaccharides varied for the two gums.

Attempts to degrade the polysaccharide of plum-bark gum to lower molecular-weight fragments with aqueous solutions of commercial enzyme preparations (i.e. enzyme 19 A.P., Carterzyme ADFP 3 and Carterzyme C.Z. 103) failed.

Periodate oxidations of crude and purified specimens of both types of gum were carried out, and the uptake of periodate and liberation of formic acid were determined at various intervals of time. A comparison of the results for both purified and crude gums (Table 4) shows that purification by alcoholic precipitation had little degradative effect on the polysaccharides, differences in results probably being due to the varying ash content of the crude gums.

Electrophoresis

The potentialities of the Tiselius electrophoresis apparatus for the separation of mixtures of acidic polysaccharides were examined. Preliminary experiments showed that plum (var. Czar)-bark

gum could be separated from gum acacia with 0.2M-borate-KCl buffer, pH 7.8. Similarly, a mixture of sugar-beet araban and plum-bark gum could be resolved with 0.07M-phosphate buffer, pH 7.0.

Electrophoresis of plum-bark gum in several buffers (0.2M-borate-KCl, pH 7.8; 0.07M-phosphate, pH 7.0; 0.1M-acetate, pH 6.0; 0.1M-citrate, pH 3.0) suggested that it was homogeneous, as single peaks were observed in all cases. The boundaries migrated towards the positive electrode and in the borate buffer the rate of movement was calculated as 3.08×10^{-5} cm²/v/sec. However, attempts to separate artificial mixtures of fruit-surface gum and bark gum in borate buffer failed.

Chromatography

Paper-chromatographic examination of cold methanolic extracts of various plant gums showed that in many cases traces of mono- and oligosaccharides were present (Table 5). At least three oligosaccharides were present in cherry-bark gum and small amounts (5–30 mg.) of each were isolated on Whatman no. 3MM paper with solvent (1). Each was completely hydrolysed to arabinose when

heated with 0.01N-H₂SO₄ for 1.5 hr. at 95°. The $R_{\text{galactose}}$ values of the three sugars in solvent (1) were 1.2, 0.65 and 0.30, and an application of the rule of Consden, Gordon & Martin (1944) suggested that oligosaccharides were a homologous series consisting of arabinobiose, arabinotriose and arabinotetraose respectively. The suspected disaccharide ($R_{\text{galactose}}$ 0.30; $[\alpha]_D^{25} + 112^\circ$) gave a phenylosazone in small yield (m.p. 190°).

The three oligosaccharides detected in gum arabic had $R_{\text{galactose}}$ values (in solvent 1) of 0.46, 0.53 and 0.73. A small amount of the fastest-moving material, again isolated by partition on thick paper, was shown to be stable to 0.01N-H₂SO₄ (95°; 1 hr.) but was completely hydrolysed to arabinose with N-H₂SO₄ under the same conditions. This oligosaccharide also co-chromatographed with an authentic specimen of 3-O-β-L-arabinopyranosyl-L-arabinose. Cold ammoniacal methanol also extracted galactose, arabinose, rhamnose and the above-named oligosaccharides from gum arabic.

Examination of a number of specimens of plum gum and fruit tissues for sugar phosphates yielded negative results. Inorganic phosphate only was detected.

The residues obtained from gum after strong acid hydrolysis yielded on oxidation with alkaline nitrobenzene a compound which co-chromatographed with vanillin and was detected on paper chromatograms with a 2:4-dinitrophenylhydrazine spray reagent. Both bark gum and fruit-surface gum reacted similarly.

Phenolic and amino compounds

Paper chromatographic examination of the ether and ethyl acetate extracts of an aqueous solution of plum-bark gum showed the presence of four main components (*D*, *E*, *F* and *G*) with the properties listed in Table 6. Traces of these compounds were also detected in the residual aqueous solution. The properties of compound (*F*) were identical with those of phloroglucinol, both compounds giving similar u.v. spectra and having the same chromatographic behaviour. Phenolic glycosides appeared

Table 5. *Free sugars detected in plant gums*

Gum	Sugars detected
Cherry bark	Galactose, arabinose and three oligosaccharides
Arabic	Galactose, arabinose, rhamnose and three oligosaccharides
Almond bark	Arabinose and xylose
Plum bark (var. Cose Late Red)	
Sample I	Glucose, fructose and one oligosaccharide
Sample II	Sucrose, glucose and arabinose
Plum bark (var. Victoria)	Glucose, arabinose, xylose and two or three oligosaccharides

Table 6. *Properties of phenolic compounds present in plum gums*

Compounds	Ether extract		Ethyl acetate extract	
	<i>D</i>	<i>E</i>	<i>F</i>	<i>G</i>
R_F [solvent (6)]	0.79	0.90	0.68	0.63
R_F [solvent (4)]	0.79	0.87	0.72	0.66
Fluorescence with u.v. light + NH ₃	Pale blue	Pale blue	Blue	Pale blue
Colour with diazotized <i>p</i> -nitroaniline-Na ₂ CO ₃ spray reagent	Brown	Brown	Red-brown	Yellow
Reaction with diazotized sulphanilic acid spray reagent	Orange	—	Orange	—
U.v.-absorption maxima (mμ)	257	—	268	—

to be absent from the gum. Fruit-surface gum was shown to have a chromatographically similar phenolic content. Plum-bark gum gave a positive Mitchell's test for tannins (Mitchell, 1923).

Analysis of several samples of plum gum showed that they all contained a small amount of nitrogen (0.3–0.6 %). No free amino acids could be detected, but after mild acid hydrolysis of the gum several compounds, which gave a blue with ninhydrin, were liberated.

Enzymes

Qualitative spot tests revealed the presence of peroxidase activity in both bark gum and fruit-surface gum. Preliminary experiments indicated that the benzidine- H_2O_2 spot test could be adapted as a quantitative method and that for the gum peroxidase there was a linear relationship between the blue produced and the weight of gum used. With this method it was shown that a higher peroxidase activity was associated with bark gum than with fruit-surface gum (Fig. 1). The peroxidase in solutions of gum (2 ml.; 1 %, w/v) was completely inactivated by heating for 1 min. in a boiling-water bath.

Phenolase activity in gums, as shown by the reaction with benzidine in the absence of H_2O_2 or by the spot test with catechol, was very small. The tissues of the fruit and bast, however, contained a relatively high concentration of phenolase.

Fig. 1. Peroxidase activity in plum-bark gum (●) and fruit-surface gum (○).

Table 7. *Inorganic cations present in plum-stone gum and fruit-surface gum*

Cation	Fruit-surface gum	Stone gum
Ca	+++	+++
Mg	+++	+++
Na	++	++
K	++	+
Al	++	++
Sr, Ba, Be, Fe	-	-

An examination of the inorganic cations present in fruit-surface gum and stone gum of plums suggested that the two types of gum were essentially similar in mineral content. The results of this analysis are given in Table 7.

DISCUSSION

Our observations support the theory that gums are normal plant products although micro-organisms may stimulate the formation of excess of gum. Frequently excessive gum formation cannot be explained by mechanical injury or the presence of micro-organisms.

Microscopic examination of immature plum fruits showed that small amounts of gum were present in apparently sterile tissues. The hardening of the globules of gum was probably due to dehydration and not polymerization, because in a water-saturated atmosphere this increase in viscosity was prevented. In the absence of oxygen the globules remained colourless, thus suggesting that the dark colour of many gums is due to the oxidation of phenolic compounds.

The soluble sugars present in the mesocarp tissues of plum fruit included sucrose, glucose, fructose and small amounts of raffinose and xylose. All of these compounds were detected in both gumming and non-gumming fruits, but in the latter the concentration of glucose was significantly higher than in tissues showing excessive gummosis. Hexoses will be consumed in normal metabolic processes, and with gumming tissues it is probable that, in addition hexoses will also be utilized for the biosynthesis of gum polysaccharide. This may lead to their depletion, but it seems improbable that this alone would account for the fall in respiration. It seems more likely that the differences in respiration and the higher R.Q. obtained with the gumming tissues are due to the formation of a gum barrier which interferes with gaseous exchange, although partial anaerobiosis due to the thickness of the tissues would account for the high R.Q. values obtained in all cases.

It has often been stated (e.g. Hirst, 1949) that the function of the gum in the plant is to seal mechanical wounds and to 'wall-off' pockets of infection. Experiments with plum gum showed that commercial enzyme preparations, which will hydrolyse pectin and hemicelluloses, failed to degrade the gum polysaccharide. Such preparations consist of a highly reactive mixture of fungal carbohydrases. Some evidence was obtained for the presence of active bacteriostatic materials in the gum. The zones of growth inhibition which were observed when gum nodules were introduced into plate cultures of the bacillus may have been caused by the simple phenolic constituents of the gum or by

tannins. Another possibility is that the gum peroxidase has an antimicrobial action, since proteins can be oxidized and certain enzymes inactivated by this enzyme in the presence of hydrogen peroxidase (Sizer, 1953).

The monosaccharide composition of bark gums from several species of Rosaceae have been examined, e.g. cherry (Jones, 1939), damson (Hirst & Jones, 1938), egg plum (Hirst & Jones, 1947), peach (Jones, 1950) and almond (Brown, Hirst & Jones, 1948), but little has been reported on the composition of fruit-surface gums from any plant family. The Rosaceae bark gums have all been observed to contain D-glucuronic acid, D-galactose, L-arabinose and D-xylose, and some also contain D-mannose and L-rhamnose. The homogeneity of the gum polysaccharides is questionable, particularly in view of the recent work of Heidelberger, Adams & Dische (1956), who used immunological techniques to resolve gum arabic into two fractions with different monosaccharide compositions. With bark gum from egg plum, the gum nodules from different trees and varying locations have been observed to contain a constant monosaccharide composition (Hirst & Jones, 1947). Stone (1890) compared the galactose and pentose contents of the fruit-surface gum and bark gum of peach and concluded that they were similar, although the analytical methods used are open to criticism.

Analysis of plum-bark gum and fruit-surface gum revealed that the monosaccharides in both were essentially the same (4-O-methylglucuronic acid was not detected in bark gum, however), but the bark gum contained a greater proportion of hexuronic acid, mannose and arabinose than did the fruit-surface gum (Table 3). The differences in analytical data obtained for the 1 and 2 hr. hydrolyses can be explained by assuming that larger proportions of L-arabinose and D-mannose in the bark gum are linked to the glycosidic carbons of hexuronic acids than in the fruit-surface gum. Acid-stable aldobiouronic acids would be among the first products of hydrolysis and these units would be cleaved only by vigorous hydrolytic conditions. Support for this theory is given by the fact that acidic oligosaccharides were detected in the partial hydrolysis products of both types of gum.

The relative proportions of the monosaccharides present after hydrolysis for 1 hr. suggest that some of the D-mannose and L-arabinose units exist in a similar state of combination in both gums.

Periodate oxidation of the two gums also showed a distinct structural difference (Table 4). The results indicate that the bark-gum polysaccharide has a greater proportion of pyranosyl non-reducing end groups than the fruit-surface gum.

Qualitative analysis of the stone gum showed that it had a monosaccharide composition similar to that of the fruit-surface gum. Quantitative analysis was not possible because of the difficulty in isolating the gum free from pectin and other fruit polysaccharides. The stone gum was less soluble in water than the fruit-surface gum and it was suspected that this was due to a difference in the inorganic content of the two gums. Paper-chromatographic analysis, however, showed that the same cations were present in essentially similar proportions (Table 7). The solubility difference may be a purely physical phenomenon rather than structural differences in the macromolecules.

Colvin, Cook & Adams (1952) and Isherwood (1949) have studied the electrophoresis of polysaccharides. The former workers were able to separate an artificial mixture of sodium alginate and pectin and Isherwood was able to resolve mixtures of hemicelluloses obtained from pear cell wall. Our own preliminary experiments indicated that plant gums could be examined electrophoretically, but the length of time used for separations was limited owing to the rapid diffusion of the boundaries. With a phosphate buffer, pH 7.0, plum-bark gum was readily separated from sugar-beet araban and it could also be separated from gum arabic with borate buffer, pH 7.8. Bark gum appeared to be homogeneous in a variety of buffers of varying pH, but despite the obvious differences in the structures of bark gum and fruit-surface gum, an artificial mixture of the two could not be resolved electrophoretically. The homogeneity of plum gums is therefore still doubtful.

Small amounts of mono- and oligo-saccharides were detected in a number of different gums. Free sucrose, glucose, fructose and xylose were detected in the plant tissues; hence the gums could easily have become contaminated with these sugars. Arabinose may have arisen by autohydrolysis of arabofuranose end groups, which are common in many plant polysaccharides. In view of the mild extractive conditions, however, arabinose and the other oligosaccharides probably arose as a result of photochemical degradation of the gum polysaccharides whilst on the tree and are not substrates for gum biosynthesis.

The lability to acid of the arabinose oligosaccharides extracted from cherry gum suggested that arabofuranose units were present (Hirst, 1949). The disaccharide was probably 3-O- or 4-O-L-arabofuranosyl-L-arabinose. A 1 → 5 linked disaccharide is improbable owing to the high positive optical rotation. 2:5-Di-O-methyl-L-arabinose has been isolated from methylated cherry gum (Jones, 1947), and this could arise from 1 → 3 linked arabofuranose units.

The available evidence suggests that one of the oligosaccharides extracted from gum arabic is 3-O- β -L-arabopyranosyl-L-arabinose, a disaccharide which has been isolated from the partial hydrolysis products of several gums (Andrews, Ball & Jones, 1953; Charlson, Nunn & Stephen, 1955). The detection of this disaccharide in gum arabic is of interest in relation to the structural studies of Dillon, O'Ceallachain & O'Colla (1953), who state that one of the side chains in the gum-polysaccharide molecule must contain two arabinose units, in addition to the galactose end group, instead of a single unit as was originally suggested (Jones & Smith, 1949).

The small amount of nitrogen present in plum gums is probably all proteinaceous. Phenolase (oxidase) and peroxidase have been observed in gum arabic (Laursen, 1932) and various colorimetric tests have confirmed the presence of peroxidase in plum gums, although the phenolase activity appeared to be low.

The bark gum is invariably darker in colour than the fruit-surface gum and this may be due to the higher peroxidase activity in the former, which results in an increase in coloured phenolic oxidation products. In this connexion it is interesting that oxidized phenolic compounds can be antimicrobial (R. J. W. Byrde, A. H. Fielding & A. M. Williams, unpublished results).

Paper chromatography showed that many of the phenolic materials found in the fruit-mesocarp tissues of plum were not present in bark gum or fruit-surface gum. The fruit tissues contained a large number of compounds which, on paper chromatograms, reacted with the diazotized *p*-nitroaniline-sodium carbonate spray reagent and fluoresced under u.v. light. Evidence was also obtained for the presence of a number of phenolic glycosides. The gums, however, contained only a small number of phenolic constituents and phenolic glycosides were not apparent. It is tempting to suggest that the phenolic glycosides are utilized for the biosynthesis of gum polysaccharide, with the liberation of the phenolic aglycones. There is no direct evidence for this hypothesis, however.

With regard to the biosynthetic route of gum-polysaccharide formation the analytical results show that the plum-bark gum and fruit-surface gum have different compositions and may therefore be formed by different processes, although similar repeating units, synthesized by the same pathways, could be present in both gums.

SUMMARY

1. The composition, function and formation of gum has been studied with particular reference to the plum tree (var. Victoria).

2. The polysaccharide moiety of the gum exuded on the fruit surface was composed of D-galactose, D-mannose, L-arabinose, D-xylose, L-rhamnose, glucuronic acid and probably traces of 4-O-methylglucuronic acid. The bark gum was similarly constituted but contained higher proportions of D-mannose, L-arabinose and hexuronic acid. Periodate oxidation of the two types of gum suggested structural differences though their electrophoretic mobilities were identical.

3. Crude bark gum and fruit-surface gum contain the enzyme peroxidase, phenolic compounds and a 'lignin-like' substance and some inorganic material. Gums from several different species of plants were observed to contain small amounts of mono- and oligo-saccharides.

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