770. *The Gum Component of Olibanum.*

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Two polysaaccharides designated I and II have been isolated from the gum component of olibanum, the gum oleoresin from *Boswellia carteri*. Polysaccharide I is neutral and composed of galactose and arabinose in equimolecular ratio. Polysaccharide II is acidic and composed of galactose and galacturonic acid in the molar ratio 2 : 1.

Olibanum, the gum oleoresin exuded from the incense tree *Boswellia*, has been extensively studied. Most of the work dealt with the chemistry of its resin and its essential oils and little is known of its gum component. Tschirch and Halbey [1] isolated an acidic gum after removing the essential oils and resinous matter by extraction with 90% alcohol. The gum was in the form of a salt containing calcium and magnesium. Malandkar [2] isolated from the Indian variety of olibanum, *Boswellia serrata*, a gum which was also acidic; on hydrolysis with 3% sulphuric acid it yielded arabinose, xylose, and galactose. The present work had as its objective the isolation of the pure gum components and a study of their structures.

Commerical East African olibanum from *Boswellia carteri* was used. The essential oils and resins were removed by ether-extraction, leaving a water-soluble gum, completely precipitated by 3 volumes of ethanol and containing 11% of inorganic matter, mainly calcium. Demineralisation with cation-exchange resin then gave an acidic gum mixture. Precipitation with 3 volumes of ethanol yielded a neutral gum, polysaccharide I (26%), and the major portion (59%) was precipitated only after further addition of 3 volumes of acetone, yielding an acidic gum, polysaccharide II. Both polysaccharides were purified by repeated precipitation with alcohol and acetone.

Polysaccharide I had $[\alpha]_D^{20}$ $-14{\cdot}7°$. Its mean reducing power determined by the methods of Meyer, Noelting, and Bernfeld,[3] and Jendrassik and Polgar [4] was 4·1%, corresponding to an average molecular weight of 4400 (30 monosaccharide molecules). The reducing power after hydrolysis, determined as glucose by Jendrassik and Polgar's method, reached a maximum value of 99·2% after 6 hours' hydrolysis with 0·5N-hydrochloric acid. Colour reactions indicated the presence of an aldopentose and the absence of ketoses, amino-sugars, and uronic acids. Paper chromatograms of the acid hydrolysate showed spots corresponding to galactose and arabinose; the two aldoses were separated on a large scale by means of broad paper chromatograms, and were thus obtained crystalline. D-Galactose was characterised as its *N*-methyl-*N*-phenylhydrazone and phenylosazone; L-arabinose as its *N*-benzyl-*N*-phenylhydrazone and *N*-methyl-*N*-phenylhydrazone. By using known amounts of hydrolysate and Meyer, Noelting, and Bernfeld's [3] and Jendrassik and Polgar's [4] methods of analysis it was shown that there were formed equimolar proportions of galactose and arabinose: after 82 hours 2·0 mols. of periodic acid were consumed and 0·22 mol. of formic acid was produced per two aldose molecules (formaldehyde could not be detected). Methylation and hydrolysis followed by fractionation on paper chromatograms yielded 2 : 4-di- (identified as aniline derivative), 2 : 3 : 4-tri- (identified as aniline derivative and phenylhydrazide), and 2 : 3 : 4 : 6-tetra-*O*-methylgalactose (identified as aniline derivative); no methylated arabinose could be isolated in crystalline form.

Polysaccharide II had $[\alpha]_D^{20}$ $-9{\cdot}2°$. Its mean reducing power was 3·25%, corresponding to an average molecular weight of 5500 (33 monosaccharide molecules). The reducing power determined as glucose by Jendrassik and Polgar's method [4] reached a maximum value of 93% after 6 hours' hydrolysis with 0·5N-hydrochloric acid. Colour reactions indicated the presence of uronic acid and absence of ketoses, amino-sugars, and pentoses.

[1] Tschirch and Halbey, *Arch. Pharm.*, 1898, **236**, 487.
[2] Malandkar, *J. Indian Inst. Sci.*, 1925, **8**, *A*, 240.
[3] Meyer, Noelting, and Bernfeld, *Helv. Chim. Acta*, 1948, **31**, 103.
[4] Jendrassik and Polgar, *Biochem. Z.*, 1940, **304**, 271.

Paper chromatograms of the acid hydrolysate of polysaccharide II showed spots corresponding to galactose and uronic acid. The two components were separated and D-galactose was identified as above. Galacturonic acid was identified as brucine salt and by conversion into mucic acid. The galactose was estimated after quantitative separation on paper chromatograms and galacturonic acid by titration and by estimation of carbon dioxide produced on decarboxylation with 12% hydrochloric acid.[5] The ratio of galactose and galacturonic acid was found to be 2 : 1. Polysaccharide II consumed 3·0 mols. of periodic acid in 56 hours and 0·46 mol. of formic acid was produced per 3 monosaccharide molecules (formaldehyde could not be detected).

Methylation and hydrolysis followed by fractionation on paper chromatograms yielded the same methylated galactose derivatives as from polysaccharide I, suggesting the same type of galactose linkage. No methylated derivative of galacturonic acid could be isolated in a crystalline form. In both polysaccharides the amount of 2 : 4-di-*O*-methylgalactose isolated was considerable, suggesting highly branched structures.

Malandkar[2] claimed the presence of xylose in the hydrolysate of his gum; paper chromatography of polysaccharide I and II and of the crude gum showed no traces of this pentose. The xylose present in Malandkar's gum is due either to parts of the plant remains present in commercial olibanum or to a difference in the composition of the gum obtained from the Indian variety and our East African variety.

Experimental

Isolation and Purification of the Gum Mixture.—Continuous extraction of powdered olibanum (160 g.) with ether for 26 hr. completely removed the essential oil and the resin components, leaving a residue (42 g.) of a white water-soluble gum. This was dissolved in warm water (900 ml.), and a small insoluble residue (mainly bark and plant remains) was rejected. The clear brown filtrate was treated with 3 vols. of ethanol and kept overnight at 0° and the precipitate collected by centrifugation. This product was again dissolved in warm water (500 ml.), filtered, and precipitated with 3 vols. of ethanol to give a colourless gum mixture (33·5 g.) containing 11% of ash.

Separation of the Two Polysaccharides.—A solution of the gum mixture (29 g.) in water (250 ml.) was passed several times through Zeocarb. Polysaccharide I was precipitated from the solution by the addition of 3 vols. of ethanol and collected by centrifugation after storage overnight at 0°, yielding 7·6 g. of a pale brown glass, extremely difficultly soluble in water but readily soluble in dilute alkalis.

The supernatant liquid from the alcohol precipitation was treated with 3 times its volume of acetone and kept overnight at 0°. The precipitated polysaccharide II was a colourless glass (17 g.), readily soluble in water.

Both products still contained a small amount of ash.

Polysaccharide I.—(a) *Purification.* A solution of polysaccharide I (7·6 g.) in 0·5N-sodium hydroxide (80 ml.) under nitrogen was acidified with acetic acid, filtered from a small insoluble residue (which was rejected), and treated with 3 vols. of ethanol. The precipitate was collected by centrifugation after storage overnight at 0°. It was treated twice more in the above manner, the last time with 5 vols. of ethanol. The final product was dialysed at 0° against frequent changes of distilled water until free from acetate ions and evaporated to dryness. Pure polysaccharide I was a pale cream-coloured friable glass, difficult to dissolve in water. It had $[\alpha]_D^{20} = -14·7°$ (*c*, 1·09 in 1 : 3 pyridine–water) and gave no ash on combustion [Found: C, 44·8, 44·7; H, 6·3, 6·3; N, 0·0. $(C_6H_{12}O_6 + C_5H_{10}O_5)_{15} - (H_2O)_{29}$ requires C, 44·7; H, 6·1%].

(b) *Reducing power before and after hydrolysis.* (1) By the method of Meyer, Noelting, and Bernfeld.[3] The reference sugar curve for glucose was made by treating standard solutions (3 ml.) containing 0·6—2·0 mg. of glucose with 1·5% aqueous 3 : 5-dinitrosalicylic acid (1 ml.) and 6N-sodium hydroxide (1 ml.) and heating them in a thermostat at 65° for 30 min. On cooling, 10 ml. of water were added and the colour determined in a Spekker absorptiometer (a 1-cm. cell and Ilford Spectrum Green Filter No. 604).

The reducing power of polysaccharide I before hydrolysis was determined on 3 ml. of solutions

[5] Norris and Resch, *Biochem. J.*, 1935, **19**, 1590.

containing 10 mg. Duplicate readings gave for the unhydrolysed polysaccharide a reducing power of 4·0%.

(2) By the method of Jendrassik and Polgar.[4] The reducing power of the unhydrolysed polysaccharide was determined directly on 10 mg. in warm water (10 ml.). For the hydrolysed polysaccharide, solutions in 0·5N-hydrochloric acid (*ca.* 1 mg./ml.) were heated at 100° in sealed tubes for different times. The tubes were cooled and the contents accurately neutralised with 0·5N-sodium carbonate and made up to 10 ml. with water. For each determination 1 ml. of this solution was used after dilution with 10 ml. of water. Results were :

Time (hr.)	0	1	2	3	6	8
Reducing power as glucose (%)	4·2	62·0	80·6	97·7	99·2	99·2

(c) *Paper chromatography of hydrolysate.* The polysaccharide (40 mg.) was hydrolysed for 6 hr. at 100° in a sealed tube with 0·5N-sulphuric acid. Sulphate ions were removed by boiling the mixtures with excess of barium carbonate, and the filtrate was evaporated to dryness and redissolved in 0·5 ml. of water. One-dimensional paper chromatograms were run on Whatman No. 1 filter paper and developed with the upper layer of butan-1-ol–ethanol–water–ammonia (40 : 10 : 49 : 1),[6] and sprayed with ammoniacal silver nitrate.[7] For complete separation of the components, the chromatograms were allowed to run for 48 hr.; two spots were obtained with R_F 0·14 and 0·19, controls on the same paper showing identical values for galactose and arabinose respectively.

(d) *Separation of the sugar components of hydrolysate.* The polysaccharide (0·5 g.) was hydrolysed with 0·5N-sulphuric acid (4 ml.) as above and the products were redissolved in 1·2 ml. of water. One-dimensional paper chromatograms were run on Whatman No. 1 filter paper with the hydrolysate evenly distributed along the whole length of the line of origin (0·1 ml./10 cm.); they were left to run for 48 hr. with the developing solvent mixture described in (c). Three strips, each 1 cm. wide, were cut through the length of each chromatogram in its right, left, and middle portions and sprayed with ammoniacal silver nitrate. The positions of sugar bands thus revealed were marked on the unsprayed portions of the chromatograms, which were cut off and eluted with water. Combined eluates from the upper bands gave, on drying in a vacuum-desiccator, crystals of galactose, m. p. 117° (lit., 118°), and those from the lower bands gave arabinose, m. p. 155—156·5°.

(e) *Identification of galactose.* Galactose obtained from the combined upper bands was recrystallised from hot alcohol, yielding colourless crystals, m. p. 164° not depressed on admixture with α-D-galactose. It had $[\alpha]_D^{20}$ +75° (*c* 0·24) (lit., +140° ⟶ 81·7°). A portion (0·1 g.) was heated in water (1 ml.) at 100° for 5 min. with acetic acid (0·5 ml.) and *N*-methyl-*N*-phenylhydrazine (5 drops). The crystals which separated, when recrystallised from alcohol, had m. p. 188—189° not depressed on admixture with D-galactose *N*-methyl-*N*-phenylhydrazone, m. p. 188—189°.

This galactose was also converted into the phenylosazone, m. p. and mixed m. p. 184°.

(f) *Identification of arabinose.* The product from the combined lower bands of the chromatograms, recrystallised from alcohol, had m. p. 157°, alone or mixed with β-L-arabinose. It had $[\alpha]_D^{20}$ +101° (final value; *c* 1 in H_2O) (lit., +191° ⟶ +105°). This product (0·1 g.) in water (1 ml.) was heated with *N*-benzyl-*N*-phenylhydrazine (5 drops) in acetic acid (0·5 ml.) for 1 min. in a water-bath. The product, recrystallised from alcohol, had m. p. 171° alone or mixed with L-arabinose *N*-benzyl-*N*-phenylhydrazone. The *N*-methyl-*N*-phenylhydrazone, similarly prepared and recrystallised, had m. p. and mixed m. p. 160°.

(g) *Estimation of the components.* The components were estimated after separation on paper chromatogram. The polysaccharide (125 mg.) was hydrolysed for 6 hr. at 100° with 0·5N-sulphuric acid, and the mixture neutralised with barium carbonate, filtered, and evaporated. The residue was dissolved in 0·5 ml. of water. One-dimensional paper chromatograms (22 cm. wide) were run with the hydrolysate (0·2 ml.) evenly distributed on the line of origin except for 5 cm. on each side. A drop of the hydrolysate was put in these spaces 2 cm. from the edges and the chromatograms developed for 48 hr. with the solvent mixture described in (c). The sugars were located as in the previous experiment. The aldoses were estimated by the methods of Jendrassik and Polgar[4] and Meyer, Noelting, and Bernfeld.[3] 0·1 ml. of eluate was used in the first method and 1 ml. in the second, diluted to 10 ml. and 3 ml. respectively. Standard sugars used were galactose and arabinose run on similar chromatograms. Correction was made for

[6] Hirst, Hough, and Jones, *J.*, 1949, 931.
[7] Partridge, *Biochem. J.*, 1948, **42**, 238.

loss of reducing power of these sugars after 6 hours' heating at 100° with 0·5N-sulphuric acid. The results were :

Method	Galactose (%)	Arabinose (%)
Jendrassik and Polgar	61·0	49·4
Meyer, Noelting, and Bernfeld	61·1	49·8

(h) *Oxidation with periodate.* Polysaccharide I (20 mg.) was dissolved in water (10 ml.); 1-ml. portions of this solution were kept at room temperature with 0·0056N-sodium periodate (5 ml.) and acetate buffer of pH 5·2 (0·5 ml.). Here and below unused periodate was determined by titration. The results, expressed in terms of units of mol. wt. 294 (1 galactose + 1 arabinsoe − 36) were :

Time (hr.)	1	3	5	23	32	48	72—82
Mols. of IO_4^- consumed per sugar unit	0·5	0·8	0·9	1·3	1·8	1·9	2·0

For the estimation of formic acid, polysaccharide I (100 mg.) was treated in water (50 ml.) with 1·5% aqueous potassium periodate (30 ml.) and left in the dark for 96 hr. and the excess of periodate destroyed with ethylene glycol. Formic acid was titrated against 0·09N-barium hydroxide (methyl-red) : 0·22 mol. was produced per unit of mol. wt. 294.

(i) *Methylation.* Preliminary experiments with polysaccharide I showed that after 14 consecutive treatments with methyl iodide and sodium in liquid ammonia [8] the methoxyl content reached 29·9%. Methylation with dimethyl sulphate in 35% aqueous sodium hydroxide [9] 16 consecutive times gave a product having 34·0% of OMe. Partially methylated polysaccharide I (6 g.) obtained by the above two methods was acetylated with acetic anhydride and pyridine, then methylated with dimethyl sulphate in 35% aqueous sodium hydroxide.[10] The process was repeated 14 times and the product dialysed against frequent changes of distilled water until free from sulphate ions and then evaporated to dryness, giving a pale brown gum (1·35 g.) [Found : OMe, 41·2. $C_6H_7O_2(OMe)_3,C_5H_6O_2(OMe)_2$ requires OMe, 42·6%]. The methylated polysaccharide I was hydrolysed first with 1% methanolic hydrogen chloride, then with 0·5N-hydrochloric acid, finally neutralised with silver carbonate, and a portion of this hydrolysate run for 10 hr. on paper chromatograms with the solvent mixture described in (c). Three spots were obtained, having R_G, 0·41, 0·64, and 0·88 respectively (R_G = relative to tetramethyl glucose). The methylated sugars (0·8 g.) were separated on large paper chromatograms as described in (d). Three bands were obtained; the eluate from the upper band yielded on evaporation 2 : 4-di-*O*-methylgalactose as its hydrate, m. p. 102° (77 mg.), and was converted into the aniline derivative, m. p. and mixed m. p. 212°. The eluate (88 mg.) from the second band gave on boiling with alcoholic aniline a derivative, m. p. 164—165°, identical with that from 2 : 3 : 4-tri-*O*-methylgalactose. Another portion of this band was treated with bromine water and the acid formed converted into the phenylhydrazide, m. p. 170° not depressed on admixture with 2 : 3 : 4-tri-*O*-methylgalactonic phenylhydrazide. From the third band 2 : 3 : 4 : 6-tetra-*O*-methylgalactose (35 mg.) was obtained and identified by its conversion into the aniline derivative, m. p. and mixed m. p. 184—186°.

Polysaccharide II.—(a) *Purification.* Polysaccharide II (7·5 g.) was passed in water (250 ml.) through Zeocarb, filtered, and treated with 3 vols. of ethanol; a small amount of polysaccharide I separated (1·5 g.) and was removed by centrifugation after storage overnight at 0°. The supernatant liquid after the alcohol precipitation was treated with 3 vols. of acetone and left overnight at 0° and the precipitated polysaccharide II collected by centrifugation and dried. On further treatment as above no precipitate separated on addition of 3 vols. of ethanol; with acetone 5·5 g. of product were obtained. For final purification this product (5 g.) was dialysed in water (100 ml.) at 0° against frequent changes of distilled water, then treated with 3 vols. of ethanol; again no precipitation occurred and the solution was treated with 5 vols. of acetone and kept overnight at 0°; pure polysaccharide II was collected by centrifugation and dried. It (4·9 g.) formed a white powder, readily soluble in water, $[\alpha]_D^{20}$ −9·2° (*c*, 1·1), [Found : C, 43·5, 43·6; H, 6·2, 6·1; N, 0·0; ash, 0·0. $(C_6H_{10}O_7 + 2C_6H_{12}O_6)_{11} - (H_2O)_{32}$ requires C, 43·1; H, 5·6%].

(b) *Reducing power before and after hydrolysis.* The reducing powder of the unhydrolysed polysaccharide II was determined by the method of Meyer, Noelting, and Bernfeld.[3] Duplicate

[8] Muskat, *J. Amer. Chem. Soc.*, 1934, **56**, 2449.
[9] Haworth, *J.*, 1915, **107**, 8.
[10] Haworth and Percival, *J.*, 1932, 2279.

readings gave a reducing power of 3·2%. The reducing power before and after hydrolysis determined by Jendrassik and Polgar's method [4] was :

Time (hr.)	0	1	2	4	6	8
Reducing power as glucose (%)	3·3	62·0	77·5	89·9	93·0	93·0

(c) *Paper chromatography of the hydrolysate.* The polysaccharide was hydrolysed and chromatographed as described for polysaccharide I. Two spots were obtained having R_F values 0·03 and 0·14; controls on the same paper showed identical values for uronic acid and galactose (galacturonic and glucuronic acid showed very similar R_F values and could not be separated from an artificial mixture of the two).

(d) *Separation of the sugar components of the hydrolysate.* The hydrolysis products were separated on broad paper chromatograms as described for polysaccharide I. The combined eluates from the upper bands gave, when evaporated in a vacuum desiccator, glassy galacturonic acid; those from the lower bands gave crystalline galactose.

(e) *Identification of galacturonic acid.* A portion (150 mg.) of the dry eluate from the upper bands of the chromatograms was heated in water (1 ml.) at 100° with the equivalent amount of brucine. After 15 min. the solution was cooled and extracted several times with chloroform to remove unchanged brucine. On drying of the aqueous layer in a vacuum-desiccator crystals of the brucine salt of galacturonic acid were obtained and recrystallised from alcohol. The pure salt had m. p. 176—178°, not depressed on admixture with brucine galacturonate.

The uronic acid (150 mg.) was treated in water with saturated aqueous bromine (25 ml.), left at room temperature for 4 days, and evaporated to dryness, and the residue dissolved in N-sodium hydroxide (2 ml.). Mucic acid was precipitated by addition of concentrated hydrochloric acid and, recrystallised from ethanol–ether, had m. p. and mixed m. p. 213°.

(f) *Identification of galactose.* The product obtained from the combined lower bands, recrystallised from alcohol, gave galactose, m. p. and mixed m. p. 164°, converted into the *N*-methyl-*N*-phenylhydrazone and phenylosazone as described for polysaccharide I.

(g) *Estimation of the components.* Galactose was estimated after separation on paper chromatograms as in the case of polysaccharide I (methods of Jendrassik and Polgar [4] and Meyer, Noelting, and Bernfeld [3]).

Galacturonic acid was estimated by Norris and Resch's method.[5] The polysaccharide (97 mg.) was heated with 12% hydrochloric acid at 140—150° under nitrogen, and the carbon dioxide evolved absorbed in N-barium hydroxide. The amount of carbon dioxide, from the decarboxylation of the uronic acid, was determined by titrating the barium hydroxide with 0·1N-acid. Further, galacturonic acid in the polysaccharide was estimated directly by titration against standard alkali. All the results are shown in the annexed Table.

Galactose : Jendrassik and Polgar	71·7%
Meyer, Noelting, and Bernfeld	70·1%
Galacturonic acid : Norris and Resch	38·0%
Titration	39·1%

(h) *Oxidation with periodate.* This was conducted as for polysaccharide I. The results expressed in terms of sugar units of mol. wt. 500 (2 galactose + 1 galacturonic acid − 54) were :

Time (hr.)	1	4	6	10	14	24	44—56
Mols. of IO_4^- consumed per sugar unit	0·3	0·9	1·1	1·6	1·8	2·4	3·0

Polysaccharide II (175 mg.) in water (50 ml.) was treated with 1·5% potassium periodate solution (30 ml.), left in the dark for 60 hr., and treated with ethylene glycol. Formic acid was titrated against 0·09N-barium hydroxide (methyl-red); 0·46 mol. of formic acid was produced per sugar unit of mol. wt. 500.

(i) *Methylation.* Polysaccharide II (2·5 g.) was methylated as was polysaccharide I. The final methylated product had OMe, 45·9% [$C_6H_5O_3(OMe)_3,2C_6H_7O_2(OMe)_3$ requires OMe, 44·6%]. The methylated polysaccharide II (1·1 g.) was hydrolysed and the products were separated on large paper chromatograms as described for polysaccharide I. Three bands were obtained : the eluate from the upper band yielded on evaporation 2 : 4-di-*O*-methylgalactose as its hydrate, m. p. 102° (73 mg.), and was converted into the aniline derivative, m. p. and mixed m. p. 212°. The eluate from the second band (117 mg.) gave with boiling alcoholic aniline a derivative, m. p. 164—165°, identical with that from 2 : 3 : 4-tri-*O*-methylgalactose : another portion of this band was converted by bromine water, etc., into the phenylhydrazide, m. p. 170° alone or mixed with that of 2 : 3 : 4-tri-*O*-methylgalactonic acid. From the third

band 2 : 3 : 4 : 6-tetra-*O*-methylgalactose (96 mg.) was obtained and identified by its conversion into the aniline derivative, m. p. and mixed m. p. 184—186°.

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