Characterization of Polysaccharides Isolated from Forest Soils

By B. BERNIER*

Department of Forestry, University of Oxford

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The present investigation has concerned itself with the differences associated with the development of the two contrasted types of forest humus. These are known as mull and mor, and are related to the nature of the vegetation. One of the characteristics differentiating soils associated with these two morphologically different types of humus is the development of crumb structure in mull soils, but not in soils associated with mor. There is a considerable body of opinion which holds that the development of crumb structure in soil is, in part at least, associated with polysaccharides produced by soil micro-organisms (Martin, 1945; Geoghegan & Brian, 1946). This is also supported by the work of Swaby (1949), who observed that microbial gums vary widely in their ability to aggregate soil.

The isolation from the soil of carbohydrate material that might in some way be related to those carbohydrates which have been found to induce aggregation has also attracted attention. A polysaccharide material was isolated from various soils by Forsyth (1950). For each soil this material was composed of the following sugars: galactose, glucose, mannose, arabinose, xylose and an aldo-biuronic acid containing glucuronic acid. The selective method of isolation of this polysaccharide might be thought to lead to a fairly homogeneous product. Duff (1952*a*) isolated from the soil a

water-soluble polysaccharide complex which gave, on hydrolysis, the sugar units found previously in the material obtained by Forsyth (1950) and, in addition, rhamnose and at least two methylated sugars. Fucose was later identified (Duff, 1952*b*) among the sugars present in the hydrolysates. The methyl sugars have been partially identified as 2:6- or 2:7-di-*O*-methyl aldoheptoses (Duff, 1954). Duff (1954-55) has shown that one of the methylated sugars from soil extract is identical with an *O*-methyl sugar which was found to be a constituent of a polysaccharide produced by *Bacillus megaterium* (Forsyth, 1954). Dubach, Zweifel, Bach & Deuel (1955) and Whistler & Kirby (1956) have also isolated a polysaccharide complex from the soil. However, no comparative study of these constituents in forest soils associated with various types of humus has yet been made. It was therefore of interest to try to ascertain whether different kinds, and possibly different amounts, of polysaccharides are present in soils associated with mull and mor respectively.

MATERIALS AND METHODS

Soils

Representative samples of three mull soils and two soils with mor layers have been used for the investigation. Some of their characteristics are presented in Table 1. An identification number has been assigned to each soil and is used throughout the text.

* Present address: Faculty of Forest Engineering, Laval University, Quebec, Canada.

Table 1. *Characteristics of the soils used in the investigation*

Identification no.	Parent mineral material	Nature of vegetation	Horizon sampled	Degree of aggregation*
Mull soils				
1	Calcareous grit	<i>Ulmus glabra</i> Huds.	Mull layer (pH 5.7)	17
2	Calcareous clay	<i>Quercus</i> spp., <i>Fraxinus excelsior</i> L.	Mull layer (pH 6.4)	46
3	Plateau gravel (non-calcareous)	<i>Fagus sylvatica</i> L., <i>Quercus</i> spp.	Mull layer (pH 5.6)	29
Soils with mor layers				
4	Non-calcareous tertiary gravel	<i>Fagus sylvatica</i> L.	Uppermost mineral horizon (pH 4.6)	3
5	Non-calcareous tertiary gravel	<i>Calluna vulgaris</i> (L.) Hull.	Uppermost mineral horizon (pH 3.5)	Nil

* Mean wt. (g.) of aggregates > 1 mm./50 g. of soil on an oven-dry wt. basis.

Determination of aggregates in the soils. This was done by the wet-sieving technique described by Swaby (1949). Only macroaggregates (>1 mm.) were estimated.

Methods used in the examination and analysis of the polysaccharides

Material for analysis. Intensive drying of the polysaccharides was avoided. Air-dried preparations contained 10–15% of moisture. Samples for analysis were dried *in vacuo* at 60° over phosphorus pentoxide.

Total nitrogen, phosphorus and acetyl determinations. Acetyl, phosphorus and most of the nitrogen determinations were carried out by Weiler and Strauss, Oxford. Other nitrogen determinations were made by the semi-micro-Kjeldahl method.

Ash. The ash content was determined by incineration at 550° .

Uronic acid determination. Uronic acids were determined before hydrolysis by the Boas (1955) modifications of the carbazole method of Dische (1947).

Specific rotation. When samples of only a few milligrams were available the rotations were determined by Weiler and Strauss, Oxford. Otherwise this was determined by the use of a polarimeter having an accuracy of $\pm 0.1^\circ$.

Hydrolysis. Hydrolysis of polysaccharides was usually carried out in sealed tubes on 10–20 mg. samples in the presence of 0.5–1.0 ml. of $\text{N-H}_2\text{SO}_4$ for 7 hr. Neutralization was carried out with barium carbonate. N-HCl was also used and was removed in a vacuum desiccator containing solid NaOH .

Chromatography. Except where otherwise stated, chromatography was carried out on Whatman filter papers no. 1 and no. 3MM, with butanol-pyridine-water (10:3:3, by vol.) (Jones & Wise, 1952), or ethyl acetate-acetic acid-water (3:1:3, by vol.) (Jermyn & Isherwood, 1949) as solvent systems. The position of the sugars on the chromatograms was revealed by spraying with *p*-anisidine hydrochloride (Hough, Jones & Wadman, 1950) or with aniline phthalate (Partridge, 1949). The term R_f used in the text refers to the rate of movement of the spots relative to glucose.

Infrared spectra. These were obtained by the use of a Perkin-Elmer double-beam spectrophotometer, over the range 2–15 μ . The Nujol (liquid paraffin)-mull technique was used.

Acetylation. Acetylation of polysaccharides was carried out by the modifications of the method of Carson & MacLay (1948), proposed by Adams & Castagne (1952). Deacetylation was also effected according to the latter authors.

Viscosity. Measurements of relative and intrinsic viscosities were made with a Fenske-type viscometer (no. 50). The system was maintained at a temperature of $25 \pm 0.05^\circ$. The time of flow was measured to 0.1 sec. and at least three readings were made for each solution. For determinations 5 ml. of solution was used. For intrinsic-viscosity measurements, solutions containing 0.5% of polysaccharide were prepared, from which dilutions containing 0.25, 0.125 and 0.0625% were prepared. The terminology for the viscosity functions used in the text are those proposed by Cragg (1946).

Paper electrophoresis. This was carried out in an apparatus similar to that described by Kunkel & Tiselius (1951), with Whatman no. 1 paper (3.5 cm. $\times$ 45 cm.). The position of acidic polysaccharides was revealed by staining with a

solution of toluidine blue (Pasternak & Kent, 1952). In an attempt to reveal the presence of neutral polysaccharides, Schiff's reagent was used according to the method described by Köiw & Grönwall (1952).

Free-boundary electrophoresis. This was carried out at 4° by standard techniques, with the standard Hilger equipment and the optical system of Philpot (1938).

Precipitin reactions. Polysaccharide solutions (0.1 ml., about 0.1–0.4%) in physiological saline were layered over the pneumococcal antisera (0.1 ml.) in capillary tubes. The tubes were incubated at 37° for 1 hr. and the results read after standing at 4° overnight.

EXPERIMENTAL AND RESULTS

Isolation and purification of the polysaccharides

From preliminary attempts to isolate polysaccharide material from soil with dilute alkali, sodium pyrophosphate, hot water or buffer solutions as extracting agents, it was found that buffer solution was most suitable since it extracted less non-dialysable non-carbohydrate material. The yield of polysaccharide extracted was about the same as for hot-water extraction but without the disadvantage of heating. Moreover, the polysaccharides isolated in this way were found to have a higher viscosity than those isolated by other methods and may therefore be relatively undegraded.

In the method finally adopted, polysaccharides were extracted with buffer, $\text{m}/15\text{-Na}_2\text{HPO}_4$ and $\text{m}/15\text{-KH}_2\text{PO}_4$, pH 7.0, containing 5% (w/v) of KCl. Buffer solution (2 l.) saturated with toluene was used per kilogram of air-dried soil and extraction effected by vigorous stirring at room temperature for 10 hr. The solutions were filtered and the residues re-extracted for a further 6 hr. Two extracts, each from 1 kg. of soil, were bulked and dialysed at 4° against distilled water. The dialysed extracts were concentrated under reduced pressure at 25° (to 500 ml.), again dialysed (4°) and then poured into 4 vol. of ethanol containing 5% (v/v) of acetic acid. The resulting precipitate was centrifuged, washed with ethanol and redissolved in water. Solution and reprecipitation were repeated until the supernatant was colourless (usually three precipitations were required). The precipitate was then washed with ethanol of increasing strength, and then with ether. It was dried in the air until most of the ether had evaporated, and then *in vacuo* over CaCl_2 . The material from all sources gave a positive biuret test.

After many attempts to purify the extracted polysaccharides without degradation, the following purification procedure was finally adopted. The crude polysaccharide material was dissolved in water (0.5% solution) and gave a yellowish, opalescent solution. This was saturated with toluene and decolorized by passing (500 ml.)

through a bed (3.75 cm. $\times$ 2.5 cm. diam.) of activated charcoal (British Drug Houses Ltd.) previously thoroughly washed with water. The filtrate did not show any apparent change in viscosity. The charcoal bed was then washed with water (100 ml.), followed by mm-sodium tetraborate solution (usually 500 ml.), which removed almost quantitatively the part of the polysaccharide complex that had been adsorbed by the charcoal. Excess of tetraborate, or borate of higher concentration, was avoided since this resulted in the elution of some of the non-carbohydrate coloured material. The combined water and borate eluates were concentrated to the original volume and dialysed at 4°.

Proteins were removed by the method proposed by Laidlaw & Reid (1952). A slurry of 0.6 g. of CdSO₄ in 5 ml. of N-NaOH/g. of crude polysaccharide was added to the neutral solution (at 60°) and stirred vigorously for 1 min. The suspended material was centrifuged off, resuspended in water and again centrifuged. The combined supernatants were clarified by centrifuging for 30 min. at 15 000 g. Further protein was removed by the formation of a water-insoluble CHCl₃-protein gel (Sevag, Lackman & Smolens, 1938). This was followed by shaking the polysaccharide solution (to which acetic acid had been added so as to give a N solution) for 2 hr. with fuller's earth (300 mg. of adsorbent/g. of crude polysaccharide). The adsorbent was removed by centrifuging and washed with N-acetic acid. The combined washings and supernatant were dialysed and poured into 4 vol. of ethanol. The precipitate was washed repeatedly with ethanol and ether, and dried *in vacuo* over

CaCl₂ to give a white amorphous product. The yields and properties of the polysaccharides from the various soils are summarized in Table 2.

The polysaccharides gave no coloration with iodine and did not reduce Fehling's solution. They all gave a positive carbazole test (Dische, 1947) for uronic acids. The biuret test was negative. No characteristic ketose coloration was formed on treating with the anthrone reagent of Johanson (1953), thereby indicating the absence of levans. After hydrolysis, paper chromatography indicated that the various polysaccharide preparations were very similar in composition. The sugars identified were glucuronic acid (or its lactone), galactose, glucose, mannose, arabinose, xylose, fucose (absent in polysaccharides from soils 4 and 5) and rhamnose. A spot (R_f 2.76) observed in chromatography of the hydrolysates of at least four soil polysaccharides before the purification procedures was eliminated in the hydrolysates of the purified materials.

Procedures used in the fractionation of polysaccharide materials and examination for homogeneity

Attempted fractionation by ethanol precipitation. In typical experiments, about 500 mg. of a soil-polysaccharide preparation was dissolved in 100 ml. of 1% potassium acetate. Cold ethanol was added gradually while the solution was being stirred. When the solution became opalescent it was allowed to stand for a short time in the cold (5°) and any precipitate formed was centrifuged off, and washed with ethanol and ether and dried. Details of the yields and properties of the various fractions of the polysaccharide preparation from soil 1 are listed in Table 3. Fractionation by ethanol precipitation was carried out on polysaccharide preparations from all five soils. Results did not indicate any marked heterogeneity of the polysaccharide materials, as judged from the specific rotations of the fractions and their uronic acid content. This became more evident after hydrolysis (3–10 mg.) of each fraction followed by chromatography of the hydrolysates. All the sugars previously identified as constituents of the unfractionated polysaccharides were present in each fraction

Table 2. *Yields and properties of the polysaccharide preparations from the various soils*

Soil no.	Yield (g./kg. of dry soil)	$[\alpha]_D^{20} (\pm 1^\circ)$ in water (c, 1.0)	N (%)	Ash (%)
1	0.21	-18°	0.41	2.2
2	0.39	-12	0.60	2.7
3	0.17	-19	0.49	1.9
4	0.10	-11	0.26	2.1
5	0.11	-7	0.37	1.8

Table 3. *Properties of the various fractions obtained on fractional ethanol precipitation of the polysaccharide preparation from soil 1*

The polysaccharide (0.431 g.) was precipitated from 0.5% solution.

Fraction no.	Concn. of ethanol (% v/v)	Yield (% of total)	$[\alpha]_D^{20} (\pm 1^\circ)$ in water (c, 1.0)	Uronic acid (%)
1	59	48.9	-16.9°	7.7
2	70	18.8	-18.0	7.7
3	84	17.2	—	7.4
Recovery		84.9%		

and apparently in the same proportions, although no quantitative estimation of the various sugars was carried out.

Attempts to obtain significantly different fractions from the polysaccharide preparations by precipitation with ethanol from formamide solutions were unsuccessful. Cetylpyridinium bromide (Scott, 1956) also proved unsuccessful as a fractionating agent.

Paper electrophoresis. Polysaccharide materials isolated from the various soils moved as single spots at different rates towards the anode in the following buffers: borate (pH 9.05, I 0.06; 0.01M- $\text{Na}_2\text{B}_4\text{O}_7$ -0.005M- H_3BO_3), barbiturate (pH 8.6, I 0.075; Durrum, 1955) and phosphate (pH 8.0, I 0.1; 0.0326M- Na_2HPO_4 -0.0022M- KH_2PO_4). Tailing was more pronounced in acetate buffer (pH 5.1, I 0.1; 0.03M-acetic acid-0.07M-sodium acetate), particularly when high voltage (500-600v) was applied. With one exception all the fractions isolated by fractional ethanol precipitation had the same mobility as the original polysaccharide in borate buffer, a fact which is in agreement with the similarity of the other characteristics of the various fractions. With the polysaccharide preparation from soil 2 there appeared to be a large proportion of a stationary component, at least when borate buffer was used. Paper-electrophoresis patterns are shown in Fig. 1.

By application of the chromatographic method proposed by Kerby (1953) and with a solution of propanol (35%) in phosphate buffer (pH 6.4;

0.018M- Na_2HPO_4 -0.048M- KH_2PO_4) as the eluting agent, it was possible to separate the polysaccharide complexes from all the soil samples as two distinct spots, one fast-moving (R_f 0.76) and one stationary. Separation of 10 mg. quantities of these materials was effected by using several sheets of Whatman no. 3MM paper. The materials thus obtained were hydrolysed and chromatographed, which revealed that both the moving and the stationary fractions contained the same sugars in apparently the same proportions as the unfractionated polysaccharide preparation. In paper-electrophoresis experiments the material which separated into two spots on the chromatograms moved as a single spot (Fig. 1). It is possible that the moving component on the chromatogram arises from the original polysaccharide, although it is a fraction having a lower molecular weight, but the same charge. However, this fraction was only partly eliminated after prolonged dialysis. It remained stationary on a chromatogram run for 90 hr. with propan-2-ol (75%)-acetic acid (9:1, v/v) (Bird & Hopkins, 1954), a solvent which is particularly suitable for the rapid separation of oligosaccharides.

Attempted fractionation of acetylated polysaccharides. Polysaccharide preparations from soils 1 and 2 were selected for acetylation. The main fractions of both these polysaccharide preparations were insoluble in CHCl_3 , acetone, pyridine and dioxan after acetylation. A similar observation has been made with the acetylated complex polysaccharide produced by the blue-green alga *Anabaena cylindrica* (Bishop, Adams & Hughes, 1954). The dry acetylated polysaccharides were dissolved in CHCl_3 (1%, w/v) and the soluble material fractionally precipitated with light petroleum (b.p. 60-80°). All fractions were reprecipitated once from CHCl_3 soln. and the precipitates repeatedly washed with ether and dried *in vacuo* over phosphorus pentoxide. They were recovered as dry friable solids. The characteristics of these fractions are given in Table 4.

After deacetylation, 10-20 mg. of each fraction was hydrolysed and the neutralized hydrolysates were analysed by paper chromatography. All fractions from the polysaccharide preparation from soil 1 gave qualitatively identical chromatograms having approximately the same proportions of the various sugar constituents as in the original unfractionated preparation, except that arabinose tended to be absent from fractions 2 and 3.

Fractions of the deacetylated polysaccharide material from soil 2 also gave on hydrolysis the same sugars as were found in the original unfractionated preparation. However, there was some indication that the proportions of the sugar constituents differed in the various fractions and that some

Fig. 1. Paper-electrophoresis patterns of soil-polysaccharide preparations in borate buffer (pH 9.05, I 0.06; potential gradient: 12.5 v/cm. (a) Soil 1. 2 hr.: (1) spot of polysaccharide which was stationary on chromatogram (see text); (2) spot of polysaccharide which was mobile on chromatogram. (b) Soil 1. 5 hr.: (1) and (2), fractions 1 and 2 from polysaccharide fractionally precipitated by ethanol. (c) Soil 2. 4 hr.: (1) and (2), fractions 2 and 3 from polysaccharide fractionally precipitated by ethanol. (d) Soil 3. 3 hr.: unfractionated polysaccharide preparation. (e) Soil 5. 4 hr.: unfractionated polysaccharide preparation.

Table 4. *Yields and properties of the acetylated polysaccharide preparations from soils 1 and 2 after fractionation from chloroform solution by light petroleum*

Fraction no.	Concn. of light petroleum (% v/v)	Percentage of total wt.	Acetyl (%)	$[\alpha]_D^{20}$
Soil 1				
Insoluble	—	60.7	—	—
1	46.7	10.1	32.1	+15.7°
2	51.5	5.9	33.0	+8.46
3	67.5	3.3	—	—
Recovery		80.0%		
Soil 2				
Insoluble	—	41.1	—	—
1	44.9	10.4	35.3	—
2	47.4	14.9	35.7	—
3	50.9	7.3	36.6	No rotation found
4	60.8	11.4	35.95	+19.1
5	65.3	2.8	35.4	+23.7
6	86.5	2.6	36.1	+33.5
Recovery		90.5%		

Table 5. *Electrophoretic data of preparations of soil polysaccharide*

Soil no.	Buffer (pH; I)	$10^5 \times$ Average mobility (μ) (cm. ² v ⁻¹ sec. ⁻¹)
1	Borate (9.1; 0.12)	-6.3
2	Phosphate (7.8; 0.14)	(α) -0.9; (β) -3.6
	Acetate (5.1; 0.24)	(α_1) -1.3*; (α_2) -2.8*
3	Borate (9.1; 0.12)	-7.9
4	Borate (9.1; 0.12)	-5.1
5	Borate (9.1; 0.12)	-5.5

* Measured on the ascending boundary.

fractionation of the polysaccharide complex had been achieved. Glucose appeared in much greater concentration in the hydrolysates of the first fractions than in those of the last. Arabinose was absent from the first two fractions but gradually increased in quantity in the last fractions. There was no apparent change in the relative concentrations of the other sugars as compared with those of the unfractionated preparation, except that fucose was almost absent from fractions 5 and 6.

Free-boundary electrophoresis. The concentration of the polysaccharide preparations in the buffer solutions varied from 0.5 to 2%. Spreading of the boundaries was rather rapid and it was clear that the material was highly polydisperse. Mobility measurements were computed from the descending boundary and are given in Table 5. Because of the polydispersity of the material, the mobility figures quoted were derived from measurements on the maximum of the schlieren-boundary curves and are therefore average values.

Electrophoretic patterns of the polysaccharide preparation from soil 1 are shown in Fig. 2. The

Fig. 2. Electrophoretic patterns of polysaccharide preparation from soil 1. A, Borate buffer (pH 9.1, I 0.12); potential gradient: 4.7 v/cm.; 1% of polysaccharide; exposure at 39 min. B, Phosphate buffer (pH 7.8, I 0.14); 1% of polysaccharide; exposure at 37 min. Direction of movement anodic as shown by arrows; (a), ascending boundary, (d), descending boundary.

large stationary boundaries were found to be artifacts produced by unusually large δ and ϵ boundary anomalies. Similar observations have been reported for well-known acidic polysaccharides (Wolfrom, Madison & Crow, 1952; Record & Grinstead, 1953). The causes of the spreading of initially sharp boundaries have been discussed by Ogston (1946). In the present system spreading of boundaries in both limbs was reversed by reversal of the current, which would exclude the possibility of its being due to thermal diffusion, although the boundaries did not completely recover their initial sharpness. It is likely that the polysaccharides are mixtures of components having different mobilities, symmetrically distributed about a mean value.

Polysaccharides from soils 3 and 5 also gave a single moving boundary (Fig. 3). The polysaccharide preparation from soil 2 proved to be electrophoretically heterogeneous in the following buffer solutions: borate (pH 9.1, I 0.12; 0.02M- $\text{Na}_2\text{B}_4\text{O}_7$ -0.01M- H_3BO_3); phosphate (pH 7.8, I 0.14; 0.046M- Na_2HPO_4 -0.003M- KH_2PO_4); acetate (pH 4.7, I 0.1; 0.05M-acetic acid-0.05M-sodium acetate); barbiturate (pH 8.6, I 0.075; Durrum, 1955). Electrophoretic patterns are shown in Fig. 4. Attempts to isolate the different fractions by preparative electrophoresis procedures were unsuccessful. However, it had been observed in previous experiments that when the polysaccharide complex, dissolved in NaOH (approx. N), was treated with Benedict's solution, only part of it formed a precipitable copper complex. This method of fractionation proved to be successful as it enabled one component (α) to be separated from the remainder of the polysaccharide complex (β). The following procedure was used. The polysaccharide preparation (1.270 g.) was dissolved in 100 ml. of cold 4% NaOH and the solution was quickly treated with an equal volume of Benedict's solution. This was vigorously stirred for about 1 min. and a little acetone added to hasten the settling of the precipitate, which was separated by centrifuging, and washed with 20-30 ml. of 0.01M-NaOH and again centrifuged. The precipitation procedure was repeated twice. The precipitate was then suspended in water (100 ml.) by vigorous stirring. Cold N -HCl was added to decompose the copper complex and a slightly opalescent solution was obtained. The polysaccharide was then precipitated by pouring the solution into 3 vol. of cold ethanol. The white flocculent precipitate was immediately centrifuged and washed with slightly

acidified ethanol-water mixture (7:3, v/v), in order to remove the copper completely, and then with the unacidified mixture to remove the acid and finally with ethanol and ether (recovery 0.809 g., 63.7%). When dissolved in water this component (α) gave an opalescent and viscous solution. The intrinsic viscosity was almost unaffected by addition of electrolytes: $[\eta]=4.16$ (concn. g./100 ml.) in acetate buffer, pH 5.1, I 0.24; 0.1M-NaCl-0.1M-sodium acetate-0.04M-acetic acid. In acetate buffer (pH 5.1, I 0.24) this substance migrated slowly and only after electrophoresis had proceeded for a long time the boundary showed, besides a main component (α_1), the presence of a small amount of an additional component (α_2). The two components probably have similar molecular shape and weight since they are not separated significantly by ultracentrifuging (see Addendum).

That part of the polysaccharide preparation (β) which did not form a copper complex probably contained also a small amount of the component α , as judged by electrophoretic patterns obtained in acetate buffer (pH 5.1).

Fig. 3. Electrophoretic patterns of soil-polysaccharide preparations in borate buffer (pH 9.05, I 0.12); potential gradient 4.7 v/cm. A, Soil 3; 0.8% of polysaccharide; exposure at 37 min. B, Soil 5; 1% of polysaccharide; exposure at 58 min. (a), Ascending boundary; (d), descending boundary.

Fig. 4. Electrophoretic patterns of polysaccharide preparation from soil 2. A, Acetate buffer (pH 4.7, I 0.1); potential gradient 2.2 v/cm.; 0.74% of polysaccharide; exposure at 29 min. B, Phosphate buffer (pH 7.8, I 0.14); potential gradient 2.8 v/cm.; 0.5% of polysaccharide; exposure at 37 min. C, α -Polysaccharide fraction in acetate buffer (pH 5.1, I 0.24); potential gradient 2.2 v/cm.; 1.2% of polysaccharide; exposure at (1): 49 min., (2): 152 min. (a), Ascending boundary; (d), descending boundary.

Chemical composition and physicochemical characteristics of the soil polysaccharides

Polysaccharides from soils 1, 3, 4 and 5 were isolated from that section of the electrophoresis cell to which the single displacing boundary had moved. These materials were used for further chromatographic analysis.

The degree of polymerization (P) was derived from intrinsic viscosity measurements with Staudinger's equation $(\eta_{sp}/c)_{c \rightarrow 0} = K_m P$, where c is expressed in g./l. of solution and the K_m factor $= 5 \times 10^{-4}$ (Husemann, 1940). Estimates of the molecular weights (Table 6) were obtained by multiplying P by the average molecular weight of anhydrosugar residues, assuming an equimolecular ratio of pentoses and hexoses units. It is clear that the accuracy of the results is only limited. However, the estimate of molecular weight (124×10^3) obtained by the use of Staudinger's equation, for the α -polysaccharide fraction from soil 2, is in good agreement with the average molecular weight (130×10^3) derived from sedimentation measurements (see Addendum).

The polysaccharides gave on hydrolysis all the sugars detected in previous hydrolysates, and in approximately the same proportions, although quantitative estimation of the sugars was not made. Further details of the composition of the polysaccharides are given in Table 7. Graded hydrolysis was carried out by treating four separate lots of polysaccharides (20 mg.) with $N-H_2SO_4$ (1 ml.) at $97-100^\circ$ for 1, 2, 4 and 7 hr. respectively. Examina-

tion of the partial hydrolysates by chromatography showed that arabinose was released quickly, which would indicate that it is probably present in the chains in the furanoside form.

It was possible to isolate an aldobiuronic acid from the α -polysaccharide from soil 2, by pouring the hydrolysate (4 hr. hydrolysate, neutralized to methyl-red paper with $BaCO_3$) into 5 vol. of ethanol. The suspension was centrifuged and the precipitate of barium salts dried with ethanol and ether. The barium salt (about 4 mg.) was further hydrolysed in a sealed tube with $2N-H_2SO_4$ (0.4 ml.) for 6 hr., and from this glucose and glucuronic acid (or its lactone) could be detected on the chromatograms. Because of the nature of its aldobiuronic acid groups, this polysaccharide was used in precipitin tests with a variety of pneumococcal antisera. No cross-reaction could be observed with any of the antisera used (types II, III, VIII, IX, XII and XXII).

Table 6. *Intrinsic viscosities and molecular weights of soil polysaccharides*

Soil no.	Intrinsic viscosity $[\eta]^*$	$10^{-3} \times$ Approx. mol.wt.†
1	0.16	47
2 (α)‡	0.42	124
4	0.035	10.3
5	0.042	12.4

* c is expressed in g./l. of solution.

† Calculated from intrinsic viscosity measurements.

‡ $[\alpha]_D^{20} = 41 \pm 1^\circ$.

Table 7. *Sugar composition of the soil polysaccharides*

M, Major component; m, minor component; t, trace. The uronic acid was tentatively identified as glucuronic acid. It was not determined in soil no. 4.

Soil no.	Hexoses			Pentoses	Methyl pentoses	Uronic acid (%)
	Galactose	Glucose	Mannose			
1	(M)	(M)	(M)	Arabinose (m) Xylose (M)	Fucose (m) Rhamnose (t)	7.7
2 (α)	Galactose (t)	Glucose (M)	Mannose (m)	Xylose (m)	Fucose (m) Rhamnose (t)	3.0
3	Galactose (M)	Glucose (M)	Mannose (m)	Arabinose (m) Xylose (M)	Fucose (t) Rhamnose (t)	7.3
4	Galactose (M)	Glucose (M)	Mannose (M)	Arabinose (m) Xylose (M)	Rhamnose (t)	—
5	Galactose (M)	Glucose (M)	Mannose (m)	Arabinose (m) Xylose (M)	Rhamnose (t)	6.5

Fig. 5. Infrared spectra of the polysaccharides isolated from soils 1 and 3.

Infrared spectra of two soil polysaccharides (nos. 1 and 3) were obtained (Fig. 5). In general they show very similar patterns to those obtained from polysaccharides produced by fungi known to be active in decomposing wood and forest litter (Bernier, 1958). The weak inflexion at 1736 cm^{-1} confirms the presence of carboxylic groups arising from the small amount of glucuronic acid residues (Orr, 1954). The broad band between 1600 and 1700 cm^{-1} is probably due to residual water rather than to monosubstituted amides, which would appear as two characteristic bands at 1648 and 1560 cm^{-1} .

DISCUSSION

It seems probable that the soluble-polysaccharide fraction of soil does vary, to a certain extent, in various forest soils and might not have such constant characteristics as was hitherto suspected. It seems clear from these results that, although the same sugars appear to occur constantly in the hydrolysates of the polysaccharide preparations from a variety of forest soils, they are probably derived from various differing polysaccharides which might therefore be a reflexion of a certain degree of differential biological activity.

From the results of the fractionation procedures it would appear that the polysaccharides in the soil are heterogeneous and perhaps constitute a mixture of complex substances not easily separated. Their degree of complexity would explain the fact that they can, to a certain extent, accumulate in an environment where the microbial activity is extremely intense. Little is known about the mechanisms involved in the breakdown of heteropolysaccharides, except that they are usually more resistant to microbial attack than are less complex polysaccharides (Manners, 1955). This is also supported by results obtained from a preliminary study of the stability of the isolated soil polysaccharides to enzyme activity, by incubating the polysaccharide solutions with a soil suspension and with snail-crop-fluid preparations (Bernier, 1956).

The fact that neutral polysaccharides have not been isolated from the forest soils investigated might be of significance. It is not certain whether organisms producing dextrans and levans are able to maintain a high rate of metabolic activity under the acidic conditions often encountered in forest soils. But in any case, soil contains organisms capable of utilizing levans and dextrans. Thus certain moulds (e.g. *Aspergillus niger* and some strains of *Penicillium*), when grown in the presence of dextran, produce dextranase extracellularly (Hultin & Nordström, 1949; Jeanes, Wilham, Jones, Tsuchiya & Rist, 1953). In addition, *Azotobacter chroococcum* can produce an enzyme which brings about the hydrolysis of levan (Hestrin & Goldblum, 1953).

It appears, from the present findings, that the polysaccharides isolated from the upper mineral horizons beneath mor are smaller in both amount and size than the polysaccharides from mull. It may well be that the polysaccharides formed in mor layer reach the mineral-soil particles in a relatively degraded condition owing to the activity of micro-organisms during their downward passage, whereas the polysaccharides from mull would presumably be formed more or less *in situ*. It is noteworthy that polysaccharides isolated from a well-aggregated mull soil (no. 2) are much more active in aggregating soil particles of a non-aggregated soil than are the polysaccharides isolated from mor (Bernier, 1956).

Further study to elucidate the factors controlling the formation and destruction of the soil polysaccharides, which are presumably of microbial origin, should throw some light on the little-understood influence of these substances in forest-soil processes.

SUMMARY

1. Polysaccharide complexes have been extracted with $m/15$ phosphate buffer (pH 7.0) from a variety of forest soils associated with both mull and mor types of humus and were purified.

2. Attempts to fractionate the polysaccharide complexes by fractional precipitation with ethanol, paper electrophoresis and fractionation of the acetylated derivatives from chloroform solutions with light petroleum were not completely successful. However, the last two methods of fractionation indicated that at least one polysaccharide preparation might be a mixture of substances.

3. This was confirmed by free-boundary electrophoresis, which indicated that the material contains at least three components. Fractionation of this heterogeneous polysaccharide preparation was partially achieved by means of formation of a copper complex with Benedict's solution.

4. By viscosimetric measurements and application of Staudinger's equation the polysaccharides were found to have molecular weights varying from 10×10^3 (preparation from a soil associated with mor) to 124×10^3 (preparation from a mull soil).

5. Hydrolysis and paper chromatography revealed that the polysaccharides isolated from all the soils investigated were complex heteropolysaccharides. Except the high-molecular-weight preparation, from a mull soil, which did not contain any arabinose and the two preparations from soils associated with mor, which did not contain fucose, all polysaccharides contained the following sugar constituents: glucuronic acid, galactose, glucose, mannose, arabinose, xylose, fucose and rhamnose. There was, however, some variation in the proportion of the sugar constituents.

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ADDENDUM

Sedimentation in the Ultracentrifuge

By A. G. OGSTON

Department of Biochemistry, University of Oxford

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Sedimentation of the α -polysaccharide fraction from soil 2 (Bernier, 1958) was observed in a Svedberg oil-turbine ultracentrifuge by the optical system of Baldwin (1953); the sedimentation coefficients were calculated by the method of Cecil & Ogston (1948). All measurements were made at

900 rev./sec. and at 27–28°. The solutions were in buffer, 0.1M-sodium chloride, 0.1M-sodium acetate, 0.04M-acetic acid. The concentration of polysaccharide was measured refractometrically (Cecil & Ogston, 1951) assuming a specific refractive increment of 1.4×10^{-3} .

Fig. 1. Sedimentation diagrams of polysaccharide at 900 rev./sec. and 27–28°; for conditions, see text. *a*: 1.6 g./100 ml., 34 min. after full speed; *b*: 0.8 g./100 ml., 53 min. after full speed; *c*: 0.4 g./100 ml., 24 min. after full speed. Sedimentation occurs from right to left.

The material showed a single sedimentation boundary, whose breadth and asymmetry, particularly at lower concentrations where self-sharpening was less marked, showed that the material is considerably polydisperse. No attempt was made to measure the degree of polydispersity. The sedimentation coefficients quoted were derived from measurements on the maximum of the schlieren boundary curves, and are average values. Inspection of the diagrams shows that there may be a smaller, polydisperse component sedimenting faster than the main component (Fig. 1). Values for the sedimentation coefficients, corrected to water at 20°, are given in Table 1. Because of the breadth of the boundary, the values are not very precise, particularly at the lower concentrations; the plot of $1/S_{20,w}$ against concentration is shown in Fig. 2. The values obtained from this are: $S_{20,w}^0$ (zero concentration) = 2.92×10^{-13} ; $d(1/S)/dc = 0.06 \times 10^{13}$.

Combining these values with the intrinsic viscosity (4.16) (Bernier, 1958), the following values are obtained by the method of Ogston (1953): molecular weight $M = 130 \times 10^3$; hydro-

Table 1. Sedimentation coefficients of α -polysaccharide fraction

Concn. (g./100 ml.)	$10^{13} \times S_{20,w}$
1.6	2.28
0.8	2.46
0.4	2.86
0.2	2.76

Fig. 2. Plot of $1/S_{20,w}$ against concentration, for extrapolation to zero concentration. The scatter of experimental points is exaggerated by the wide scale of the ordinate.

dynamic specific volume $V' = 1.0$ ml./g.; axial ratio = 80.

These dimensions, which should be regarded as only approximate, indicate that the (average) particle approximates in configuration to an elongated, stiff and little-solvated rod. In this it differs strikingly from the dextrans (Ogston & Woods, 1953, 1954).

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