three solvents, but could not be removed entirely. It should be noted that, of the lignin fraction, approximately $^2/_3$ is dioxane-soluble, an extremely high figure, even for eugenol lignins as formed in tissue slices.

Isolation of Klason (72% sulfuric acid) lignin after final dioxane extraction yielded 1–3 mg./g. of paper of a dark brown material, insoluble in all solvents employed, and giving positive color tests for lignin. The characteristic reversible darkening of lignin-containing tissues in the acid was observed also in papers yielding positive color tests, but not in control samples.

In the extension of this study, methylcellulose was selected as the first of a large series of substances, principally polysaccharides, but also intended to include synthetic polymers, mucoproteins and fibers. It will be the aim of this series to relate efficacy in the formation of lignin and other aromatic polymers to composition, configuration and molecular size. Comparison of the data in Table I with those obtained with methylcellulose (Table II) shows that in proportion to the amount of cellulosic material present, methylcellulose provides a far more efficient framework for polymerization than does paper. It has not been deemed advisable to attempt theoretical interpretation of these differences in results at present, although the particular distinction between paper and methylcellulose may reside in the inaccessibility of all but the most superficial fibers in the filter paper mat. The failure thus far met in replacing celluloses with alumina, or silica gel, does indicate, however, that the phenomena associated with polymer formation involve a more or less specific association between the eugenol, enzyme and a macromolecule. In experiments with methylcellulose, the dioxane-soluble fraction of lignin approximated 40%, in agreement with findings for eugenol synthetic lignin formed in tissue slices or cell wall material. In order to compare previous and current work it may be noted that tissue slices and cell wall fragments gave, for rate of lignin deposition, 3.46 mg./100 mg. dry matter/hr. and 2.68 mg./100 mg./hr., respectively.

TABLE II

EFFECT OF METHYLCELLULOSE ON THE PEROXIDATION OF EUGENOL[a]

	Control		With methylcellulose	
Product soluble in	Wt. (mg.)	Lignin test	Wt. (mg.)	Lignin test[b]
Chloroform	88	−	79	−
Ethanol	0		6	±
Dioxane	0		3	+
Residual	0		5	+
Total	88		93	
Total as % initial eugenol supplied	53.6		56.7	

[a] Product deposited in solution (control) or together with 3.9 mg. of methylcellulose; initial eugenol 1.0 mmole, H_2O_2, 2.0 mmoles in 30 ml. reaction volume. [b] Color reactions employing both phloroglucinol–HCl and chlorine–sodium sulfite reagents. Color tests are definite (+), weak (±), negative (−).

On the whole, the behavior of model systems as described mimics remarkably the sequence of events which has been observed in organized tissues and cell wall preparations, suggesting that genuine advantage is to be had in using such models in the study of lignification and kindred processes.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Composition and Behavior of Soil Polysaccharides[1,2]

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A soil polysaccharide concentrate was isolated from a Brookston type agricultural soil and quantitatively analyzed. It contains D-galactose, D-glucose, D-mannose, L-arabinose, D-xylose, L-rhamnose, glucuronic acid and possibly ribose and glucosamine. Examination shows that it is likely a mixture of polysaccharides possibly derived from the cells of microörganisms. The material is found to have significant soil aggregation properties. The rate of decomposition of the polysaccharide in the soil is much slower than that of most plant polysaccharides and bacterial gums.

Polysaccharide concentrates from various British soils and a Trinidad soil have been examined chemically.[3] This Laboratory also has undertaken the examination of the soil polysaccharides. Since polysaccharides from an American soil have not been subject to close scrutiny, Brookston soil of a typical Midwest agricultural region was selected for examination. Polysaccharide material can be isolated from this soil in approximately equivalent yield by extraction with either water or 2% sodium hydroxide solution. Quantitative analyses for anhydrosugar units in the separated polysaccharide fraction are shown in Table I. By actual isolation in the form of crystalline derivatives the sugars are found to be D-galactose, D-glucose, D-mannose, L-arabinose, D-xylose and L-rhamnose. The uronic acid is undoubtedly glucuronic acid since it gives the typical color reaction[4] with thioglycolic acid, its barium salt possesses the expected optical rotation and its lactone has the expected chromatographic flow rate.

Ribose and glucosamine are present in such small

(1) Journal Paper No. 910 of the Purdue Agricultural Experiment Station, Lafayette, Indiana.

(2) Paper presented before the Division of Agricultural and Food Chemistry at the 128th Meeting of the American Chemical Society in Minneapolis, Minnesota, September, 1955.

(3) (a) W. G. C. Forsyth, *Biochem. J.*, **46**, 141 (1950); (b) R. B. Duff, *J. Sci. Food Agr.*, **3**, 140 (1952); (c) R. B. Duff, *Chemistry and Industry*, 1104 (1952); (d) 1513 (1954).

(4) Z. Dische, *J. Biol. Chem.*, **171**, 725 (1947).

amounts as to be indicated by chromatographic values only. The equivalent weight by titration corresponds with the value expected from the amount of glucuronic acid present. From an examination of the analytical data in Table I it is evident that approximately half of the polysaccharide concentrate is not carbohydrate. This unknown organic contaminant is not removed by ordinary purification procedures. A similar observation is made by Duff[3b] and the data of Forsyth[3a] suggest the presence of non-carbohydrate substances in his soil polysaccharide material. It is interesting, furthermore, to note that the composition of the polysaccharide material from Brookston soil is similar to the compositions of polysaccharide materials isolated from quite different soils (Table I).

TABLE I

COMPARATIVE COMPOSITION AND PROPERTIES OF SOIL POLYSACCHARIDE CONCENTRATES

Androsugar units, etc.	Brookston soil, %	Craigie-buckler[3b] soil, %	Gardeners[3a] turfy loam, %
Galactose	6.2	10.5	13.3
Glucose	7.9		13.8
Mannose	6.9	8.7	14.5
Arabinose	3.9		7.8
Xylose	4.7	3.0	15.7
Rhamnose	5.3	3.2	....
Ribose	Trace	1.3	1.2
Glucosamine	Trace	....	....
Unknown sugars	2.4[a]	2.0	....
Uronic acid	9.1	20.1	15.8
Nitrogen	0.34	1.6	0.34
Methoxyl	2.4	2.0	Nil
Equiv. wt.[b]	1945	1000	1185
$[\alpha]_D$	+36.8°(25°)	....	−5°(20°, as sodium salt)

[a] Combination of three unknowns; unknown 1 estimated as arabinose and unknowns 2 and 3 estimated as an average of all hexose curves. [b] Neutralization equivalent.

The variety of simple sugars obtained on hydrolysis of the polysaccharide portion strongly suggests the presence of a polysaccharide mixture. So far no polysaccharide is known[5] which contains more than 5 or 6 types of monosaccharide units in its structure and it is doubtful that polysaccharides of more complex composition occur in nature. Evidence of the heterogeneous nature of the polysaccharide material is also indicated by the broad band obtained upon electrophoresis in borate buffer and by the shape of the precipitation curve obtained upon gradient addition of ethanol to a 1% solution of the polysaccharide material in water (Fig. 1).

The low proportion of D-xylose and D-glucose units in the polysaccharide concentrate may be taken as evidence that the soil polysaccharides do not directly originate from higher plants. Instead it is probable that the polysaccharides are derived from microörganisms. In part, the polysaccharides may be products of microbial metabolism but it seems probable that for the most part they are components of microbial tissue derived from the recently dead cells of soil microörganisms. It has been stated that the microbiological inhabitants of soils are rather uniformly distributed throughout the world.[6] If this statement were accepted it would lend support to the apparent uniformity in composition of polysaccharide material from soils of different parts of the world.

The polysaccharide concentrate exhibited considerable soil aggregate stabilizing power when tested with a kaolin–sand mixture by conventional methods.[7] If the non-carbohydrate components of the mixture have little effect on aggregation, the polysaccharide effect becomes even more significant. Inference that the non-carbohydrate components have little aggregation effect is obtained from the observation that the related humic material isolated from the crude extract has very little aggregate stabilizing action as shown in Table II.

TABLE II

AGGREGATION OF KAOLIN–SAND MIXTURE (90:10) WITH SOIL POLYSACCHARIDE CONCENTRATE AND HUMIC MATERIAL

	Humic material		Soil polysaccharide concentrate	
Screen opening, mm.	0.1% concn.	1.0% concn.	0.1% concn.	1.0% concn.
	——Aggregates retained on screen, %——			
2.000	0.0	0.0	0.0	2.4
1.000	.0	.0	.0	5.8
0.500	.0	1.0	1.7	27.3
0.250	1.6	1.0	4.0	16.9

A polysaccharide material has recently been extracted from a Wisconsin Spencer silt loam[8] and was found to have aggregate stabilizing action when sprayed on soil. Since the polysaccharide material was extracted by methods similar to those used here it may be assumed to have contained about 50% non-carbohydrate substance and hence the polysaccharide portion very likely had much greater aggregate stabilizing action than the tests demonstrated.

Persistence in the soil of an aggregate stabilizing agent is, of course, of importance if the agent is to exhibit lasting effect. When the soil polysaccharide concentrate is tested for decomposition rate as measured by carbon dioxide evolution, it is observed to decompose at a rather steady rate, but much slower than many plant polysaccharides tested.[9] Comparison of the rate of decomposition of soil polysaccharide concentrate with that of pectin is shown in Table III. Levans, bacterial gums and other polysaccharide by-products of microorganisms seem to have such short persistence in the soil as to suggest that they have only a limited and inconsequential aggregate stabilizing action. Since it is evident that soil polysaccharide material is slowly but continually decomposing in the soil, the aggregate stabilizing action of any particular material must be regarded as transient. Consequently, soils with good natural aggregation must have the polysaccharides which contribute to aggregation continually renewed. Such renewal from

(5) R. L. Whistler and C. L. Smart, "Polysaccharide Chemistry," Academic Press, Inc., New York, N. Y., 1953, p. 18.

(6) (a) H. Jenny, "Factors of Soil Formation," McGraw-Hill Book Co., New York, N. Y., 1941, p. 200; (b) Jack Major, *Ecology*, **32**, 397 (1951); (c) D. Feher and B. Frank, *Erdeszeti Kiserletek*, **46**, 299 (1947).

(7) R. E. Yoder, *J. Am. Soc. Agron.*, **28**, 337 (1936).

(8) D. A. Rennie, Emil Truog and O. N. Allen, *Soil Sci. Soc. Amer. Proc.*, **18**, 399 (1954).

(9) L. R. Frederick, H. E. Parker and R. L. Whistler, unpublished results.

TABLE III

DECOMPOSITION RATE OF SOIL POLYSACCHARIDE CONCENTRATE AND PECTIN IN SOIL; % CARBON EVOLVED AS CARBON DIOXIDE

Days	Soil polysaccharide Concentrate C, %	Pectin C, %
1	0	8
2	2	15
3	4	20
5	4	24
6	4	25
8	5	30

disintegrating microbial tissue is a reasonable expectation. These considerations apply only to molecular or nearly molecular material and not to more or less intact parts of microörganisms such as mycelial filaments which mechanically bind soil particles together while alive, though perhaps again in a different way when dead and disintegrated to molecular levels. As molecular disintegration proceeds, the aggregate stabilizing action of a particular molecule probably diminishes until it is lost by molecules becoming too short for effective action.

While much aggregate stabilizing action has often been attributed to plant material plowed into or mixed with soil, it is difficult to visualize that extensive stabilizing action is directly obtained. Large pieces of plant tissue can have little or no effect on soil aggregation. To produce an important effect large molecules would need to become loosened and transported or disseminated in the soil. However, it is unlikely that more or less intact plant molecules such as cellulose and hemicelluloses can be removed from dense plant tissue without simultaneous degradation to small ineffectual fragments. It is much more satisfying to assume that higher plant material when mixed with soil serves primarily to feed the soil microörganisms and it is tissue from migrating organisms which produces significant aggregate stabilizing effects. It has already been demonstrated that the addition of sugar to the soil increases markedly the soil aggregation.[10]

Experimental

Preparation of Soil Sample.—A Brookston silty clay loam was sampled from the top six inches, air-dried, sieved and ground to pass a 2 mm. opening. The soil had been in sod for three years and a crop of sweetclover was harvested prior to sampling in December. Analysis showed *p*H, 6.6; organic matter, 3.38% (modified Walkley–Black[11]); nitrogen, 0.15%.

Extraction Methods.—To compare extraction methods, similar 3-kg. portions of soil were extracted by four different procedures. In the first, the soil was autoclaved at 15 p.s.i. with 4500 ml. of water for 30 minutes. In the second, soil was extracted with 4500 ml. of water by mixing in a revolving bottle on a roller in a nitrogen atmosphere for 72 hr. In the third, the soil was stirred in 4500 ml. of water at 85° in an atmosphere of nitrogen for 4 hr. In the fourth, soil was stirred in 10 l. of 0.5 *N* sodium hydroxide for 48 hr., the settled soil removed and re-extracted for 48 hr. with another 10-l. portion of 0.5 *N* sodium hydroxide and finally with 10 l. of water.

Extracts from the first three procedures were centrifuged and filtered through a medium porosity sintered glass funnel. The extracts were concentrated to 400 ml. and the polysaccharide precipitated by addition to 3 volumes of acetone.

The combined extracts from the fourth procedure were centrifuged, filtered through a sintered glass funnel and the humic material precipitated by adjusting to *p*H 3 with hydrochloric acid. The supernatant liquid was passed through a charcoal pad on a büchner funnel and the pad was eluted with 3 l. of 10% water in acetone. The polysaccharide was extracted with about one l. of distilled water and precipitated by addition to 3 volumes of acetone.

Each of the above precipitates was further purified by dissolution in approximately 60 ml. of water and pouring into 3 volumes of acetone. After three such reprecipitations the precipitates were washed with dry acetone, followed by ether and dried in a vacuum desiccator over calcium chloride. The precipitate from the fourth procedure was white; all the others were somewhat brown. Yields from the various procedures were: first, <5 mg.; second, 19 mg.; third, 202 mg.; fourth, 209 mg.

Qualitative Paper Chromatography.—One hundred-mg. portions of the precipitates from procedures three and four were each hydrolyzed in separate 3-ml. portions of *N* sulfuric acid by heating on a steam-bath. Periodically samples were withdrawn and chromatographed on Whatman No. 1 paper using ethyl acetate, pyridine and water (8:2:1 v./v.). The papers were irrigated in descending chromatographic equipment, removed, dried and sprayed with *p*-anisidine hydrochloride to locate the sugars. Both precipitates showed identical components. After hydrolysis for 1.5 hr., three unidentified spots could be observed on the chromatograms. Use of the reagents of Elson and Morgan[12] indicated that a trace of glucosamine was present in the hydrolyzate. Irrigation of paper chromatograms in butanol-1, ethyl acetate and water (4:1:5 v./v.) followed by drying and spraying with ninhydrin showed that amino acid residues were also present. A typical chromatogram obtained after 8 hr. hydrolysis gave indication of the substances shown in Table IV. R_{xylose} is the distance traveled for a sugar compared to the distance traveled by D_{xylose}. Unknown 1 colored pink when sprayed with *p*-anisidine hydrochloride indicating a pentose whereas unknowns 2 and 3 colored yellow indicating hexoses or 6-deoxyhexoses. These data indicate that in addition to the unknown substances there are seven monosaccharides, glucosamine and a uronic acid present. Since water seems to extract the same amount and type of soil polysaccharide as alkali and is undoubtedly a much milder solvent, additional amounts of soil were extracted by a slight modification of procedure three.

TABLE IV

SUBSTANCES INDICATED IN THE HYDROLYZATE FROM SOIL POLYSACCHARIDE CONCENTRATE

	R_{xylose} values Known	From procedure 3	From procedure 4
Glucuronic acid	0.15	0.15	0.15
Galactose	.63	.66	.63
Glucose	.70	.74	.70
Mannose	.80	.81	.81
Arabinose	.91	.96	.93
Xylose	1.00	1.04	1.02
Ribose	1.13	1.12	1.12
Rhamnose	1.20	1.22	1.20
Glucosamine	..	Trace	Trace
Unknown 1	..	1.30	1.35
Unknown 2	..	1.41	1.44
Unknown 3	..	1.52	1.55
Amino acid	..	Trace	Trace

Preparation of Polysaccharide Material.—Thirty-two kg. of soil was extracted in 4-kg. portions. Each portion was stirred in 6000 ml. of oxygen-free water under an atmosphere of nitrogen at 85° for 6 hr. At the end of this period the mixture was cooled to room temperature and the supernatant liquid clarified by centrifugation and filtration through

(10) (a) M. J. Geoghegan and R. C. Brian, *Nature*, **158**, 837 (1946); (b) W. N. Haworth, F. W. Pinkard and M. Stacey, *ibid.*, **158**, 836 (1946); (c) T. C. Peele, *J. Am. Soc. Agr.*, **32**, 204 (1940); (d) J. P. Martin, *Soil Sci.*, **59**, 163 (1945).

(11) (a) A. Walkley and I. A. Black, *Soil Sci.*, **37**, 2938 (1934); (b) A. Walkley, *J. Agr. Sci.*, **25**, 598 (1935).

(12) (a) L. A. Elson and W. T. J. Morgan, *Biochem. J.*, **27**, 1824 (1933); (b) S. M. Partridge, *ibid.*, **42**, 238 (1948).

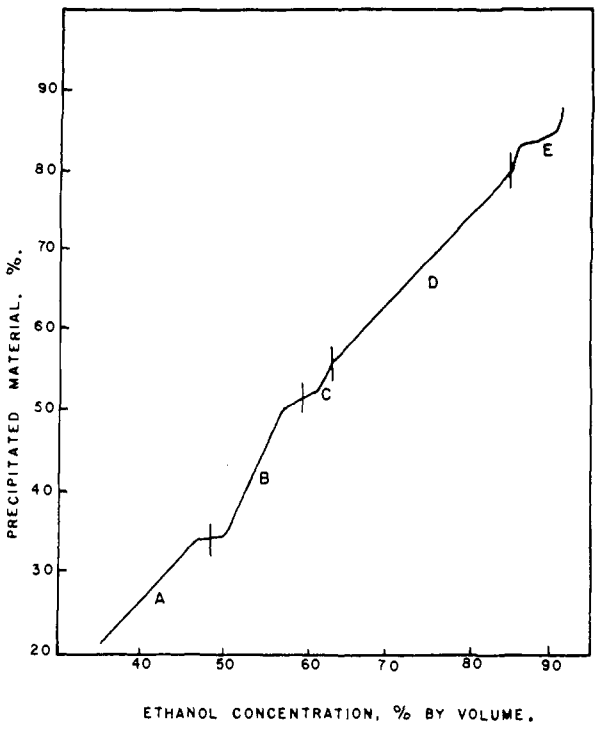

Fig. 1.

glass wool and a medium porosity sintered glass funnel. The straw colored filtrate of each batch was concentrated under vacuum to 120 ml. and poured into 3 volumes of acetone. The filtered precipitate was redissolved in approximately 500 ml. of distilled water and reprecipitated by pouring into 1500 ml. of ethanol–glacial acetic acid (3:1). The dissolution in water and precipitation in ethanol–glacial acetic acid was repeated four successive times. The final precipitate, dissolved in 500 ml. of water, was dialyzed in two successive 48-hr. periods, first against tap water and then against distilled water. The final solution on freeze-drying produced a light tan colored product. The combined yields totaled 15.7 g. equivalent to 1.45% of the soil organic matter or 0.05% of the air dried soil; $[\alpha]^{26}D$ +36.8° (*c* 0.1, in water); ash, 8.32%; nitrogen (micro-Kjeldahl), 0.34%; carbon, 39.39%; hydrogen, 5.53%; uronic anhydride,[13] 9.14%; methoxyl,[14] 2.42%; equivalent weight, 1945; intrinsic viscosity, 0.72; material becoming insoluble in hot *N* sulfuric acid, 21%. Qualitative tests showed phosphate absent and a slight sulfate test in the ash.

Quantitative Estimation of Monosaccharides.—One-half g. of the polysaccharide material was hydrolyzed in 25 ml. of *N* sulfuric acid for 24 hr. on a steam-bath after which it was adjusted to *p*H 6 and filtered through Whatman No. 42 paper. A black insoluble residue appeared during hydrolysis which was removed by filtration (0.104 g. equivalent to 21%). The material was insoluble in 42% hydrochloric acid but was slowly soluble in 2% sodium hydroxide. The material may thus be humic substances. Fifty-microliter portions of hydrolyzate were pipetted in a narrow line near the top of a chromatogram of Whatman No. 1 paper. Locator spots were placed near the edge of each paper.[15] Irrigation in ethyl acetate, pyridine and water (8:2:1 v./v.) separated the slower moving components in 40 hr. while 10 to 16 hr. was sufficient to separate arabinose, xylose, rhamnose and the unidentified sugars. After drying, the locator strips were cut away, sprayed with *p*-anisidine hydrochloride and sections cut from the center section in position relative to the known spots on the locator strip. Each unknown area was cut into small pieces, eluted with 5 ml. of distilled water in a small beaker and transferred to a 25 × 200 mm. test-tube. The elution with water was repeated and the volume made to 12 ml. Sugars were determined using the method of Hagedorn and Jensen.[16] Each sugar was compared to a standard curve established for it. Recovery experiments eluting known amounts from paper showed D-galactose, 96.1% and D-glucuronic acid, 90%. Results of analysis are shown in Table I. The molar ratios of the individual components are D-galactose, 1.26; D-glucose, 1.60; D-mannose, 1.41; L-arabinose, 1.00; D-xylose, 1.18; L-rhamnose, 1.18; uronic acid, 1.70.

Fractionation of the Polysaccharide Material.—A 0.5% solution of polysaccharide material was not precipitated by the addition of potassium chloride to 0.8 molar.

Gradient addition of ethanol in 3-ml. increments to 20 ml. of a 1% solution of polysaccharide material at *p*H 1.85 brought about separation of insoluble material as indicated in Fig. 1. This precipitation curve, typical of a heterogeneous polysaccharide, is obtained only at low *p*H's where ionization of the carboxyl group is suppressed. At higher *p*H's there is obtained only a smooth curve. Quantitative analysis of the five fractions by the above method corresponding to the lettered portions of the curve in Fig. 1 are shown in Table V.

TABLE V

COMPOSITION OF ETHANOL PRECIPITATED FRACTIONS, %

	A	B	C	D	E
Galactose	1.52	2.22	0.23	1.65	0.80
Glucose	0.80	1.62	1.46	1.06	3.45
Mannose plus arabinose	1.06	1.82	2.65	1.75	2.82
Xylose	0.93	0.33	0.66	0.66	2.42
Rhamnose	0.73	0.46	0.53	1.72	0.90
Uronic acid	2.29	0.36	1.59	2.22	2.35
Precipitate during hydrolysis	20.3	6.80	0.20	1.00	0.10

After centrifuging a 1% solution 10 minutes at 30,000 r.p.m., the mobility as observed in a Tiselius electrophoresis apparatus showed a single low peak indicating an extremely heterogeneous mixture. The test was made in *p*H 9.9 borate buffer of 215 ohms resistance at 8 ma. for 4300 seconds.

Analysis of Humic Material.—Humic material was that precipitated as in procedure four by adjusting the sodium hydroxide soluble extract of soil to *p*H 3 with hydrochloric acid. The precipitate was removed by filtration and dissolved in 1 l. of 2% sodium hydroxide solution and precipitated by addition of hydrochloric acid to *p*H 3. After two further dissolutions and reprecipitations the material was dried in vacuum over calcium chloride. A yield from 4000 g. of air-dried soil was 115 g. equivalent to 2.9%. Ten g. of humic material (ash 67.2%) was hydrolyzed with constant stirring in 100 ml. of *N* sulfuric acid for 24 hr. on a steam-bath. After filtration the residue was 5.855 g. The hydrolyzate was neutralized with barium carbonate and chromatograms prepared for quantitative analysis in the manner described above. It contained galactose, 0.06%; glucose, 0.09%; mannose, 0.07%; arabinose, 0.02%; xylose, 0.11% and rhamnose, 0.06%.

Aggregation Properties.—Sand passing a 100 mesh screen was mixed with kaolin (10% by weight). Humic material prepared as above was added to the kaolin-sand mixture in 0.1 and 1.0% concentrations. Soil polysaccharide from procedure three was added to separate kaolin-sand mixtures in 0.1 and 1.0% concentrations. To each of the four preparations distilled water was added slowly with continuous mixing to the sticky point. Each sample was pressed through a number 4 mesh screen into a petri dish, covered and allowed to age at room temperature for 24 hr. The artificial aggregates were then dried at 50° for 24 hr. Wet sieving using a modification of the method of Yoder[7] followed. Twenty-five-g. portions of each preparation were placed on the top sieve of a series of four sieves with openings 2.000, 1.000, 0.500 and 0.250 mm. stacked in order of decreasing size from the top. The samples and sieves were placed in water for 30 minutes after which the mechanical up and down motion (37 cycles per minute; 3 cm. stroke) was started and

(13) R. L. Whistler, A. R. Martin and M. Harris, *J. Research Natl. Bur. Standards*, **24**, 13 (1940) RP1268.

(14) J. Niederl and V. Niederl, "Organic Quantitative Micro-Analysis," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 187.

(15) R. L. Whistler and J. L. Hickson, *Anal. Chem.*, **27**, 1514 (1955).

(16) H. C. Hagedorn and B. N. Jensen, *Biochem. Z.*, **135**, 46 (1923), as described in F. J. Bates, "Polarimetry, Saccharimetry and the Sugars," Natl. Bur. Standards Circ. c440, 1942, p. 198.

continued for 30 minutes. The screens were removed, dried and the amount of aggregates remaining on each screen determined. Results of analysis are shown in Table II.

Decomposition Rate in Soil.—To 25 g. of fresh Brookston soil (approximately 60% water saturated) in a 125-ml. erlenmeyer flask was added 0.25 g. of the soil polysaccharide concentrate and the mixture stirred with a rod for 10 minutes. Carbon dioxide evolved was measured according to a slight modification in the procedure of Fred and Waksman.[17] The gases evolved in the flask were conducted below the surface of 25 ml. of 0.167 *N* sodium hydroxide solution. Daily the sodium hydroxide solution was replaced by a fresh sample and the removed portion titrated with standard hydrochloric acid. A control flask containing only 25 g. of soil was assembled in the same manner. The results in Table III are compared to the carbon dioxide evolved from 0.25 g. of pectin mixed into 25 g. of soil; the values represented are corrected for controls.

Separation of Monosaccharides.—Three grams of the freeze-dried polysaccharide from procedure three was hydrolyzed in 150 ml. of *N* sulfuric acid with constant stirring for 24 hr. on a steam-bath. The hydrolyzate was neutralized to *p*H 6 with barium carbonate and the insoluble material was centrifuged and re-extracted twice with water. The combined extracts were concentrated *in vacuo* to a sirup which was soaked up in powdered cellulose and placed on a cellulose column 40 × 600 mm. A mixture of butanol-1, ethanol, water and ammonium hydroxide (40:10:49:1 v./v.) was used to develop the column. Fractions from the column were subjected to further chromatographic separation on sheets of Whatman No. 1 paper with ethyl acetate, pyridine and water (8:2:1 v./v.) as an eluent. Guide strips at the two edges and the center were cut out and sprayed with *p*-anisidine hydrochloride to locate the individual sugar bands. Paper strips containing individual monosaccharides were cut into pieces 7 × 7 cm. and each extracted with 200 ml. of distilled water. The extracts were concentrated *in vacuo*, then evaporated to dryness in a vacuum desiccator over calcium chloride.

After removal of the monosaccharides from the cellulose column the barium uronate was removed by a water wash and concentrated to a sirup. Paper chromatography using ethyl acetate, acetic acid, formic acid and water (18:3:1:4 v./v.) as an irrigant and *p*-anisidine hydrochloride as a spray reagent suggested the presence of glucuronic acid only.

D-Galactose *p*-Nitrophenylhydrazone.[18]—Reaction of 78 mg. of galactose from the chromatographed hydrolyzate with 78 mg. of *p*-nitrophenylhydrazine produced crystals with $[\alpha]^{25}D$ +70° (*c* 0.25, in ethanol–pyridine); m.p. 192°. These are correlative with literature values.[19]

D-Glucose *p*-Nitrophenylhydrazone.[18]—Reaction of 84 mg. of glucose from the chromatographed hydrolyzate with 84 mg. of *p*-nitrophenylhydrazine produced crystals with $[\alpha]^{25}D$ −88° (*c* 0.5, in ethanol–pyridine); m.p. 188°.

D-Mannose Phenylhydrazone.[20]—Reaction of 61 mg. of mannose from the chromatographed hydrolyzate in 1.5 ml. of water with 0.5 ml. of phenylhydrazine reagent produced crystals with $[\alpha]^{25}D$ +26.6 (*c* 0.5, in pyridine); m.p. 198°.

L-Arabinose *p*-Nitrophenylhydrazone.[18]—Reaction of 41 mg. of arabinose from the chromatographed hydrolyzate with 41 mg. of *p*-nitrophenylhydrazine produced crystals with $[\alpha]^{25}D$ +22.6 (*c* 1.0, in ethanol–pyridine); m.p. 181°.

D-Xylose Dimethyl Acetal Dibenzylidene.[21]—The reagent was prepared by adding 8 ml. of redistilled benzaldehyde to a mixture of 6 ml. of 1.6 *N* methanolic hydrogen chloride and 22 ml. of dry methanol. Reaction of 35 mg. of xylose from the chromatographed hydrolyzate with 1 ml. of reagent produced crystals with $[\alpha]^{25}D$ −7° (*c* 1.0, in chloroform); m.p. 211°.

L-Rhamnose *p*-Nitrophenylhydrazone.[18]—To 35 mg. of rhamnose obtained from the cellulose column was added 35 mg. of *p*-nitrophenylhydrazine. The crystals produced had $[\alpha]^{25}D$ −43° (*c* 0.1, in ethanol–pyridine); m.p. 188°.

Glucuronolactone.—The barium glucuronate from the cellulose column was dissolved in 5 ml. of water and ethanol was added to 90% concentration to obtain a pure product. The white precipitate was removed by filtration and washed successively with absolute ethanol and ether before it was dried in a vacuum desiccator over calcium chloride; $[\alpha]^{25}D$ +20° (*c* 0.5, in water). A water solution was mixed at 80° with IR120 H resin in a beaker to remove the barium. The effluent was concentrated *in vacuo*, the glucuronic acid lactone dissolved in 5 drops of water and a portion placed on a paper chromatogram which was irrigated in ethyl acetate, pyridine and water (8:2:1 v./v.) for 12 hr. A spot corresponding to a known sample of glucuronolactone appeared after spraying the paper with *p*-anisidine hydrochloride and heating for 1 minute at 130°. A qualitative test using the Dische reagent[22] was positive for glucuronic acid. A further colorimetric test[4] using thioglycolic acid gave a specific red color indicating glucuronic acid.

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