for $C_{28}H_{42}O_5$: C, 73.32; H, 9.23. Found: C, 73.04; H, 9.15.

Formaldehyde Test.—Prior to adding water to the periodate reaction mixture, a 2-ml. aliquot was removed and added to a solution containing 0.06 ml. of 2 *N* sulfuric acid in 8 ml. of water. The solution was distilled slowly until 3 ml. of distillate were collected. The distillate was mixed with 5 ml. of chromotropic acid reagent and heated on a steam-bath. A purple color developed indicating formaldehyde was present. A similar test run on a reagent blank treated identically to the periodate oxidation of the steroid gave a negative test.

(b) A solution of 0.5 g. of II in 30 ml. of ethyl acetate was cooled to −60° in a Dry Ice–acetone-bath. Ozone was passed through the solution until a persistent blue color was noted. The solvent was then removed *in vacuo*. The residue was taken up in benzene and chromatographed on Florisil. The benzene eluates contained 0.08 g. of IV, identical to the product obtained by periodate oxidation of III.

Conversion of III to I.—A suspension of 0.15 g. of III in 0.3 ml. of pyridine was warmed. To this was added 0.15 g. of *p*-toluenesulfonyl chloride and the mixture heated on a steam-bath until all the solids dissolved. The mixture was allowed to stand overnight at room temperature, and decomposed by addition of four drops of water followed by heating on the steam-bath. The mixture was taken up in ether, washed successively with dilute hydrochloric acid, sodium carbonate, and water and then dried over anhydrous sodium sulfate. A clear, white oil was obtained which was characterized by infrared spectrum as being the crude 21-tosylate of compound III. This oil, designated as VI, was dissolved in 25 ml. of dry ether and added dropwise to a refluxing suspension of 0.4 g. of lithium aluminum hydride in 25 ml. of ether. Refluxing was continued for four hours and the lithium aluminum hydride decomposed by addition of water followed by 10% sodium hydroxide solution. The aqueous suspension was extracted with ether in the usual manner. The product was acetylated with pyridine–acetic anhydride at room temperature. Following the usual work-up, methanol crystallization gave 0.07 g. of product, m.p. 234–235°, identical to I.

PHILADELPHIA 18, PENNA.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Hydrolysis Products from Methylated Arabinoxyloglycan and Arabinogalacto-mono-*O*-methylglucuronoxyloglycan of Corn Cobs[1]

BY ROY L. WHISTLER AND G. E. LAUTERBACH

RECEIVED NOVEMBER 14, 1957

Hydrolysis of methylated arabinoxyloglycan from corn cobs yields 2,3,5-tri-*O*-methyl-L-arabinose, 3,5-di-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose and 2-*O*-methyl-D-xylose in the molar ratios 2:3:2:26:4 which with other properties suggests that the polymer is a xylan chain with L-arabinose units attached in short linear side chains. Hydrolysis of methylated arabinogalacto-mono-*O*-methylglucuronoxyloglycan from corn cobs yields 2,3,5-tri-*O*-methyl-L-arabinose, 3,5-di-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose, D-xylose, 2,3,4,6-tetra-*O*-methyl-D-galactose and methylated aldobiouronic acid in a ratio of 2:4:2:16:8:1:1:5, which with other characteristics suggests that the molecule is extensively branched.

Availability of corn cobs in mammouth quantities assures their eventual industrial use as a polysaccharide source. While much work has been done to characterize oligosaccharides[2] obtained from cob polysaccharides by partial hydrolysis, little examination has been directed toward the intact polysaccharides. Cellulose which constitutes approximately 40% of cobs is similar to wood cellulose while the xylan which constitutes approximately 30% of cobs seems to be a more or less linear molecule with perhaps a D-glucuronic acid unit at one end. Approximately 10% of corn cobs are additional cell wall polysaccharides which are extractable by alkaline solutions and which remain soluble when the extract is neutralized. These polysaccharides seem to be branched heteroglycans. Two have been isolated in pure form[2] by alkaline extraction of cob holocellulose and subsequent fractional precipitation from several systems. One polymer is a neutral diheteroglycan containing approximately 100 sugar units of which 90% are xylose and 10% are arabinose units. The second is a tetraheteroglycan containing approximately 150 sugar units of which 60% are xylose, 22% are arabinose, 11% are monomethylglucuronic acid and 8% are galactose units.

Information as to the possible arrangement of sugar units in these two polysaccharides is now obtained by hydrolysis of the fully methylated polysaccharides. The molar ratios of the various hydrolytic products are shown in Table I. Although the 3,5-di-*O*-methyl-L-arabinose has not been confirmed by isolation of a crystalline derivative its identity seems certain on the basis of its formation of a borate complex, of expected electrophoretic movement, its susceptibility to alkaline degradation and its optical rotation.

From what is now known of the arabinoxyloglycan it might be looked upon as a linear 1 → 4 linked xylose chain with 4 side chains totaling 7 units for each 30 units of the xylose chain. An extended, somewhat linear structure is suggested by its film-forming properties. L-Arabinofuranose units probably occur in the side chains since L-arabinose is the first sugar obtained on hydrolysis. Some of the L-arabinose side chains may be terminated with D-xylose units since on partial hydrolysis of cob polysaccharides there is isolated the disaccharide 2-*O*-α-D-xylopyranosyl-L-arabinose.[3] None of the chain units bears more than a single branch since no non-methylated sugars were obtained on hydrolysis.

The tetraheteroglycan must be highly branched, since the polymer, though it has a degree of polymerization of 150, forms only a brittle film and since hydrolysis of the methylated polymer yields considerable monomethyl-D-xylose, presumably

(1) Journal Paper No. 1194 of the Purdue Agricultural Experiment Station.

(2) For a list of earlier publications from this Laboratory see: R. L. Whistler and G. E. Lauterbach, *Arch. Biochem. Biophys.*, in press (1958). See also I. Ehrenthal, R. Montgomery and F. Smith, THIS JOURNAL, **76**, 5509 (1954).

(3) R. L. Whistler and D. I. McGilvray, *ibid.*, **77**, 1884 (1955).

TABLE I

Sugar	Diheteroglycan, molar ratio	Tetraheteroglycan, molar ratio
2,3,5-Tri-*O*-methyl-L-arabinose	2	2
2,3,4-Tri-*O*-methyl-D-xylose	2	2
2,3,4,6-Tetra-*O*-methyl-D-galactose		1
3,5-Di-*O*-methyl-L-arabinose	3	4
2,3-Di-*O*-methyl-D-xylose	26	16
2-*O*-Methyl-D-xylose	4	8
D-Xylose		1
Methylated aldobiouronic acid[a]		5

[a] Calculated from amount isolated from the column.

arising from the sites of a single branch, and D-xylose supposedly arising from the sites of a double branch. All of the D-galactose and possibly all the mono-*O*-methyl-D-glucuronic acid occur as end units. Likewise some D-xylose and L-arabinose units serve as non-reducing end units. As expected the number of end units equals the number of branch points. There seem to be 10 side chains for every 44 sugar units and one mono-*O*-methyl-D-glucuronic unit[2] for each 9–11 sugar units. No direct information is available to prove whether the polymer is a chain structure with straight side chains or whether it is a branch-on-branch structure. However, one might tentatively infer a branch-on-branch structure from comparison with the somewhat closely related plant gums.

Experimental

Polysaccharides.—Isolation and purification of the polysaccharides used in this investigation have been previously described.[2] The first of these (component I) was a diheteroglycan of molecular weight 13,700, $[\alpha]^{25}D$ −87.0°, with 10.6% arabinose and 89.4% xylose. The second (component III) was a branched tetraheteroglycan of molecular weight 21,900, $[\alpha]^{25}D$ −77.0°, equivalent weight 1560 and with 59.1% xylose, 21.9% arabinose, 11.3% monomethylhexuronic acid and 7.6% galactose.

Methylation.—Both polysaccharides were subjected to four successive methylations with dimethyl sulfate[4] and potassium hydroxide at room temperature and were twice methylated in N,N-dimethylformamide with methyl iodide and silver oxide at 25° for 16 hr.[5]

Separation of Sugar Units in Methylarabinoxyloglycan.—Methylated arabinoxyloglycan at 12% concentration in chloroform was fractionated by stepwise addition of *n*-heptane. Although fractions 2 through 6 were of similar methoxyl content and specific optical rotation (Table II) only

TABLE II

FRACTIONATION OF METHYLATED ARABINOXYLOGLYCAN

Heptane, %	Weight, g.	Methoxyl, %	$[\alpha]^{25}D$
54.5	0.03	26.7	.....
73.6	0.47	35.6	−65.4°
76.2	3.46	36.1	−71.5
78.3	0.76	36.4	−70.9
80.8	.93	36.0	−72.5
83.9	.42	36.6	−69.7
Supernatant	.77	33.1	−49.8

fraction 3 was methanolized at 4% concentration in methanol containing 5% hydrogen chloride for 6 hr. to a constant specific rotation of +92°. The silver carbonate neutralized solution was concentrated to a sirup to remove methanol and was taken up in 80 ml. of half-saturated barium hydroxide solution. After standing 16 hr. at 30° carbon dioxide was bubbled through the solution and the barium carbonate removed by filtration. The filtrate was deionized by ion exchange resins[6] IR-120 (H) and IR-4B (OH). This solution was concentrated, redissolved in 1 *N* sulfuric acid and hydrolyzed 4.5 hr. at 98° to constant rotation. The hydrolyzate was neutralized with barium carbonate and barium ions removed with IR-120 (H) resin. The hydrolyzate was then evaporated to a sirup *in vacuo* and dried by azeotropic distillation with benzene:ethanol (2:1). On dissolution in butanone–water azeotrope (irrigant A), the mixture was placed on a cellulose column (43 × 485 mm.), irrigated with the same solvent and the effluent collected in 15-ml. aliquots. Paper chromatograms with the same irrigant permitted the grouping together of aliquots with the same compositions. In such manner 4 fractions were obtained. The first group (202 mg.) appeared to be a mixture of trimethylpentoses, while the second group contained some of group I as well as two other zones. Group III (951 mg.) seemed to be dimethylxylose and group IV (215 mg.) appeared to be monomethylxylose.

Group I was chromatographed on sheets of Whatman No. 1 filter paper with irrigant A. Appropriate zones indicated by marker strips at the side of the paper were cut out and the components extracted with methanol. The most rapid component Ia (R_g 1.01)[7] and the slower-moving component Ib (R_g 0.98) were evaporated to sirups and identified as described later.

Group II was reseparated on a smaller cellulose column (25 × 430 mm.) with irrigant A. The first fractions were chromatographically identical to group I. The second set of fractions contained dimethylpentoses. The third set of fractions were chromatographically identical to group III with which they were combined. The second set of fractions, above, were rechromatographed on the cellulose column with the irrigant A to obtain the major component IIa which gave a single spot on paper chromatography.

2,3,5-Tri-*O*-methyl-L-arabinose.—Group Ia (R_g 1.01; 15 mg.) was oxidized 4 days with 2 ml. of bromine water, aerated to remove excess bromine, neutralized with silver carbonate, filtered and treated with resin IR-120 (H). The solution was evaporated to a sirup which was held at 80° for 2 hr. The product was dissolved in methanol saturated with ammonia, held at 7° for 16 hr., and the solution concentrated to a sirup which, when nucleated, crystallized to give 2,3,5-tri-*O*-methyl-L-arabonamide, m.p.[5] 128–131° undepressed with authentic sample. The X-ray diagram was identical with that of a known specimen.

2,3,4-Tri-*O*-methyl-D-xylose.—Group Ib (R_g 0.98; 18 mg.), $[\alpha]^{25}D$ +18.3° (*c* 0.5 in water) was dissolved in 2 ml. of anhydrous methanol with 5 drops of aniline and allowed to stand 3 days at room temperature. It was evaporated to a sirup and desiccated over sulfuric acid. Nucleation gave crystals of N-phenyl-D-xylosylamine 2,3,4-trimethyl ether, m.p.[9] 98–99° undepressed by admixture of authentic sample, and the X-ray diagram was identical with that of a known specimen.

3,5-Di-*O*-methyl-L-arabinose.—Material IIa showed one spot corresponding to dimethyl-L-arabinose (R_g 0.78; 14 mg.), $[\alpha]^{25}D$ +15.4° (*c* 0.7 in methanol) and showed arabinose to be the only sugar freed by demethylation with 48% hydrobromic acid[10] when the reaction mixture was chromatographed in either irrigant A or ethyl acetate–pyridine–water (10:4:3, v./v.).

Ionophoresis[11a] of the borate complex of IIa indicated two zones; the major zone moved at a rate such as to suggest no substitution of the hydroxyl on C2 and after alkaline degradation in lime water[11b] the major component almost disappeared, again suggesting the lack of a methoxyl group at carbon C2. Since the borate ionophoresis rate is not that for authentic 3,4-di-*O*-methyl-L-arabinose, the major component seems most likely to be 3,5-di-*O*-methyl-L-arabinose.

(4) W. N. Haworth, *J. Chem. Soc.*, **107**, 8 (1915).

(5) R. Kuhn, H. Trishman and I. Loew, *Angew. Chem.*, **67**, 32 (1955); J. Saarnio, Doctor's Dissertation, Helsinki, 1956.

(6) Products of Rohm and Haas Co., Resinous Products Division, Washington Square, Philadelphia 5, Pa.

(7) *Rg* is the ratio of the paper chromatographic flow-rate relative to 2,3,4,6-tetra-*O*-methyl-D-glucose in irrigant A.

(8) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 506 (1946).

(9) D. I. McGilvray, *ibid.*, 2577 (1953).

(10) L. Hough, J. K. N. Jones and W. H. Wadman, *ibid.*, 1702 (1950).

(11) (a) A. B. Foster, *Chemistry & Industry*, 828 (1952); A. B. Foster and M. Stacey, *J. Chem. Soc.*, 1778 (1955). (b) R. L. Whistler and W. M. Corbett, THIS JOURNAL, **78**, 1003 (1956).

2,3-Di-*O*-methyl-D-xylose.—Group III (951 mg.), $[\alpha]^{25}D$ +23.0° (*c* 1 in water), R_g 0.61, treated with aniline in methanol, produced a crystalline derivative upon vacuum desiccation. Recrystallization from ethyl acetate–*n*-hexane yielded a product, m.p.[12] 145–146°, which gave the same X-ray diagram as authentic N-phenyl-D-xylopyranosylamine 2,3-dimethyl ether.

2-*O*-Methyl-D-xylose.—Group IV (215 mg.), $[\alpha]^{25}D$ +21.4° (*c* 1.8 in water), R_g 0.23, when nucleated gave crystals which were recrystallized from ethyl acetate and methanol, m.p.[13] 130–131°. Its X-ray diagram was identical with that of an authentic specimen.

Separation of Sugar Units in Methylated Arabinogalacto-4-*O*-methylglucuronoxlyoglycan.—The methylated polysaccharide at 9% concentration in chloroform was separated into seven fractions by stepwise addition of hexane. More than 80% of the material precipitated in five fractions between 75 and 83% n-hexane (Table III). The five fractions had nearly the same methoxyl content and specific optical rotation and were combined. This group was methanolized at 2% concentration in anhydrous methanol containing 5% hydrogen chloride. The specific rotation increased and in 8 hr. became constant at +46.5°. After neutralization with silver carbonate, the filtered solution was dissolved in 100 ml. of 0.5 *N* hydrochloric acid and hydrolyzed 4 hr. at 98°. After treatment with silver carbonate the hydrolyzate was treated with Amberlite IR-120 (H) cation exchange resin. The filtered solution of methylated sugars was concentrated to a sirup which was dried by azeotropic distillation with a benzene–ethanol (2:1) solution.

TABLE III

FRACTIONATION OF METHYLATED ARABINOGALACTO-4-*O*-METHYLGLUCURONOXYLOGLYCAN

Hexane, %	Precipitate, mg.	Methoxyl, %	$[\alpha]^{25}D$
50.0	38	15.0	.....
57.1	20	26.7	.....
75.0	155	35.4	−40.3°
77.0	991	35.4	−40.6
78.5	461	35.9	−40.5
81.2	659	36.1	−40.4
83.2	340	35.8	−40.0
Supernatant	576	35.4	−40.0

The sirup (1.99 g.) was dissolved in the mixture ethyl acetate–pyridine–water (8:2:1 v./v.), and the solution placed on a cellulose column (25 × 430 mm.) where it was irrigated by the same mixture. The eluate was collected automatically[14] in 25-ml. aliquots. Paper chromatograms showed that the eluate could be grouped to produce five fractions which differed in the types of sugars present. Fractions I, II and V were mixtures while fractions III and IV were subsequently shown to be pure compounds.

Fraction I was separated on sheets of Whatman No. 1 filter paper with irrigant A. When the guide strips were sprayed with *p*-anisidine hydrochloride[15] the colors produced upon heating suggested slightly overlapping zones of two different colors characteristic of 2,3,5-tri-*O*-methyl-L-arabinose and 2,3,4-tri-*O*-methyl-D-xylose.[16] The portion of the zones corresponding to that which showed a single color were cut out and eluted with methanol. This process was repeated until chromatographically pure Ia and Ib were obtained.

The major fraction (II, 922 mg.) was rechromatographed on another cellulose column using irrigant A. Paper chromatograms of the eluates with irrigant A showed the first eluates to be chromatographically identical to fraction I with which these were combined. The next group of eluates contained a mixture of five sugars, while the last group contained only one compound which was chromatographically identical to fraction III and was combined with it. The group of five sugars was separated by repeated preparative paper chromatography until the two fractions IIa and IIb were obtained chromatographically pure.

Fraction V appeared chromatographically to be a mixture of uronic acids and xylose. Fraction Va was obtained by deionizing an aqueous solution of fraction V with Amberlite resins IR-120 (H) and IR-4B (OH). The material retained by the IR-4B (OH) anion exchange resin was eluted (Vb) with 30 ml. of 1 *N* sulfuric acid. The acid was neutralized with barium carbonate, filtered and the uronic acid converted to the acid form with resin IR-120 (H).

2,3,5-Tri-*O*-methyl-L-arabinose.—Fraction Ia (15 mg.) when treated on paper chromatograms with aniline trichloroacetate[15] and *p*-anisidine hydrochloride[15] gave color reactions typical of trimethylarabinofuranose. The fraction was oxidized 48 hr. in the dark with bromine water, excess bromine removed by aeration and the solution neutralized by addition of silver carbonate. After treatment with Amberlite IR-120 (H) the solution was filtered and evaporated to a sirup under reduced pressure. The residue was taken up in acetone, filtered and again evaporated to a sirup and held at 60° for 2 hr. This sirup was dissolved in 5 ml. of ammonia-saturated methanol and held at 8° overnight. Upon removal of the solvent, crystallization occurred after nucleation and storing several days in a vacuum desiccator over sulfuric acid. The crystals had a m.p.[8] of 131–133° and had the same X-ray powder diffraction pattern as an authentic sample of 2,3,5-tri-*O*-methyl-L-arabonamide.

2,3,4-Tri-*O*-methyl-D-xylose.—Fraction Ib (23 mg.; R_g 0.98) contained some extraneous material from the paper which was insoluble in ethyl acetate–petroleum ether. Upon filtration and evaporation, a sirup was obtained which crystallized upon nucleation. The crystals were placed on porous tile and triturated with cold ethyl acetate and dried to give a m.p. of 87–88°, undepressed when mixed with authentic material (reported m.p.[17] 91–92°) and gave an X-ray powder diagram identical to that of authentic 2,3,4-tri-*O*-methyl-D-xylose.

2,3,4,6-Tetra-*O*-methyl-D-galactose.—Fraction IIa (42 mg.) $[\alpha]^{25}D$ +95° (*c* 2 in water) had the same R_g (0.82) as authentic 2,3,4,6-tetra-*O*-methyl-D-galactose. Conversion to the aniline derivative produced a crystalline material melting[18] at 196° and having an X-ray powder diffraction pattern identical to that of a known specimen.

3,5-Di-*O*-methyl-L-arabinose.—Fraction IIb (15 mg.), $[\alpha]^{25}D$ −12.4° (*c* 0.8 in water), gave a single spot (R_g 0.78) on paper chromatograms developed with irrigant A or with ethyl acetate–pyridine–water (10:4:3 v./v.). Demethylation showed arabinose to be the only free sugar on chromatograms developed with either irrigant just mentioned. Ionophoresis on paper in borate buffer showed this fraction to be a mixture of two materials. The major component moved at a rate suggestive of a free hydroxyl at C2. After alkaline degradation in lime water at room temperature the major component almost completely disappeared which again suggests the lack of a methoxyl group at C2. The specific rotation of the sample suggests that the major component is a furanose rather than a pyranose sugar. Since the ionophoretic movement of 3,4-di-*O*-methyl-L-arabinose is different from that of the principal component, it seems likely that fraction IIb is mostly 3,5-di-*O*-methyl-L-arabinose accompanied by possibly 2,5-di-*O*-methyl-L-arabinose. The latter compound is suggested because of its lack of movement on electrophoresis in borate buffer.

2,3-Di-*O*-methyl-D-xylose.—Fraction III, $[\alpha]^{25}D$ +21.4° (*c* 1 in water) was converted to the aniline derivative by refluxing 100 mg. with 10 drops of aniline in anhydrous methanol for 2 hr. Removal of the solvent and desiccation was followed by spontaneous crystallization. After recrystallization from methanol–ethyl acetate, the m.p.[19] 123–124° and X-ray diffraction were identical to the values for authentic N-phenyl-D-xylopyranosylamine 2,3-dimethyl ether.

2-*O*-Methyl-D-xylose.—Fraction IV (304 mg.; R_g 0.23) crystallized completely upon standing overnight. After recrystallization from methanol–ethyl acetate the material had a m.p.[13] of 131–132° and an X-ray diffraction pattern

(12) H. A. Hampton, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 1739 (1929).

(13) G. S. Robertson and T. H. Speedie, *ibid.*, 824 (1934).

(14) J. L. Hickson and R. L. Whistler, *Anal. Chem.*, **75**, 1425 (1953).

(15) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 1702 (1950).

(16) R. Montgomery and F. Smith, THIS JOURNAL, **77**, 3325 (1955).

(17) A. F. Carruthers and E. L. Hirst, *J. Chem. Soc.*, **121**, 2299 (1922).

(18) J. C. Irvine and D. McNicoll, *ibid.*, **97**, 1449 (1910).

(19) G. O. Aspinall and R. S. Mahomed, *ibid.*, 1731 (1954).

identical to the values for a known sample of 2-*O*-methyl-D-xylose. *Anal.* Calcd. for $C_6H_{12}O_5$: C, 43.9; H, 7.36. Found: C, 44.0; H, 7.36.

D-Xylose.—Fraction Va was chromatographically identical to D-xylose on chromatograms developed with ethyl acetate–pyridine–water (10:4:3 v./v.) and with irrigant A. The material was allowed to react with benzaldehyde in methanolic hydrogen chloride[20] to produce D-xylose dibenzylidine dimethyl acetal, m.p.[21] 210°, having an X-ray diffraction pattern identical to the known material.

Uronic Acids.—Fraction Vb (350 mg.) was shown to be a mixture on paper chromatography. Papers developed in ethyl acetate–acetic acid–formic acid–water (18:3:1:4 v./v.) show three major zones at R_g 0.78, 0.58 and 0.15 with a trace of material at 0.09. Conversion to the methyl glycoside–methyl ester by refluxing with anhydrous methanol containing 5% hydrogen chloride for 4 hr. was followed by neutralization with silver carbonate, filtration and evaporation to a sirup. The mixture was dissolved in anhydrous ether and reduced with 100 mg. of lithium aluminum hydride[22] dissolved in ether. The excess reagent was decomposed with ethyl acetate and water and then extracted continuously with chloroform for 16 hr. A sirup remaining after reduction (250 mg.), $[\alpha]^{25}D$ +40.3° (*c* 1.2 in water), was hydrolyzed for 4 hr. in 1 *N* sulfuric acid at 98°. The hydrolyzate was neutralized with barium carbonate, filtered and deionized with resins IR-120 (H) and IR-45 (OH). Paper chromatograms in irrigant A showed a major elongated zone of R_g 0.70 to 0.80 which corresponded to a mixture of known 2,3,4-tri-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-xylose. A second zone at R_g 0.22 corresponded to 2-*O*-methyl-D-xylose, and a trace of material was present at R_g 0.03. Ionophoresis[10] in borate buffer gave four zones having M_G values of 0.96, 0.88 (trace), 0.32, and 00.0. M_G is the ratio of movement compared to that of D-glucose corrected for electroendosmotic flow.

Quantitative Paper Chromatography.—The methylated polysaccharides (100 mg.) were hydrolyzed in sealed tubes and chromatographed with irrigant A in essentially the same way as described in an earlier report,[23] except that the hydrolyzate was deionized with resins IR-120 (H) and IR-45 (OH) before concentrating and evaporating. Whatman No. 1 filter paper extracted with water and ethanol was used for the chromatograms. The sugar zones were eluted from the paper chromatogram with methanol and the molar ratios determined by alkaline hypoiodite[24] after correction for paper blanks. The results are shown in Table I.

(20) L. F. Wise and E. K. Ratliff, *Anal. Chem.*, **19**, 691 (1947).

(21) L. J. Breddy and J. K. N. Jones, *J. Chem. Soc.*, 738 (1945).

(22) M. Abdel-Akher and F. Smith, *Nature*, **166**, 1037 (1950); B. Lythgoe and S. Trippet, *J. Chem. Soc.*, 1983 (1950).

(23) R. L. Whistler and J. N. BeMiller, THIS JOURNAL, **78**, 1163 (1956).

(24) S. K. Chanda, E. L. Hirst, J. K. N. Jones and E. G. V. Percival, *J. Chem. Soc.*, 1289 (1950).

LAFAYETTE, IND.

[CONTRIBUTION NO. 334 FROM THE DEPARTMENT OF ORGANIC CHEMISTRY AND ENZYMOLOGY, FORDHAM UNIVERSITY]

Investigations on Lignins and Lignification. XIX.* The Mode of Incorporation of *p*-Hydroxyphenylpyruvic Acid into Lignin

BY SAMUEL N. ACERBO, WALTER J. SCHUBERT AND F. F. NORD

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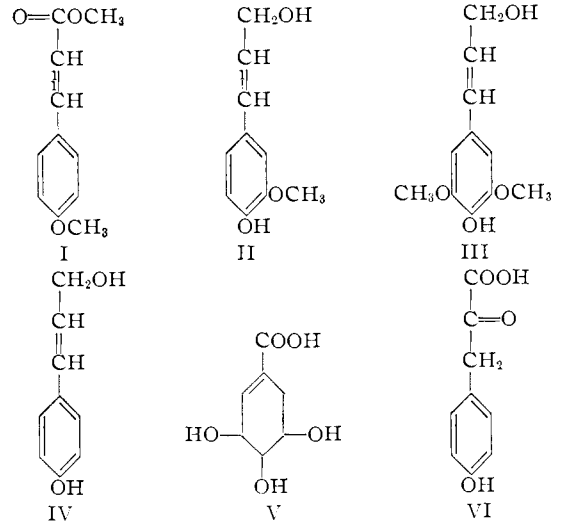
Carboxyl-labeled *p*-hydroxyphenylpyruvic acid was incorporated into a living sugar cane plant. Seventy-one per cent. of the introduced radioactivity was located in the isolated lignin. Alkaline nitrobenzene oxidation of the lignin to (nonradioactive) vanillin and alkaline fusion degradation to (radioactive) oxalic acid indicated that the *p*-hydroxyphenylpyruvic acid was utilized by the plant as a unit in the course of its conversion to lignin.

Introduction

The mechanism of the formation *via* shikimic acid of methyl *p*-methoxycinnamate (I) by *Lentinus lepideus* has been found to be related to that of the biogenesis of certain aromatic amino acids.[1] Due to the structural similarity of I with the postulated building stones of lignin, namely, coniferyl alcohol (II), sinapyl alcohol (III) and *p*-hydroxycinnamyl alcohol (IV), this finding also establishes a relationship with the biogenesis of lignin.

During the course of investigations of the metabolism of *Lentinus lepideus*, shikimic acid and five keto-acids, including *p*-hydroxyphenylpyruvic acid, were detected in the culture medium. Shikimic acid (V) is now regarded as a direct precursor of the aromatic rings of methyl *p*-methoxycinnamate[2] and of lignin.[3] Detection in the medium of *p*-hydroxyphenylpyruvic acid (VI), which is structurally related to the postulated building stones of lignin (II, III, IV), prompted an investigation of the possible



role of this acid in the mechanism of lignification.[4] The results to be reported here present evidence

* For the previous paper of this series see W. J. Schubert, S. N. Acerbo and F. F. Nord, THIS JOURNAL, **79**, 251 (1957). For a factual and critical review of the chemistry of lignins and the mechanism of lignification the reader is referred to: (a) F. F. Nord and W. J. Schubert, *Tappi*, **40**, 285 (1957); (b) W. J. Schubert and F. F. Nord, *Advances in Enzymology*, **18**, 349 (1957); (c) F. F. Nord and Geo. de Stevens, *Handbuch d. Pflanzenphysiologie*, **10**, 389 (1958), Springer Verlag, Heidelberg.

(1) G. Eberhardt and F. F. Nord, *Arch. Biochem. Biophys.*, **55**, 578 (1955).

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