in the aromatic ring of vanillin which agrees well with the original distribution of $C^{14}$ in the six-membered ring of the incorporated shikimic acid, *i.e.*, the results give evidence that the cyclohexene ring of the acid was converted directly into the aromatic rings of lignin without randomization of the carbon atoms.

TABLE II

DISTRIBUTION OF ACTIVITY IN THE VANILLIN

| | Activity, cts./min. | Percentage distribution of the total activity |
|---|---|---|
| Vanillin | 58 | 100 |
| C-6 | 204 | 44 |
| C-2 | 190 | 41 |
| C-5 | 0 | 0 |

## Conclusions

After absorption of specifically $C^{14}$-labeled shikimic acid through the leaves of a sugar cane plant, it has been established that some of this compound was metabolized by the plant and was incorporated into a non-water extractable component of the stem of the plant. The analytical evidence indicates that the activity was incorporated to a great extent in the lignin. The degradation of the lignin, *via* vanillin, discloses that the activity, located in the aromatic ring, is comparable to the distribution of the activity in the incorporated shikimic acid.

From these results, it can be concluded that shikimic acid is an intermediate on the pathway from carbohydrates[13] formed by photosynthesis, to the aromatic rings of the lignin building stones.

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[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

# Structure of Corn Hull Hemicellulose. I. Partial Hydrolysis and Identification of 2-*O*-(α-D-Glucopyranosyluronic Acid)-D-xylopyranose[1,2]

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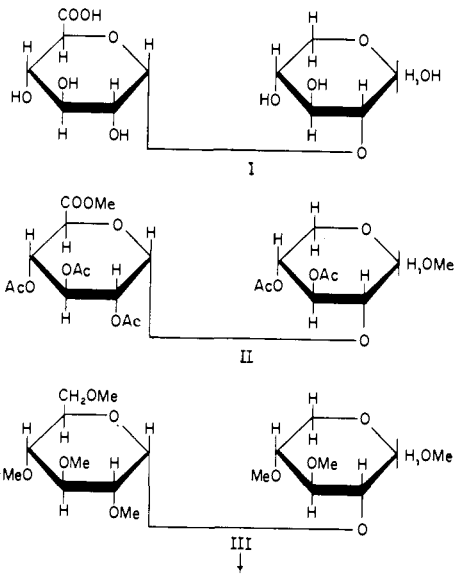
Graded hydrolysis of the acidic hemicellulose from the hulls of the kernels of maize (*Zea mays*) with dilute sulfuric acid yields arabinose, xylose, galactose and an aldobiouronic acid fraction. By treating the aldobiouronic acid fraction first with methanolic hydrogen chloride and then with acetic anhydride, two crystalline acetates of a methyl aldobiouronoside methyl ester have been produced. One of these acetates (II or B, m.p. 178–180°, $[\alpha]_D$ + 163° in chloroform) has been shown to be derived from 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylopyranose. Methylation of II followed by reduction and remethylation gave methyl 2-*O*-(2,3,4,6-tetra-*O*-methyl-D-glucopyranosyl)-3,4-di-*O*-methyl-D-xylopyranoside (III), the structure of which has been proved by the fact that upon hydrolysis it yields 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose.

Corn hulls which are the outer coverings of the kernels of maize (*Zea mays*) are obtained as a by-product in the manufacture of corn starch by the wet-milling process. The major component of the corn hull is a hemicellulose which can be extracted with dilute alkalies. Previous studies[3] have shown that it is composed of xylose (48%), arabinose (35%), galactose (7%) and uronic acid (10%).

Since the corn hulls represent a very cheap by-product and since the hemicellulose component of the hulls appears to show promise as an adhesive, thickener or stabilizer, a detailed study of the structure of the corn hull hemicellulose has been undertaken as part of a project[2] designed to establish in a general way the relationship between the structure and physical properties of polysaccharide gums. A preliminary report of the methylation studies on




2,3,4,6-Tetra-*O*-methyl-D-glucose
3,4-di-*O*-methyl-D-xylose

(1) Paper No. 3481, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota.

(2) This research was done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract was supervised by the Northern Utilization Research Branch of the Agricultural Research Service.

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the hemicellulose itself has already been published.[4] This paper is concerned with the isolation of two aldobiouronic acids (as their crystalline methyl ester methyl glycoside pentaacetates) and identification of one of them as 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylopyranose (I), a compound previously isolated from corn cobs.[5]

Graded hydrolysis of the corn hull hemicellulose with *N* sulfuric acid at room temperature has recently been found to give xylose, arabinose, 3-*O*-α-D-xylopyranosyl-L-arabinose and L-galactopyranosyl-(1 → 4)D-xylopyranosyl (1 → 2)-L-arabinose.[6] On a boiling water-bath the hydrolysis of the hemicellulose with *N* sulfuric acid produces arabinose, galactose, xylose and an aldobiouronic acid. When the aldobiouronic acid portion was boiled with methanolic hydrogen chloride and subsequently acetylated,[7] a crystalline product was obtained. Fractional crystallization of the latter afforded two crystalline methyl ester methyl glycoside aldobiouronic acid pentaacetates: A, m.p. 255–257°, $[\alpha]^{22}D$ +103° in chloroform, and B, m.p. 178–180°, $[\alpha]^{22}D$ +163° in chloroform. The acetate of the methyl aldobiouronoside methyl ester (B or II) has been shown to be methyl 2-*O*-[methyl(2,3,4-tri-*O*-acetyl-α-D-glucopyranosid)uronate]-3,4-di-*O*-acetyl-D-xylopyranoside by the following experimental evidence. Upon deacetylation of II and subsequent methylation with Purdie reagents, a partially methylated aldobiouronic acid was obtained. Since this methylated aldobiouronic acid proved to be difficult to methylate completely, it was reduced with lithium aluminum hydride[8,9] to give a partially methylated neutral disaccharide which readily underwent methylation with methyl iodide and silver oxide and afforded the fully methylated disaccharide, methyl 2-*O*-(2,3,4,6-tetra-*O*-methyl-α-D-glucopyranosyl)-3,4-di-*O*-methyl-D-xylopyranoside (III). The structure of III followed from the fact that upon hydrolysis it yielded: (a) crystalline 2,3,4,6-tetra-*O*-methyl-D-glucose[10] which, in turn, gave the corresponding crystalline anilide[11] and (b) 3,4-di-*O*-methyl-D-xylose which was identified by bromine oxidation to the crystalline 3,4-di-*O*-methyl-D-xylono-δ-lactone.[12] The relatively high positive rotation of I, II and III indicates that the biose linkage is of the α-type.

## Experimental

**Partial Hydrolysis of Corn Hull Hemicellulose and Isolation of Two Crystalline Acetates of Methyl Aldobiouronoside Methyl Ester.**—A solution of crude corn hull hemicellulose (100 g.) in *N* sulfuric acid (3000 ml.) was heated on a boiling water-bath for 5 hr. (complete hydrolysis requires 11.5 hours). The resulting solution, while still hot, was neutralized with barium carbonate, cooled and filtered. The filtrate was concentrated under reduced pressure to approximately 750 ml. and the solution was slowly poured with stirring into ethanol (3000 ml.) when a brown sirup was precipitated. Reprecipitation of the sirupy product containing the barium salts from aqueous solution with ethanol gave a colorless amorphous powder (3.3 g.). The free acids were liberated from the barium salts by dissolving them in water (30 ml.) and passing the solution first through the cation exchange resin Amberlite IR-120[13] and then through the anion exchange resin Duolite A4.[14] The aldobiouronic acid was displaced from the resin with *N* sodium hydroxide and transformed into the free acid by passing the aqueous alkaline solution through the cation exchange resin Amberlite IR-120. Concentration of the solution to dryness *in vacuo* gave the acid fraction designated $F_1$. The mother liquor from the initial precipitation of the barium salts of $F_1$ was concentrated to a small volume and the aqueous solution was poured with stirring into ethanol as before. The gummy product thus precipitated was treated as described above with ion exchange resins to give the acid components (3.5 g.) designated $F_2$. The mother liquor from the precipitation of the barium salts of $F_2$ did not contain barium, thus showing that all the acid components had been recovered from the hydrolysis products of the hemicellulose.

The acid fractions $F_1$ and $F_2$ were each refluxed with 2% methanolic hydrogen chloride (50 ml.) for 7 hr. after which time the hydrogen chloride was neutralized with basic lead carbonate. After filtration, the methanolic solution was concentrated at room temperature *in vacuo* to a sirup which was dissolved in pyridine (100 ml.) and treated with acetic anhydride (40 ml.). The reaction mixture was heated at 60° for 5 hr., cooled and poured with stirring into ice-water. In each case the acetate solidified. The acetates were filtered, washed thoroughly with water and then with small volumes of methanol and methanol–ether, the organic washings being collected separately, combined and concentrated *in vacuo* to solid residues.

The acid fraction $F_1$ afforded 1.43 g. of acetate, $[\alpha]^{22}D$ +113° in chloroform (*c* 0.9), which was insoluble, and 0.59 g. of acetate, $[\alpha]^{22}D$ +139° in chloroform (*c* 3), which was soluble in the combined methanol and methanol–ether washings. The acid fraction $F_2$ afforded 1.67 g. of acetate, $[\alpha]^{22}D$ +114° in chloroform (*c* 0.7), which was insoluble, and 1.05 g. of acetate, $[\alpha]^{22}D$ +131° in chloroform (*c* 3.1), which was soluble in the combined methanol and methanol–ether washings.

Repeated recrystallization of these four fractions from ethanol, acetone–ethanol and chloroform–ethanol afforded only two acetates: acetate A, m.p. 255–257°, $[\alpha]^{22}D$ +103° in chloroform (*c* 1.2). *Anal.* Calcd. for $C_{28}H_{32}O_{16}$: C, 48.9; H, 5.7; $OCH_3$, 11.0; glycoside $OCH_3$, 5.5. Found: C, 49.2; H, 5.9; $OCH_3$, 10.8; glycoside $OCH_3$ (by saponification with barium hydroxide, determination of the ether methoxyl content of the residual dried salt and subtracting this value from the original methoxyl value),[15] 5.5. Acetate B, m.p. 158–160° with previous sintering at 150–151°, $[\alpha]^{22}D$ +163° in chloroform (*c* 1.0), m.p. 178–180° after heating *in vacuo* at 56°. *Anal.* Calcd. for $C_{28}H_{32}O_{16}$: C, 48.9; H, 5.7; $OCH_3$ 11.0; glycoside methoxyl, 5.5. Found: C, 48.7; H, 5.2; $OCH_3$, 10.9; glycoside $OCH_3$ (determined as for A), 5.6.

**Deacetylation and Methylation of Acetate B.**—Acetate B, m.p. 178–180° (0.37 g.), was suspended in ethanol (20 ml.) at 0° and *N* sodium hydroxide (4 ml.) added. The mixture was slowly brought to room temperature and allowed to stand for 12 hr. to complete solution after which time the ethanol was distilled off at room temperature *in vacuo*, the total volume being maintained at 24 ml. (approx.) by the periodic addition of water. The resulting aqueous solution was heated at 40–50° for 2 hr. and then passed through Amberlite IR-120 ion exchange resin. The acidic effluent was evaporated to dryness *in vacuo* and the viscous sirupy residue dissolved in the minimum amount of anhydrous methanol. The resulting methanolic solution was treated with methyl iodide and silver oxide, first at room temperature with shaking for about 2 hr. and then under reflux for 6 hr. The sirupy product (0.27 g.), isolated by extraction with methanol, was subjected to two further methylations with silver oxide and methyl iodide to give a sirup (0.29 g.) which was distilled, b.p. (bath temp.) 165–175° (0.015 mm.) (found: $OCH_3$, 38.0). Three additional methylations with silver oxide and methyl iodide failed to increase the methoxyl content.

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**Reduction of the Partially Methylated Aldobiouronic Acid.**—The partially methylated methyl aldobiouronoside methyl ester (0.20 g.) obtained above was dissolved in ether (20 ml.) and the solution added dropwise to finely ground lithium aluminum hydride (0.30 g.) in ether (20 ml.). The resulting solution was heated under reflux for 3 hr., cooled and the excess lithium aluminum hydride decomposed with ethyl acetate. After acidification of the solution with glacial acetic acid, it was evaporated to dryness and the residue treated with acetic anhydride (50 ml.) for 3 hr. on a steam-bath to liberate the product from inorganic complexes.[16,17] The acetic anhydride was removed by distillation *in vacuo* and to the residue water (20 ml.) was added. After standing overnight the product was extracted with chloroform several times. The combined chloroform extracts were washed free of acid with water, evaporated to dryness *in vacuo* and the residue (0.20 g.) was dissolved in a mixture of acetone (20 ml.) and 0.1 *N* sodium hydroxide (10 ml.). The deacetylation was carried out at 60° for 3 hr. and the resulting solution deionized by passing through Amberlite IR-120 and Duolite A4 ion exchange resins in this order. The neutral effluent was evaporated to dryness *in vacuo* to give a sirupy product (0.20 g.).

**Methylation of the Partially Methylated Disaccharide and Isolation of Methyl 2-*O*-(2,3,4,6-Tetra-*O*-methyl-α-D-glucopyranosyl)-3,4-di-*O*-methyl-D-xylopyranoside (III).**—The partially methylated disaccharide (0.20 g.) was subjected to 4 treatments with silver oxide and methyl iodide, acetone being added only in the first treatment to dissolve the partially methylated disaccharide. The final product was a sirup (0.17 g.) which distilled giving methyl 2-*O*-(2,3,4,6-tetra-*O*-methyl-α-D-glucopyranosyl)-3,4-di-*O*-methyl-D-xylopyranoside, a colorless liquid, b.p. (bath temp.) 130–140° (0.01 mm.), $[\alpha]^{22}D$ +146° in methanol (*c* 1.9). *Anal.* Calcd. for $C_{18}H_{34}O_{10}$: $OCH_3$, 52.9. Found: $OCH_3$, 50.2.

**Hydrolysis of Methyl 2-*O*-(2,3,4,6-Tetra-*O*-methyl-α-D-glucopyranosyl)-3,4-di-*O*-methyl-D-xylopyranoside.**—A solution of the methylated disaccharide (117 mg.) in *N* sulfuric acid (5 ml.) which showed $[\alpha]^{21}D$ +140° (*c* 2.3) was refluxed for 13 hr. The brown colored reaction mixture was neutralized ($BaCO_3$), centrifuged and the supernatant liquid evaporated to dryness *in vacuo*. The residue was extracted with methanol, the extract treated with charcoal, filtered and evaporated *in vacuo* to give a pale yellow sirup (74 mg.) which had $[\alpha]^{22}D$ +59.6° in methanol (*c* 1.5). Chromatographic analysis of this sirup on paper using methyl ethyl ketone:water azeotrope[18] and benzene–ethanol–water (200:47:15—upper layer)[19] as irrigants and *p*-anisidine trichloroacetate[20] as spray reagent revealed two spots with $R_f$ values corresponding to 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose. With methyl ethyl ketone:water azeotrope as the solvent, these two methylated sugars have almost identical $R_f$ values but they are readily separated with benzene:ethanol:water; *R*(tetra-*O*-methyl-D-glucose) values: 2,3-di-*O*-methyl-D-xylose, 0.27; 3,4-di-*O*-methyl-D-xylose, 0.35. Moreover, they give different colors with the *p*-anisidine spray. Since 3,4-di-*O*-methyl-D-xylose forms a borate complex while the 2,3-di-*O*-methyl derivative does not do so to any extent, the two sugars are readily separated by paper electrophoresis using a borate buffer (*p*H 9.2), the former moving 4.5 cm. and the latter 0.3 cm. with respect to 2,3,4,6-tetra-*O*-methyl-D-glucose in 2 hr. at 600 volts without cooling.[21]

The two methylated sugars were separated by chromatographing the sirupy mixture (74 mg.) on large sheets (22″ × 18.5″) of Whatman No. 1 filter paper using methyl ethyl ketone:water. After location of the two sugars by spraying three one-quarter inch marking strips, the appropriate zones of the chromatograms were extracted with methanol and the sugars identified as described below.

**Identification of 2,3,4,6-Tetra-*O*-methyl-D-glucose.**—The component (45 mg.) with the higher $R_f$ value corresponding to 2,3,4,6-tetra-*O*-methyl-D-glucose and having $[\alpha]^{23}D$ +55° in methanol (*c* 1) was purified by dissolving it in water and treating the solution with a small amount of charcoal. The solution obtained after filtration was concentrated *in vacuo* to a sirup which crystallized. The crystals of 2,3,4,6-tetra-*O*-methyl-D-glucose[10] had m.p. and mixed m.p. 86–88° (after one recrystallization from ether). The mixture of crystals and sirup (37 mg.), recovered from the mother liquors, was dissolved in absolute ethanol (5 ml.) and aniline (200 mg.) was added. The solution was refluxed for 6 hr., evaporated *in vacuo* to remove the excess of the alcohol and the aniline. The resulting sirup readily crystallized and after recrystallization from ether the anilide of 2,3,4,6-tetra-*O*-methyl-D-glucose[11] had m.p. and mixed m.p. 132–134°.

**Identification of 3,4-Di-*O*-methyl-D-xylose.**—The sirupy di-*O*-methyl-D-xylose component (28 mg.) had $[\alpha]^{23}D$ +23.6° in methanol (*c* 0.5) (literature value[17] $[\alpha]D$ +25° in methanol). The sirup (27 mg.) was dissolved in water (2 ml.) and bromine (10 drops) was added. The reaction mixture was kept in the dark at room temperature. After 5 days, when chromatography showed that the oxidation was complete, the reaction mixture was freed of bromine by aeration, neutralized ($Ag_2CO_3$), filtered and passed through Amberlite IR-120 resin. The acidic effluent was evaporated *in vacuo* to a sirup (17 mg.) which was lactonized by heating in high vacuum at 80–90° for 1 hr. Upon nucleation, crystallization of the colorless liquid followed immediately and after resublimation the 3,4-di-*O*-methyl-D-xylono-δ-lactone[12] had m.p. and mixed m.p. 66–67°, $[\alpha]^{23}D$ −50° changing to −15° in water (*c* 0.26).

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ST. PAUL, MINNESOTA

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