*cis*-I-Phenylundecapentaenal was obtained from the chromatogram described in the next Section. Since it could not be rechromatographed unchanged, the following data were determined upon iodine catalysis. $E^{mol.}_{1cm.}$ $8.08 \times 10^4$ at $\lambda_{max}$ 389 mμ (in hexane); $7.30 \times 10^4$ at $\lambda_{max}$ 401 mμ (in benzene).

*cis*-II-Phenylundecapentaenal.—Seventeen milligrams of pure all-*trans* crystals was dissolved in 15 ml. of benzene on a water-bath and, after cooling to 20°, exposed to intense diffuse daylight on a window sill. After 2 hr. the solution was developed on a 22 × 3.5 cm. column:

| | |
|---|---|
| 20 | orange-yellow (golden-yellow): unchanged all-*trans* |
| 5 | pale yellow (dull dark brown): *cis*-I |
| 5 | empty interzone |
| 23 | yellow (golden light yellow): *cis*-II |
| 7 | pale yellow (dull brown): *cis*-III |
| 160 | empty section |

The eluate of the 23-mm. zone was transferred to benzene, dried, concentrated and rechromatographed as described on a 18 × 2 cm. column. The developing process was continued until a narrow but clear interzone appeared between the *cis*-II and *cis*-III zones. The benzene solution obtained from the former was evaporated completely, the crystalline residue dissolved at 25° in the minimum amount of benzene and crystallized by cautious addition of hexane; yield 4 mg. (in all, 20 mg. were prepared). Fine, orange-yellow needles grouped in sheaves or fan-like forms, m.p. 103–105°, or short needles, m.p. 108–110°; $E^{mol.}_{1cm.}$ $7.49 \times 10^4$ at $\lambda_{max}$ 390 mμ (in hexane); $6.2 \times 10^4$ at $\lambda_{max}$ 401 mμ (in benzene).

*Anal.* Calcd. for $C_{17}H_{16}O$: C, 86.93; H, 6.83. Found: C, 87.25; H, 7.39.

*cis*-III-Phenylundecapentaenal.—The 7-mm. zone of the above chromatogram was treated as described for *cis*-II and combined with seven similar zones originating from parallel experiments. After rechromatographing (column, 22 × 3.5 cm.) the benzene solution (50 ml.) was evaporated to 1 ml. at 10° (oil pump, receiver in acetone–Dry Ice). Upon cautious addition of hexane at 20°, this concentrate deposited crystals. Recrystallization in the same manner yielded 3 mg. of small, sturdy, yellow spears. The sample sintered at about 103° and melted at 176–178°, *i.e.*, somewhat below the m.p. of the all-*trans* isomer; evidently, this determination was disturbed by spatial changes in the fused state. The following values were obtained upon iodine catalysis: $E^{mol.}_{1cm.}$ $6.91 \times 10^4$ at $\lambda_{max}$ 390 mμ (in hexane); $5.90 \times 10^4$ at $\lambda_{max}$ 401 mμ (in benzene).

*cis*-IV-Phenylundecapentaenal was formed, when a solution of 5 mg. of *cis*-III in 5 ml. of benzene was exposed to sunshine (*cf.* Table IC). In the chromatogram, the thin, pale yellow *cis*-IV zone was located just below *cis*-III. After rechromatographing, the following data were obtained by the iodine catalysis method: $E^{mol.}_{1cm.}$ $6.64 \times 10^4$ at $\lambda_{max}$ 387 mμ (in hexane); $5.67 \times 10^4$ at $\lambda_{max}$ 394 mμ (in benzene).

PASADENA, CALIF.

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

# Structure of Corn Hull Hemicellulose. Part VI. The Synthesis of 5-O-β-D-Galactopyranosyl-L-arabinose[1,2]

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The synthesis of 5-*O*-β-D-galactopyranosyl-L-arabinose is described.

In a previous paper[3] of this series there was discussed the isolation and characterization of 5-*O*-β-D-galactopyranosyl-L-arabinofuranose, a disaccharide formed amongst others when corn hull hemicellulose[4] is subjected to graded hydrolysis with dilute mineral acid.[3]

The formulation of the disaccharide as 5-*O*-β-D-galactopyranosyl-L-arabinose was based upon these observations: Acid hydrolysis showed the presence of galactose and arabinose, while hydrolysis of the phenylosazone of the disaccharide gave galactose, but no arabinose. Hydrolysis of the fully methylated disaccharide furnished 2,3,4,6-tetra-*O*-methyl-D-galactopyranose and 2,3-di-*O*-methyl-L-arabinose. The decision to assign a β-configuration to the galactose residue and a furanose conformation to the arabinose moiety was based upon the rules of isorotation.[3,5]

This communication is concerned with the synthesis of 5-*O*-β-D-galactopyranosyl-L-arabinose in order to confirm the structure assigned to the disaccharide from the corn hull hemicellulose. The synthesis was accomplished as follows: interaction of ethyl 2,3-di-*O*-acetyl-α-L-arabinofuranoside with 2,3,4,6-tetra-*O*-acetyl-α-D-galactosyl bromide in the presence of silver oxide gave a sirup which, when deacetylated and subjected to controlled hydrolysis, afforded 5-*O*-β-D-galactopyranosyl-L-arabinofuranose. This was purified by chromatography on a carbon:Celite column[6] taking care to avoid washing with water to remove monosaccharides, since this leads to imperfect separations.[7] In this manner two isomeric galactose–arabinose disaccharides were obtained. Methylation of the one whose rotation corresponded to that of the galactose–arabinose disaccharide isolated from the corn hull hemicellulose, first with methyl sulfate and 40% potassium hydroxide solution in the absence of air, and then with methyl iodide and silver oxide, gave methyl 5-*O*-(2,3,4,6-tetra-*O*-methyl-β-D-galactopyranosyl)-2,3-di-*O*-methyl-L-arabinofuranoside, a non-reducing sirup which showed $[\alpha]^{24}D$ −45° in methanol. Acid hydrolysis of the latter followed by paper chromatographic analysis afforded two cleavage products, namely, 2,3,4,6-

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(2) This research was done under contract with the United States 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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tetra-*O*-methyl-D-galactopyranose and 2,3-di-*O*-methyl-L-arabinose.

Separation of these components was effected by sheet paper chromatography in the usual way using butanone:water azeotrope. The 2,3-di-*O*-methyl-L-arabinose was identified as its crystalline 1,4-di-*p*-nitrobenzoate[3] while the 2,3,4,6-tetra-*O*-methyl-D-galactopyranose afforded the characteristic crystalline aniline derivative.[8]

The characterization[3] of the *O*-D-galactosyl → L-arabinoise disaccharide from the corn hull hemicellulose as 5-*O*-β-D-galactopyranosyl-L-arabinose is therefore correct.

## Experimental

The following solvents were used for partition chromatography: A, pyridine:ethyl acetate:water (1:2.5:3.5)[9]; B, 1-butanol:pyridine:water (6:4:3)[10]; C, 1-butanol:acetic acid:water (2:1:1)[11]; and D, butanone–water azeotrope.[12] The sugars were detected on the paper chromatogram by spraying with *p*-anisidine trichloroacetate[13] and/or ammoniacal silver nitrate.[14]

All evaporations were carried out under reduced pressure at 30–40°. $R_x$ represents the rate of movement of sugars with respect to D-xylose.

**Ethyl 2,3-Di-*O*-acetyl-5-*O*-trityl-α-L-arabinofuranoside.**—Ethyl α-L-arabinofuranoside[15] (1.15 g., m.p. 48–49°) was suspended in dry pyridine (5 ml.) and after the addition of triphenylmethyl chloride (1.8 g.) the mixture was shaken. The reaction mixture which became clear after 10 hr. was kept at room temperature for 20 days. To the reaction mixture, acetic anhydride (5 ml.) was added and the solution kept at room temperature for 20 hr. The mixture was poured with stirring into cold water when a viscous sirup separated. The sirup was extracted with chloroform and the extract washed successively with a dilute aqueous solution of sodium bicarbonate and water. After drying ($Na_2SO_4$), the chloroform solution was evaporated to give a viscous sirup (3.2 g.) which did not crystallize.

**Ethyl 2,3-Di-*O*-acetyl-α-L-araboinfuranoside.**—The compound (3.2 g.) obtained in the previous experiment was detritylated by dissolving it in glacial acetic acid (30 ml.) and adding to the cooled solution a saturated solution (2.5 ml.) of hydrogen bromide in acetic acid. The precipitated triphenylmethyl bromide was filtered, the residue washed with acetic acid and the combined filtrates poured into a mixture of cold water and chloroform. The chloroform layer was separated and the aqueous layer extracted twice with chloroform. The combined chloroform extracts were washed with a solution of sodium bicarbonate and then with water. The chloroform solution was dried ($Na_2SO_4$) and evaporated to give a light colored sirup (1.4 g.) which showed $[\alpha]^{20}D$ −30° in chloroform (*c* 1.7).

**2,3,4,6-Tetra-*O*-acetyl-α-D-galactosyl Bromide.**—1,2,3,-4,6-Penta-*O*-acetyl-β-D-galactose (7 g.), transformed into the glycosyl bromide in the usual way, was obtained as a liquid (6.5 g.) which showed $[\alpha]^{19}D$ +211° in benzene (*c* 6); lit.[16] $[\alpha]D$ +234°. This product was used directly.

**Interaction of Ethyl 2,3-Di-*O*-acetyl-α-L-arabinofuranoside with 2,3,4,6-Tetra-*O*-acetyl-α-D-galactosyl Bromide.**—The reagents (chloroform, silver oxide and Drierite) for the condensation reaction were prepared according to the procedure of Reynolds and Evans.[17] The reactants were dried *in vacuo* at 50–60° for 4 hr.

Sirupy ethyl 2,3-di-*O*-acetyl-α-L-arabinofuranoside (1.1 g.) was dissolved in chloroform (7 ml.) and after the addition of Drierite (5 g.) and silver oxide (2.0 g.), the mixture was shaken for 0.5 hr. A solution of iodine (0.2 g.) in chloroform (10 ml.) was added to the mixture and then 2,3,-4,6-tetra-*O*-acetyl-α-D-glactosyl bromide (1.7 g.) in chloroform (7 ml.) was added in small portions during 0.5 hr. The mixture was shaken for 36 hr., filtered and the residue washed with chloroform. The combined filtrate and washings were evaporated to give a sirup (2.2 g.).

The sirup was deacetylated by dissolving it in ethanol (50 ml.), adding 0.1 *N* potassium hydroxide (20 ml.) and heating at 60° for 1 hr. The reaction mixture was evaporated to a sirup which was dissolved in 0.01 *N* sulfuric acid (20 ml.) and the solution heated on a steam-bath for 2 hr. The inorganic ions were removed by passing the solution successively through columns of Duolite A-4[18] and Amberlite IR-100,[19] and the effluent was evaporated to a sirup (1.0 g.). Upon chromatographic analysis using solvent A the sirup was found to contain arabinose ($R_x$ 0.84), galactose ($R_x$ 0.62) and a third component with $R_x$ 0.52. This component was indistinguishable on paper chromatograms using solvents A, B and C from 5-*O*-β-D-galactopyranosyl-L-arabinose[3] isolated by graded hydrolysis of corn hull hemicellulose.

**Isolation of 5-*O*-β-D-Galactopyranosyl-L-arabinose.**—The sirupy mixture (1.0 g.) containing the galactose–arabinose disaccharide, galactose and arabinose, was dissolved in 2.5% aqueous ethanol and the solution put on a charcoal:Celite column[6] previously washed with 2.5% aqueous ethanol. The column was washed first with 2.5% aqueous ethanol[7] (750 ml.) to displace the monosaccharides and then with 10% aqueous ethanol (900 ml.), collected in six fractions of 150 ml. each, to elute the disaccharide. Fractions 1 and 2 were found to contain negligible amounts of carbohydrate material. Fraction 3, when evaporated, yielded a sirup (181 mg.) which had $[\alpha]^{25}D$ −18° in water (*c* 1). In solvents A, B and C it had the same $R_f$ as that of 5-*O*-galactopyranosyl-L-arabinose obtained from the corn hull hemicellulose. The disaccharide (10 mg.) was hydrolyzed with 0.7 *N* sulfuric acid (3 ml.) for 4 hr. on a steam-bath, neutralized ($BaCO_3$), filtered and evaporated to give a sirup which, upon chromatographic analysis, was found to contain galactose and arabinose.

Fractions 4 and 5 were combined and then evaporated to yield a sirup (217 mg.) which had the same $R_f$ as 5-*O*-β-D-galactopyranosyl-L-arabinose and showed $[\alpha]^{25}D$ −76° in water (*c* 5). Upon acid hydrolysis with 0.7 *N* sulfuric acid it also afforded galactose and arabinose. The structure of this disaccharide will form the subject of a later communication.

Fraction 6, upon evaporation, gave a sirup (6.7 mg.) which was shown to be a mixture of galactosylarabinose and higher oligosaccharides and was not further examined.

**Proof of Structure of 5-*O*-β-D-Galactopyranosyl-L-arabinose. Methyl 5-*O*-(2,3,4,6-Tetra-*O*-methyl-D-galactopyranosyl)-2,3-di-*O*-methyl-L-arabinofuranoside.**—The galactose–arabinose disaccharide (90 mg., $[\alpha]D$ −18° in $H_2O$) was methylated in an atmosphere of nitrogen with 40% potassium hydroxide (24 ml.) and methyl sulfate (8 ml.), the reagents being added in eight portions with vigorous stirring at 0° during 1.5 hr.[3] The reaction mixture was stirred for another hour and after it had attained room temperature it was methylated with 40% potassium hydroxide (9 ml.) and methyl sulfate (3 ml.) in the previous manner. The mixture was stirred for 2 hr. and after warming it to 50–60° it was methylated again with methyl sulfate (2 ml.) and 40% potassium hydroxide (6 ml.). The reaction mixture was stirred for 1.5 hr. and then heated at 80–90° for 1.5 hr. The mixture was cooled, stirred with chloroform (50 ml.), filtered and the residue washed with chloroform. The chloroform solution was separated and the remaining aqueous layer extracted twice more with chloroform. The combined chloroform extracts were washed with a saturated solution of sodium sulfate, dried ($Na_2SO_4$) and evaporated to give a sirup (52.7 mg.). This was dissolved in methyl iodide (10 ml.) and silver oxide (3 g.) added in small portions. The mixture was refluxed for 12 hr. After the reaction, the mixture was filtered, washed with chloro-

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(18) A product of the Chemical Process Co., Redwood City, Calif.

(19) A product of the Rohm and Haas Chemical Co., Philadelphia, Pa.

form, and the combined filtrates evaporated to give a sirup which was methylated twice more with methyl iodide and silver oxide in the previous manner.

After distilling the methyl iodide, the silver oxide residue was extracted with ether (five 20-ml. portions). Concentration of the ethereal extract gave methyl 5-*O*-(2,3,4,6-tetra-*O*-methyl-D-galactopyranosyl)-2,3-di-*O*-methyl-L-arabinofuranoside as a yellow, non-reducing sirup (48.5 mg.) which showed $[\alpha]^{24}D$ −45° in methanol (*c* 1.6).[3]

**Hydrolysis of Methyl 5-*O*-(2,3,4,6-Tetra-*O*-methyl-D-galactopyranosyl)-2,3-di-*O*-methyl-L-arabinofuranoside.**—A solution of the methylated disaccharide (48.5 mg.) in 0.3 *N* sulfuric acid (3 ml.) was heated at 100° for 9 hr. The reaction mixture was neutralized ($BaCO_3$) and filtered, the salts being washed thoroughly first with water, then ethanol. Concentration of the filtrate gave a yellow sirup (38 mg.) which showed $[\alpha]^{26}D$ +32° in methanol (*c* 1.3).

Analysis of the sirup by paper chromatography, using solvent D, showed the presence of two components upon spraying either with ammoniacal silver nitrate or with *p*-anisidine, namely, 2,3-di-*O*-methyl-arabinose ($R_f$ 0.43) and 2,3,4,6-tetra-*O*-methylgalactose ($R_f$ 0.68).

Separation of the methylated components was achieved by sheet paper chromatography (Whatman No. 1) using solvent D. In addition to the two components noted, a small amount of the reducing unhydrolyzed methylated disaccharide was obtained, $R_f$ 0.79.

**Identification of 2,3,4,6-Tetra-*O*-methyl-D-galactose.**—The component corresponding to 2,3,4,6-tetra-*O*-methyl-D-galactose was eluted from the filter paper and concentrated to give a chromatographically pure sirup (13 mg.) which showed $[\alpha]^{25}D$ +58° in ethanol (*c* 0.4). It was converted in the usual manner to N-phenyl-D-galactopyranosylamine 2,3,4,6-tetramethyl ether, m.p. and mixed m.p. 196–197°, $[\alpha]^{27}D$ −78° in acetone (*c* 0.4), after recrystallization from ethanol; lit.[8] m.p. 192°, $[\alpha]D$ −77° (acetone).

**Identification of 2,3-Di-*O*-methyl-L-arabinose.**—2,3-Di-*O*-methyl-L-arabinose was obtained as a colorless sirup (6.5 mg.) which showed $[\alpha]^{25}D$ +92° in water (*c* 0.3). It was characterized as its 1,4-di-*p*-nitrobenzoate, m.p. and mixed m.p. 149–152°.[3]

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[CONTRIBUTION FROM THE CEREAL CROPS SECTION, NORTHERN UTILIZATION RESEARCH BRANCH[1]]

# Starch Formate

BY I. A. WOLFF, D. W. OLDS AND G. E. HILBERT

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Formylation of starch under the conditions investigated is a reversible reaction; the extent of substitution is dependent upon the ratio of formic acid to starch and on the water content of the system. Appreciation of this fact is required by workers who use formylation of cellulose for accessibility studies and refer to amylaceous materials as standards for comparison, assuming the latter to be 100% accessible. Maximum degree of substitution of starch achieved was 2.3 acyl groups per anhydroglucose unit.

As part of a program at this Laboratory on the preparation of starch esters,[2] formyl derivatives were desired.

Gottlieb, Moe, Nickerson and their co-workers[3–5] esterified starch, using 90% formic acid at room temperature and obtained esters having a degree of substitution approximating, but slightly greater than, one formyl group per anhydroglucose unit. Tarkow and Stamm,[6] using 99% formic acid at 55° also report formation of essentially a monoformate, but in their studies a relatively low ratio of formic acid to carbohydrate was used. All of these authors indicate either exclusive reaction of the formic acid with primary hydroxyl groups or at least preferential, rapid formylation of this grouping. Browning and Sell[7] state that their work "suggests formylation of secondary hydroxyl groups," though more slowly than primary hydroxyl groups. These authors conducted their experiments with 99.3% formic acid at 51° and demonstrated increased formylation of starch at higher formic acid to starch ratios. Their conclusions are based on analytical data alone. Reaction products were not isolated. The use of data on the formylation of cellulose as a measure of its accessibility, with reference to amylaceous materials as standards,[4–6] makes an understanding of the formylation of starch particularly pertinent.

In the studies reported here, starch was esterified at ambient temperature in excess formic acid containing different amounts of water. Formyl esters were isolated by alcohol precipitation at varying time intervals and analyzed for acyl content. Our results indicate that the formylation of starch under the conditions used is best depicted as a reversible reaction

$$\frac{R(OH)_3}{\text{Starch}} + n\text{HCOOH} \rightleftharpoons \frac{R(OCHO)_n(OH)_{3-n}}{\text{Starch formate}} + n\text{H}_2\text{O}$$

in which equilibrium is reached in about 8 hr. Fortuitous choice of reaction conditions was apparently responsible for isolation of a monoformate by many earlier workers.

Anhydrous formic acid reacts with starch to give a monoformate in only one-fourth of the time needed with the 90% acid (Fig. 1). The final product obtained with this more concentrated acid approximates a diformate. Use of anhydrous starch, of higher proportions of formic acid relative to the starch or reformylation of an isolated "diformate" all raise the degree of formylation still further. The equilibrium nature of the reaction was demonstrated as follows: Sufficient water was added to the reaction mixture of dry starch with

(1) One of the Branches of the Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

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