[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Hydrolysis Components from Methylated Corn Fiber Gum[1,2]

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A homogeneous, water-soluble hemicellulose from corn fiber is found by methylation analysis to be a highly branched structure. Hydrolysis of the fully methylated hemicellulose produces 2,3,4,6-tetra-*O*-methyl-D,L-galactopyranose, 2,3,5-tri-*O*-methyl-L-arabofuranose, 2,5-di-*O*-methyl-L-arabofuranose, 2,3-di-*O*-methyl-D-xylopyranose, 3-*O*-methyl-L-arabopyranose, 2-*O*-methyl-D-xylopyranose and D-xylopyranose in the molar ratio of 2:8:2:10:1:8:2.

A hemicellulose termed "corn fiber gum,"[3] prepared from coarse corn fiber by lime water extractions followed by neutralization and alcohol precipitation is obtainable on a semi-commercial scale. Corn fiber, the hull of the corn grain, is produced in large quantities by the corn wet milling industry as a by-product in processing of corn for starch, oil and protein. A similar hemicellulose was prepared earlier by extraction of corn fiber with sodium hydroxide and sodium carbonate solutions.[4]

Ethanol fractionation of the hemicellulose suggests that isolated corn fiber gum has a single main component, Fig. 1. The fractionation curve for this hemicellulose is identical with that of a hemicellulose fraction prepared from coarse corn fiber by standard laboratory methods[5] of treatment with chlorous acid followed by alkali extraction and alcohol precipitation. The possible homogeneous nature of corn fiber hemicellulose is further suggested by its electrophoretic pattern which shows but a single peak. Ethanol fractionation of hemicelluloses from other sources, particularly from corn cob, suggest that these hemicelluloses are heterogeneous.[6]

Corn fiber gum is an acidic hemicellulose, $[\alpha]^{25}\text{D}$ −81, (*c* 1.0, water) whose monosaccharide composition is 54% anhydroxylose, 33% anhydroarabinose and 11% anhydrogalactose as determined by quantitative paper chromatographic analysis of the hydrolyzate. Analysis also indicates that 3% anhydroglycuronic acid is present in the molecule.

On complete methylation, a methylated hemicellulose is obtained with a methoxyl content of 37.6%. Hydrolysis of the fully methylated hemicellulose yields 2,3,4,6-tetra-*O*-methyl-DL-galactopyranose (2 moles), 2,3,5-tri-*O*-methyl-L-arabofuranose (8 moles), 2,5-di-*O*-methyl-L-arabofuranose (2 moles), 2,3-di-*O*-methyl-D-xylopyranose (10 moles), 3-*O*-methyl-L-arabopyranose (1 mole), 2-*O*-methyl-D-xylopyranose (8 moles) and D-xylopyranose (2 moles). The tetramethylgalactose is present as an equimolar mixture of the D and L forms. Since all of the galactose is obtained as tetramethylgalactose, the DL forms must occur in the polysaccharide as non-reducing end units. Likewise 73% of the L-arabinose in the molecule occurs as non-reducing end units, as is shown by the presence of large amounts of trimethyl-L-arabinose. The presence of dimethylxylose, monomethylxylose and xylose suggests that the molecule has a branched xylan nucleus. Upon partial hydrolysis, the L-arabofuranose units are split off leaving this xylan nucleus.[7]

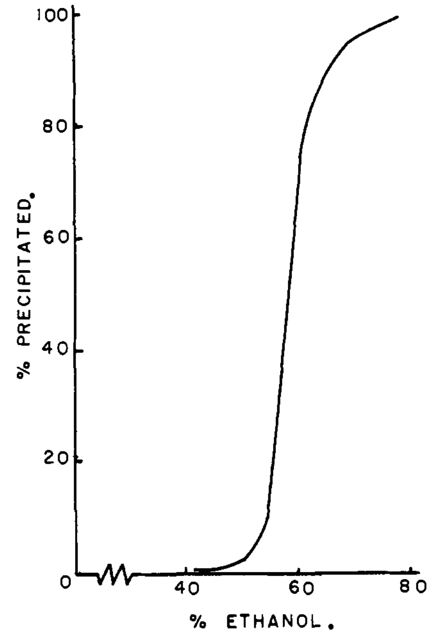

Fig. 1.—Ethanol fractionation of corn fiber gum.

Previous isolation of a trisaccharide, L-galactopyranosyl-(1 → 4)-D-xylopyranosyl- (1 → 2)-L-arabinose,[8] from the partially hydrolyzed corn fiber gum shows how the non-reducing galactose end units are linked to the nucleus. Information as to how part of the non-terminal L-arabinose units are linked to D-xylose units which are extraneous to the xylan nucleus is obtained by the isolation of a disaccharide, 3-*O*-α-D-xylopyranosyl-L-arabinose,[8] from the partial hydrolyzate.

Experimental

Hemicellulose.—The hemicellulose used was a product known commercially as "corn fiber gum."[3] This is an acidic hemicellulose which is obtained by lime water extractions of commercial corn fiber followed by neutralization and alcohol precipitation. This hemicellulose has an $[\alpha]^{25}\text{D}$ −81 (*c* 1.0, water).

Fractionation.—Fractionation of the hemicellulose was obtained by ethanol precipitation. A 2% solution was brought to a *p*H 2.0 with 0.5 *N* hydrochloric acid and the solution was equilibrated at 25° in a water-bath. This solution had an $[\alpha]^{25}\text{D}$ −80. Ethanol was then added in

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[1] Journal Paper No. 922 of the Purdue Agricultural Experiment Station.

[2] Paper presented before the Division of Carbohydrate Chemistry at the 128th Meeting of the American Chemical Society in Minneapolis, Minnesota, September, 1955.

[3] Corn Products Refining Company, Argo, Illinois.

[4] M. J. Wolf, M. M. MacMasters, John A. Cannon, E. C. Rosewall and C. E. Rist, *Cereal Chem.*, **30**, 451 (1953).

[5] Roy L. Whistler, J. Bachrach and D. R. Bowman, *Arch. Biochem.*, **19**, 25 (1948).

[6] Roy L. Whistler and G. E. Lauterbach, unpublished results.

[7] Roy L. Whistler and D. M. Corbett, unpublished results.

[8] Roy L. Whistler and D. M. Corbett, THIS JOURNAL, **77**, 6328 (1955).

successive portions and the precipitate removed by centrifugation following each addition. Figure 1 was obtained when the per cent. ethanol was plotted against the per cent. precipitate. This curve suggested the presence of a single main component which was soluble in 55% ethanol but insoluble in 60% ethanol.

A hemicellulose prepared in the laboratory from commercial corn fiber by treatment with chlorous acid followed by alkali extraction[5] showed the same solubility characteristics in aqueous ethanol. Films cast from the commercial hemicellulose were brittle indicating either a branched structure or low molecular weight.

Monosaccharide Composition.—The uronic acid composition as determined by the method of Whistler, Martin and Harris[9] showed the presence of 3% anhydroglycuronic acid units. The monosaccharide composition was determined by hydrolysis of a 1% solution of the hemicellulose in *N* sulfuric acid at 80° for 10 hr. At the end of the period the maximum reducing value was obtained. The hydrolyzate was neutralized with barium carbonate, filtered, concentrated, poured into five times its volume of ethanol and centrifuged. The centrifugate was concentrated and chromatographed. After the papers were developed with a solvent of ethyl acetate–pyridine–water (8:2:1 v./v.), the monosaccharides were eluted with water and the quantity of each was determined by the ferricyanide micro-method of Hagedorn and Jensen.[10] These values indicate the presence of 33% anhydroarabinose, 54% anhydroxylose and 11% anhydrogalactose units.

Methylation.—Twenty-five grams of hemicellulose was methylated in an atmosphere of nitrogen by dissolution in 100 ml. of water, addition of 185 ml. of oxygen-free 30% sodium hydroxide solution and subsequent alternate additions at 45-minute intervals of 95-ml. portions of 30% sodium hydroxide solution and 45-ml. portions of dimethyl sulfate until a total of 270 ml. of dimethyl sulfate had been added. The reaction flask was cooled in a water-bath at 25° until after the last addition, when the water-bath was removed and the reaction allowed to proceed at room temperature overnight. The solution was then neutralized with glacial acetic acid, dialyzed and concentrated for remethylation. The hemicellulose was methylated three times by this procedure with the exception that, during the second and third methylations, acetone was added to the solution in order to solubilize the partially methylated hemicellulose. Approximately 32 g. of partially methylated hemicellulose was recovered by chloroform extraction.

The dried material was then methylated twice by the Purdie method.[11] This reaction produced a methylated hemicellulose (34.0%, OCH_3).

Fractionation of the Methylated Hemicellulose.—The methylated hemicellulose was fractionated in chloroform–hexane (66–67°) solvent systems. Ninety per cent. of the methylated hemicellulose was soluble in a 30:70 mixture of chloroform–hexane (66–67°) but insoluble in a 20:80 mixture of the two solvents. This fractionation produced 32 g. of methylated hemicellulose with a methoxyl content of 37.6%.

Hydrolysis.—Ten grams of methylated hemicellulose in 250 ml. of boiling 5% hydrogen chloride in methanol underwent mutarotation which came to an equilibrium of $[\alpha]^{25}\text{D}$ +30.5 in 6 hr. The solution was neutralized by addition of silver carbonate, filtered and concentrated to a thick sirup. One hundred ml. of 0.5 *N* hydrochloric acid was added and the solution was heated for 3 hr. at 80°. The solution was neutralized by addition of silver carbonate, filtered and the residue washed with hot water. The excess silver ions were precipitated with hydrogen sulfide. The solution was then centrifuged and the uronic acids removed on Amberlite IR-4B anion-exchange resin.

Cellulose Column Chromatography.—The methylated sugars in the hydrolyzate were separated on a cellulose column (8.5 × 100 cm.). A butanol–ethanol–water (4:1:5 v./v., upper layer) solvent was used for resolution of the sugars. Four fractions were obtained from this column. However, fraction II contained four methylated sugars which were separated from each other by paper chromatography.

Paper Chromatography.—The methylated sugars in the hydrolyzate were separated on Whatman No. 1 filter paper by the descending method. The solvents used to develop the chromatograms were (A) methyl ethyl ketone–water azeotrope, b.p. 74–75°, and (B) 1-butanol–ethanol–water–concentrated ammonium hydroxide (40:10:49:1 v./v., upper layer). *p*-Anisidine hydrochloride[12] was used as a spray to reveal the positions of the sugars.

Fraction I.—A thin sirup (1.22 g.) which had R_g values of 0.98 in solvent A and 0.97 in solvent B was obtained first from the column. This sirup was identified as 2,3,5-tri-*O*-methyl-L-arabofuranose by oxidation with bromine water to the corresponding methylated arabonic acid which was lactonized and converted to the amide. Upon recrystallization from acetone, 90 mg. of the amide, m.p. 137°, was recovered; $[\alpha]^{25}\text{D}$ +22.0 (*c* 0.7, ethanol).

Anal. Calcd. for $C_8H_{17}O_5N$: N, 6.8. Found: N, 6.7.

Fraction IIA.—This sirup with R_g values of 0.85–0.88 in solvent A was obtained in a 63-mg. yield and had no optical rotation at 1% concentration in either water, chloroform, ethanol or benzene. The R_g value of the methylated sugar indicated a tetramethylgalactose and the optical rotation suggested a 50:50 DL mixture of tetramethylgalactoses. The carbohydrate component was identified as 2,3,4,6-tetra-*O*-methyl-DL-galactopyranose in an equimolar mixture of D and L forms by conversion to the anilide. Recrystallization from ethyl acetate produced 6 mg. of crystalline material, m.p. 179–181°, reported[13] 179–180°; no optical rotation in acetone.

Fraction IIB.—This sirup, $[\alpha]^{25}\text{D}$ −25.4 (*c* 0.79 water), was obtained in a 151 mg. yield and had R_g values of 0.76–0.80 in solvent A. It was identified as 2,5-di-*O*-methyl-L-arabofuranose by oxidation with bromine water to the corresponding methylated arabonic acid which was lactonized and converted to the amide, m.p. 132°.

Fraction IIC.—Forty-eight mg. of this methylated sugar was isolated as a sirup, $[\alpha]^{25}\text{D}$ +18.9 (*c* 0.22, water), which had an R_g value of 0.70 in solvent A. The sirup was identified as 2,3-di-*O*-methyl-D-xylopyranose by conversion to the anilide, m.p. 144–145°. This anilide was unstable and turned brown overnight.

Fraction IID.—This sirup, 144 mg., $[\alpha]^{25}\text{D}$ +96 (*c* 0.87, water), had an R_g value of 0.54 in solvent A. The sirup was identified as 3-*O*-methyl-L-arabopyranose by conversion to the anilide, m.p. 115–117°. The anilide was unstable and turned brown overnight.

Fraction III.—This sirup, 2.98 g., partially crystallized upon standing and had R_g values of 0.36 in solvent A and 0.54 in solvent B. Recrystallization from ethyl acetate containing 4% methanol produced 470 mg. of crystalline material, m.p. 137–138°, $[\alpha]^{25}\text{D}$ $-23 \rightarrow +35$ (*c* 3.0, water). The crystals, 430 mg., were converted to the anilide which identified the crystals as 2-*O*-methyl-D-xylopyranose. Recrystallization produced 204 mg. of the anilide, m.p. 125°. These crystals darkened after standing for several weeks.

Anal. Calcd. for $C_{12}H_{17}O_4N$: N, 5.9. Found: N, 6.2.

Fraction IV.—This sirup, 0.80 g., partially crystallized upon standing and had R_g values of 0.12 in solvent A and 0.26 in solvent B. Recrystallization from methanol produced 230 mg. of crystalline material, m.p. 145°. The crystals were identified as D-xylopyranose by conversion to the dibenzylidene dimethyl acetal of D-xylose.[14] The acetal was obtained in a 90-mg. yield, m.p. 211°, $[\alpha]^{25}\text{D}$ −6.70 (*c* 0.68, chloroform).

Anal. Calcd. for $C_{21}H_{24}O_6$: C, 67.7; H, 6.5. Found: C, 67.4; H, 6.8.

Table I

Methylated sugar	Molar ratio
2,3,5-Trimethylarabinose	8
2,3,4,6-Tetramethylgalactose	2
2,5-Dimethylarabinose	2
2,3-Dimethylxylose	10
3-Methylarabinose	1
2-Methylxylose	8
Xylose	2

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

[10] H C. Hagedorn and B. N. Jensen, Circular C 440 Natl. Bur. Standards, 198 (1940).

[11] T. Purdie and J. C. Irvine, *J. Chem. Soc.*, **83**, 1021 (1903).

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

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Quantitative Determination.—Fully methylated corn fiber gum, 100 mg., was heated at 100° in a sealed tube with 5 ml. of 5% hydrogen chloride in methanol for 12 hr. The solution was neutralized, filtered and the solvent removed to produce a brown sirup. This sirup was heated at 100° for 8 hr. in a sealed tube with 5 ml. of 0.5 N hydrochloric acid solution. The solution was neutralized with silver carbonate, filtered and the excess silver ions precipitated with hydrogen sulfide. The solution was concentrated to a sirup and chromatographed. Papers were developed with solvent A and the methylated sugars eluted with methanol. Quantities of the methylated sugars were determined by the alkaline hypoiodite method.[15] Amounts of each methylated sugar found in the hydrolyzate are given in Table I.

Acknowledgments.—The authors wish to thank K. W. Kirby who determined the uronic acid content of the polysaccharide and G. E. Lauterbach who made an electrophoretic pattern of the hemicellulose.

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Fluoranthene Derivatives. III. 2-Nitrofluoranthene and 2-Aminofluoranthene[1]

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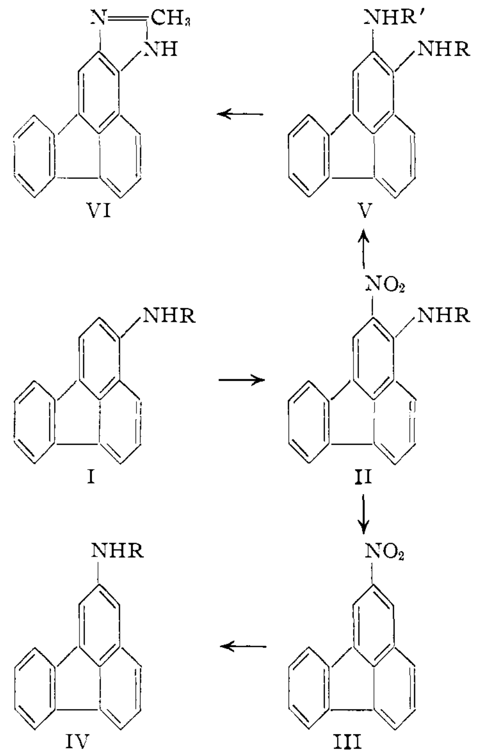
3-Acetylaminofluoranthene has been shown to undergo nitration in the 2-position. Several new 2,3-disubstituted fluoranthene derivatives have been prepared. 2-Nitrofluoranthene, 2-aminofluoranthene and several derivatives of each have been synthesized and their structures ascertained.

Since the elucidation of the structure of fluoranthene[2] numerous reports have described the preparation of 3- and 8-fluoranthyl derivatives by direct substitution of the hydrocarbon and derivatives with substituents in other positions by methods of nuclear synthesis.[3] Only two compounds have been reported, however, in which fluoranthene carries a single substituent in the 2-position. 2-Methylfluoranthene was prepared through nuclear synthesis[4] and an impure sample of 2-fluoranthenecarboxylic acid was described by Campbell and Wang.[5]

2-Nitrofluoranthene (III) has now been prepared in 39% over-all yield from commercially-available fluoranthene through a sequence involving initial nitration in the 3-position and reduction of the nitro group to the amino group. The directive influence of the latter was subsequently employed to promote nitration in the 2-position (compound II, R = $COCH_3$) and the amino group was finally eliminated by reduction of the corresponding diazonium salt. Catalytic reduction of 2-nitrofluoranthene yielded 2-aminofluoranthene (IV, R = H).



Nitration of fluoranthene was accomplished in a manner similar to that described by Garascia, Fries and Ching.[6] In addition to 3-nitrofluoranthene, we were able to isolate a small quantity of the 8-isomer from the reaction mixture. von Braun and Manz[7] indirectly demonstrated the formation of a little 8-nitrofluoranthene during nitration of fluoranthene but the compound has not been isolated heretofore. The identity of 8-nitrofluoranthene was confirmed by its conversion to the known 8-aminofluoranthene[7] and 8-acetylaminofluoranthene.[7-9]

3-Aminofluoranthene (I, R = H) formed solid salts in 96–98% yields when ether solutions of the amine were treated with hydrogen chloride, hydrogen bromide, 57% hydriodic acid or concentrated sulfuric acid. Reaction with ethyl chloroformate produced the urethan (I, R = $COOC_2H_5$). Cold acetic anhydride converted the amine to the amide (I, R = $COCH_3$) while hot acetic anhydride in the

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[1] Partially abstracted from the M.S. thesis of John H. Menkes. The numbering system for fluoranthene herein employed is that adopted by *Chemical Abstracts*.

[2] J. von Braun and E. Anton, *Ber.*, **62**, 145 (1929).

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