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

Structure of Corn Hull Hemicellulose. II. Identification of the α- and β-Forms of Methyl 2-*O*-[Methyl (2,3,4-Tri-*O*-acetyl-α-D-glucopyranosyl)-uronate]-3,4-di-*O*-acetyl-D-xylopyranoside[1,2]

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The methyl ester methyl glycoside pentaacetate (acetate I), m.p. 257°, derived from the aldobiouronic acid obtained by hydrolysis of the corn hull hemicellulose has been shown to be methyl 2-*O*-[methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl)-uronate]-3,4-di-*O*-acetyl-β-D-xylopyranoside (I). Methylation of I followed by reduction and remethylation gave methyl 2-*O*-(2,3,4,6-tetra-*O*-methyl-α-D-glucopyranosyl)-3,4-di-*O*-methyl-β-D-xylopyranoside (V) which upon hydrolysis afforded 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose. The biose linkage in both acetates I and II has been shown by enzymatic studies to be of the α-type.

Corn hull hemicellulose[3] was shown in Part I[4] to give on graded hydrolysis followed by treatment with methanolic hydrogen chloride and acetylation two methyl ester methyl aldobiouronoside pentaacetates (I, m.p. 257°, $[\alpha]_D$ +103° ($CHCl_3$); and II, m.p. 180°, $[\alpha]_D$ +163° ($CHCl_3$)). One of these acetates (II) was shown to be methyl 2-*O*-[methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl)-uronate]-3,4-di-*O*-acetyl-D-xylopyranoside, the α-biose linkage being indicated by a high positive rotation.

This paper is concerned with the elucidation of the structure of the acetate I which is now shown to be methyl 2-*O*-[methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl)-uronate]-3,4-di-*O*-acetyl-β-D-xylopyranoside by the following experimental evidence. Reduction of I with lithium aluminum hydride and concomitant deacetylation gave the methyl glycoside III of the corresponding neutral disaccharide which upon hydrolysis was found by paper chromatography to give glucose and xylose.

Methylation of I with Purdie reagents followed by reduction with lithium aluminum hydride[5,6] and remethylation gave methyl 2-*O*-(2,3,4,6-tetra-*O*-methyl-α-D-glucopyranosyl)-3,4-di-*O*-methyl-D-xylopyranoside (V) which afforded on hydrolysis the characteristic crystalline 2,3,4,6-tetra-*O*-methyl-D-glucose[7] and 3,4-di-*O*-methyl-D-xylose, the latter being identified as 3,4-di-*O*-methyl-D-xylono-δ-lactone.[8]

Since the two fully methylated disaccharide methyl glycosides, V and VI, obtained from the two corresponding acetates (I and II) by successive methylation, reduction and remethylation, were different and yet underwent hydrolysis to give the same methylated fragments, namely, 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose, it was apparent that I and II were isomers. It was not clear, however, whether the isomerization resided at C_1 of the xylose moiety or at the biose link. To resolve this problem, I and II were reduced with lithium aluminum hydride in tetrahydrofuran with simultaneous deacetylation in order to obtain the methyl glycosides of the corresponding neutral disaccharides III and IV. The latter were then treated with α- and with β-glucosidase.[9] While the two disaccharide methyl glycosides III and IV derived from I and II, respectively, were unaffected by β-glucosidase, they were cleaved by α-glucosidase to give D-glucose and β- and α-methyl D-xyloside, respectively, as shown by chromatographic analysis and glass paper electrophoresis.[10] The linkage between the glucuronic acid

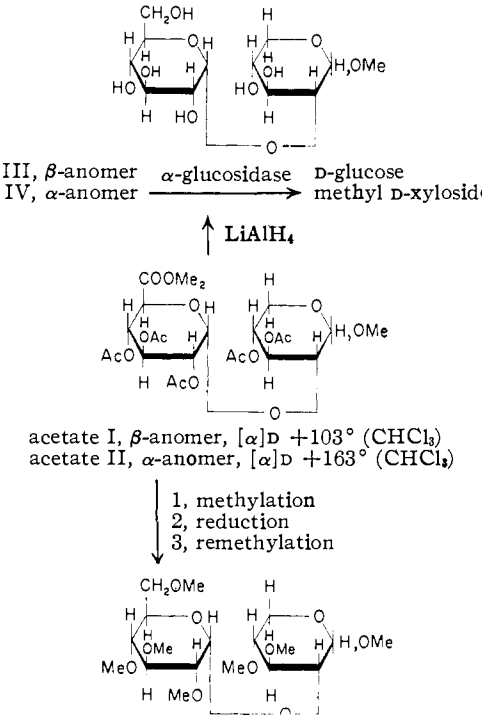

Fig. 1.

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(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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and the xylose moiety in I and II is therefore of the α type. It follows that the configuration of the glycosidic methoxyl group in I, on the basis of its lower rotation, is β whereas that of II is α. The acetate I is therefore designated as methyl 2-*O*-[methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl)-uronate]-3,4-di-*O*-acetyl-β-D-xylopyranoside and II is designated as methyl 2-*O*-[methyl (2,3,4,-tri-*O*-acetyl-α-D-glucopyranosyl)-uronate]-3,4-di-*O*-acetyl-α-D-xylopyranoside. These facts prove that the parent aldobiouronic acid is 2-*O*-α-D-glucopyranosyluronic acid-D-xylopyranose, a compound reported[11] to be present in a mixture of aldobiouronic acids isolated from corn cobs; the same aldobiouronic acid has also been obtained from chagual gum[12] and there is evidence for it in oat hull hemicellulose.[13,14]

Experimental

Methylation of Methyl 2-*O*[Methyl (2,3,4-Tri-*O*-acetyl-α-D-glucopyranosyl)-uronate]-3,4-di-*O*-acetyl-β-D-xylopyranoside (Acetate I).—The acetate I,[4] m.p. 257° (570 mg.), was suspended in cold ethanol (35 ml.) and *N* potassium hydroxide (5 ml.) added dropwise. The solution was allowed to attain room temperature gradually and then kept overnight. In order to dissolve all the substance, acetone (10 ml.) was added and the temperature raised to 50–60°. The reaction mixture was cooled to room temperature and allowed to stand for 8 hours. Acetone and ethanol were distilled off with simultaneous addition of water. The resulting aqueous solution was passed through Amberlite IR120 and the acidic effluent evaporated *in vacuo* to give a white glass. This was dissolved in dry methanol (5 ml.) and methylated with methyl iodide (10 ml.) and silver oxide (5 g.) in the usual manner. On filtration and evaporation of the filtrate, a pale yellow sirup was obtained which was remethylated by dissolving it in methanol (1 ml.) and treating it with methyl iodide (10 ml.) and silver oxide (3 g.). The product, now soluble in methyl iodide, was methylated a third time with Purdie reagents to give the methylated methyl aldobiouronoside methyl ester as a sirup (448 mg.).

Isolation of Methyl 2-*O*-(2,3,4,6-Tetra-*O*-methyl-α-D-glucopyranosyl)-3,4-di-*O*-methyl-β-D-xylopyranoside (V).—The methylated methyl aldobiouronoside methyl ester (448 mg.) obtained in the previous experiment was dissolved in dry ether (20 ml.) and the solution added dropwise to a suspension of lithium aluminum hydride (500 mg.) in ether (10 ml.). The mixture was refluxed for 0.5 hour and the excess of reagent destroyed by ethyl acetate. After acidification with glacial acetic acid, the reaction mixture was evaporated *in vacuo* and the product acetylated[15] with acetic anhydride (15 ml.) and fused sodium acetate (500 mg.) at 110–120° for 3 hours. The excess of acetic anhydride was removed by distillation and the residue acidified with *N* hydrochloric acid. The resulting aqueous solution was extracted with chloroform and the chloroform extract, after washing with water and drying (Na_2SO_4), was evaporated *in vacuo* to a light-colored viscous sirup (332 mg.) which showed $[\alpha]^{22}D$ +75° in methanol (*c* 3.3). This was saponified by dissolving it in methanol (20 ml.) and adding *N* potassium hydroxide (5 ml.) and heating the solution at 80–90° for 2 hours. The solution was then passed successively through Amberlite IR-120 and Duolite A4 resins to remove the cations and any unchanged aldobiouronic acid. The effluent was evaporated *in vacuo* to give a colorless viscous sirup (280 mg.). Three methylations of this sirup with Purdie reagents afforded methyl 2-*O*-(2,3,4,6-tetra-*O*-methyl-α-D-glucopyranosyl)-3,4-di-*O*-methyl-β-D-xylopyranoside (V) as a pale yellow mobile sirup (266 mg.), b.p. (bath temp.) 160–170° (0.005 mm.), $[\alpha]^{22}D$ +86° (*c* 3.7) in methanol.

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-β-D-xylopyranoside (V).—A solution of the methylated disaccharide (185 mg.) in *N* sulfuric acid (10 ml.) was heated for 20 hours at 100°. The solution was passed through Duolite A4 resin and evaporated *in vacuo* to a sirup. On chromatographing the sirup using benzene:ethanol:2% ammonium hydroxide (200:47:15)[16] as the irrigating solvent and *p*-anisidine trichloroacetate[17] as the spray reagent, three spots having these R_{TG} (tetra-*O*-methyl-D-glucose) values were obtained: 0.31, 0.99, 1.12. A reference sample of 3,4-di-*O*-methyl-D-xylose showed R_{TG} 0.31. The spot with R_{TG} 1.12 gave a strong pink color and was probably due to the reducing methylated disaccharide[18] since elution of it from the paper followed by hydrolysis gave spots corresponding to 2,3,4,6-tetra-*O*-methylglucose and 3,4-di-*O*-methylxylose. The hydrolyzate of the methylated disaccharide was therefore rehydrolyzed with *N* sulfuric acid (5 ml.) on the steam-bath for 10 hours and the material isolated as before. The hydrolyzate now gave only two spots corresponding to 2,3,4,6-tetra-*O*-methylglucose and 3,4-di-*O*-methylxylose on chromatographic analysis.

The mixture of 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose was resolved on sheets of Whatman No. 1 paper using methyl ethyl ketone:water[19] azeotrope as the irrigating solvent.

Identification of 2,3,4,6-Tetra-*O*-methyl-D-glucose.—The 2,3,4,6-tetra-*O*-methylglucose component obtained as a sirup (63 mg.) crystallized on nucleation and had m.p. and mixed m.p. 89–90°, $[\alpha]^{22}D$ +87° (*c* 0.3) in ethanol (after two recrystallizations from ether–petroleum ether).

Identification of 3,4-Di-*O*-methyl-D-xylose.—The 3,4-di-*O*-methylxylose component, isolated as a sirup (56 mg.) having $[\alpha]^{22}D$ +22° (*c* 0.2) in methanol, was oxidized with bromine for 5 days. The excess of bromine was removed by aeration and the solution neutralized (Ag_2CO_3). The solution, after filtration, was passed through Amberlite IR-120 and concentrated *in vacuo* to a sirup. Distillation of the latter, b.p. (bath temp.) 80–90° (0.01 mm.), gave a colorless sirup which crystallized on nucleation. The 3,4-di-*O*-methyl-D-xylono-δ-lactone thus obtained had m.p. and mixed m.p. 65–67° and $[\alpha]^{24}D$ −22° (*c* 0.3, equilibrium value) in water (after purification by sublimation).

Isolation of the α- and β-Forms of Methyl 2-*O*-(α-D-Glucopyranosyl)-D-xylopyranoside.—The acetate I (50 mg.) was suspended in dry tetrahydrofuran (5 ml.) and added gradually to a suspension of lithium aluminum hydride (100 mg.) in tetrahydrofuran (5 ml.). The mixture was refluxed for 3 hours, cooled, filtered and the filtrate after acidification with acetic acid evaporated *in vacuo* to give methyl 2-*O*-(α-D-glucopyranosyl)-β-D-xylopyranoside (III) as a colorless sirup (20 mg.). A portion of it was hydrolyzed with *N* sulfuric acid in a sealed tube for 6 hours and the reaction mixture, after neutralization ($BaCO_3$), was concentrated *in vacuo* to give a sirup which was found on chromatographic analysis to be composed only of glucose and xylose.

The acetate II, which was completely soluble in tetrahydrofuran, was reduced with lithium aluminum hydride in the same manner to give methyl 2-*O*-(α-D-glucopyranosyl)-α-D-xylopyranoside (IV).

Action of α- and β-Glucosidase on the α-(IV) and β-(III) Forms of Methyl 2-*O*-(α-D-Glucopyranosyl)-D-xylopyranoside.—The disaccharide methyl glycosides derived from acetates I and II as described above were each dissolved in sodium acetate buffer (*p*H 5.0) and incubated in m.p. tubes with β-glucosidase[9] for periods of 24 and 72 hours. Chromatographic analysis showed that the glycosides were unaffected. Control experiments showed under the same conditions cellobiose readily afforded glucose while maltose was unaffected.

When disaccharide methyl glycosides (III and IV) in aqueous solution were incubated with α-glucosidase, they were cleaved into D-glucose and β- and α-methyl D-xyloside, respectively, as indicated by paper chromatography using pyridine-ethyl acetate-water (1:2.5:3.5)[20] and by glass pa-

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per electrophoresis[10] (0.1 *M* borate buffer). Under the same conditions maltose readily gave glucose while cellobiose remained unchanged.

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[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Further Attempts to Prepare 3,4-Dimethylenethiophane and its Sulfone[1]

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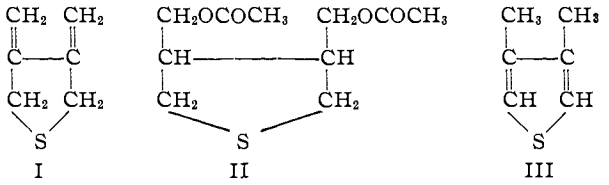
The Hofmann decomposition of the quaternary ammonium hydroxide of 3-methylene-4-dimethylaminomethylthiophane at room temperature and 5 mm. pressure has been found to give the rearranged product 3,4-dimethylthiophene in 21% yield and no 3,4-dimethylenethiophane. It has also been shown that the pyrolysis of 3,4-bis-(acetoxymethyl)-thiophane sulfone at 520 ± 5° does not lead to 3,4-dimethylenethiophane sulfone but causes decomposition with much charring to give poor yields of two products that have been tentatively identified as 3-methylene-4-acetoxymethylthiophane sulfone and 2-methyl-3-acetoxymethyl-1,3-butadiene.

A previous attempt to prepare 3,4-dimethylenethiophane (I) by the pyrolysis of 3,4-bis-(acetoxymethyl)-thiophane (II) yielded only the 3,4-dimethylthiophene (III) and none of the desired



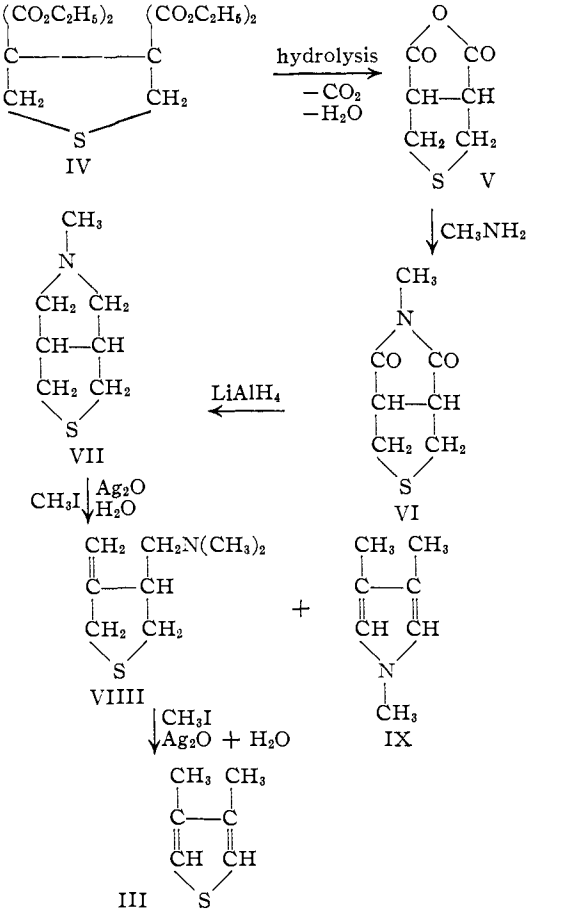
I II III

product.[3] It was thought that the rearrangement might have been caused by the high temperature encountered during the pyrolysis reaction and that the preparation of the dimethylenethiophane (I) might be accomplished if less vigorous conditions were used. It has now been shown that the Hofmann decomposition of the quaternary ammonium hydroxide of 3-methylene-4-dimethylaminomethylthiophane (VIII) at room temperature also gives the rearranged thiophene isomer III.

The steps involved in the preparation of the required quaternary ammonium hydroxide are outlined in the chart.

In the conversion of the double ring compound VII to the methiodide, some methylation of the sulfur must have occurred since in the decomposition of the quaternary hydroxide in addition to 51.5% yield of the sulfur ring compound VIII there was obtained a 23.5% yield of 1,3,4-trimethylpyrrole (IX). A third product was isolated which, while not completely characterized, appears to be a methyl mercaptan adduct of the olefinic derivative VIII.

The quaternary salt of the olefinic amine (VIII) was prepared, treated with silver oxide in water and allowed to decompose to the olefin at a temperature below 25°. The product, however, proved to be 3,4-dimethylthiophene (III). It is thus obvious that the rearrangement of the dimethylenethiophane to dimethylthiophene occurs with ease and that the higher temperature involved in the earlier pyrolysis of the diacetate was not necessarily the cause of the rearrangement.



Another approach to a five-membered sulfur ring compound with 3,4-dimethylene substitution was sought. 3,4-Diacetoxymethylthiophane (II) was prepared by modification and improvement of the method used before[3] and oxidized with 30% hydrogen peroxide in acetic anhydride to give the

(1) The work discussed herein was performed as a part of the synthetic rubber research project sponsored by the Federal Facilities Corporation and the National Science Foundation.

(2) This paper represents part of a thesis submitted by Robert M. Nowak to the Graduate School, University of Illinois, in partial fulfillment of the degree of Doctor of Philosophy, 1956.

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