Acknowledgment. This work was supported by a grant from the National Science Foundation (NSF-G581).

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Acid Resistant Portion of Corn Fiber Gum[1]

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Received February 1, 1956

Recently[2] the partial acid hydrolysis of corn fiber gum[3] was described and the structures of the two main oligosaccharides were determined. An investigation of the structure of a residual polysaccharide is here described. Complete acid hydrolysis of the residual polysaccharide produces xylose and traces of arabinose, galactose, and uronic acid material which indicates that the residual polysaccharide is essentially a xylan.

Hydrolysis of the fully methylated residual polysaccharide produces approximately 14 parts of 2,3,4-tri-O-methyl-D-xylose, 47 parts of 2,3-di-O-methyl-D-xylose, 19 parts of 2-O-methyl-D-xylose, 2 parts of D-xylose, and barium salts of methylated aldobiouronic or uronic acids.

Isolation and identification of methylated sugars from the hydrolysate of a methylated polysaccharide do not permit extensive deductions concerning the structure of the polysaccharide. Yet, in the present instance, the recovery of large amounts of 2-O-methyl-D-xylose and of some D-xylose suggests a highly branched chain. Since the initial corn fiber gum readily lost L-arabinose upon mild hydrolysis, it is presumed that L-arabofuranose units were attached to the branched chain to give an added degree of ramification. It has already been shown[2] that to some of the L-arabinose units are attached units of D-xylose and to others are attached units of D-xylose and L-galactose. No information is yet available on the location of the aldobiouronic or uronic acid units in the polysaccharide structure.

EXPERIMENTAL

Paper chromatography. Chromatographic separations were made on strips of Whatman No. 1 filter paper with one of the following commonly used irrigating solutions. (A) ethyl acetate-pyridine-water (10:4:3 v/v), (B) azeotropic mixture of methyl ethyl ketone and water, b.p. 74–75°, and (C) butanol-pyridine-water (6:4:3 v/v). The positions of the components were revealed by spraying the papers with *p*-anisidine hydrochloride.[4]

Preparation of acid-resistant residue. Corn fiber hemicellulose was hydrolyzed with *N* sulfuric acid as previously described.[2] The polysaccharide precipitated by the addition of alcohol to the neutralized hydrolysate had $[\alpha]_D^{25}$ −53.3° (*c*, 2.0 in water). Complete hydrolysis of the polysaccharide produced xylose and uronic acids together with trace amounts of arabinose and galactose as indicated by chromatographic comparison in solvent A.

Methylation of residue. A solution of 25 g. of residue in 100 ml. of water was placed in a three-neck flask and was flushed with nitrogen for 15 minutes. To the rapidly stirred solution was added 185 ml. of oxygen-free 30% sodium hydroxide solution followed by the alternate addition of six portions each of 95 ml. of 30% sodium hydroxide and 45 ml. of dimethyl sulfate. The reaction flask was cooled in a water-bath until after the last addition, and then the stirring was continued at room temperature for a further 18 hours. The solution was then neutralized with glacial acetic acid, dialyzed and concentrated for remethylation.

The polysaccharide was methylated three times by this method with the exception that during the second and third methylations, acetone was added to dissolve the partially methylated product. After the third methylation the precipitated methylated polysaccharide was freed from the neutralized solution and stirred with three 250-ml. portions of water. It was then dried by azeotropic distillation with alcohol and benzene to give 31.4 g. of a glassy product. The methylated xylan was refluxed for 18 hours with 150 ml. of methyl iodide and 20 g. of silver oxide, and then the solution was filtered from the silver oxide which was washed with hot chloroform. The combined filtrates were concentrated to a glass which was remethylated in a similar manner to give a methylated xylan having $[\alpha]_D^{25}$ −52.1° (*c*, 1.0 in chloroform); methoxyl content 36.2%. No increase in methoxyl content occurred in the final methylation treatment.

Hydrolysis of methylated residue and isolation of the components. A sample of 3.225 g. of the methylated xylan was refluxed with 60 ml. of 2% methanolic hydrogen chloride for 18 hours at which time the optical rotation was constant. The solution was neutralized with silver carbonate, filtered, and concentrated to 3.294 g. of sirup. The sirup was dissolved in 100 ml. of *N* sulfuric acid and refluxed for 3 hours when constant rotation was reached. After neutralization of the acid with barium carbonate, the filtered solution was concentrated to 2.760 g. of sirup. A sample of 0.896 g. of the sirup was separated into its components on 24 sheets of 6 × 20-inch paper, using solvent B as the irrigant. The two leading components were extracted from the paper with hot acetone, the next component with hot alcohol, and the remaining two components with water at room temperature.

The leading component was 0.099 g. of clear sirup, $[\alpha]_D^{25}$ +15.2° (*c*, 1.0 in water). In solvent B it was chromatographically identical to 2,3,4-tri-O-methyl-D-xylose. The sirup crystallized on seeding with the trimethyl ether to give crystals m.p. 83°. After recrystallization from ether-light petroleum ether the crystals had m.p. 89–90°, undepressed on admixture with an authentic sample.

The second and main component of 0.307 g. of clear sirup, $[\alpha]_D^{25}$ +21.0° (*c*, 1.0 in water) was chromatographically identical to 2,3-di-O-methyl-D-xylose in solvent B. On refluxing 0.100 g. of the sirup with 0.2 ml. of aniline in 5 ml. of methanol, 2,3-di-O-methyl-*N*-phenyl-D-xylosylamine was obtained, m.p. 124–125°, undepressed on admixture with an authentic sample.

The third component of 0.115 g. of sirup crystallized on seeding with 2-O-methyl-D-xylose. After recrystallization

(1) Journal Paper No. *925* of the Purdue Agricultural Experiment Station, Lafayette, Indiana. The corn fiber gum was kindly supplied by the Corn Products Refining Company.

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

from ethyl acetate-ethanol, the crystals had m.p. and mixed m.p. 135–137°.

The fourth component of 0.042 g. of sirup was a mixture of D-xylose and the barium salts of methylated uronic acids. An aqueous solution of the sirup was stirred with a mixture of Amberlite IR-120 (H) and IR-4B (OH) resins, filtered, and concentrated to 0.011 g. of sirup. Its chromatographic behavior in solvents (A) and (C) was identical to that shown by D-xylose.

The fifth component consisted of 0.213 g. of amorphous barium salts of methylated uronic acids, as indicated by barium analysis and chromatography of the free acids. The small yield and mixed nature made it undesirable to identify individual constituents, some of which were possibly aldobiouronic acids.

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Potential Purine Antagonists. III. Synthesis of Some 2-Methyl-6-substituted Purines[1]

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Received February 6, 1956

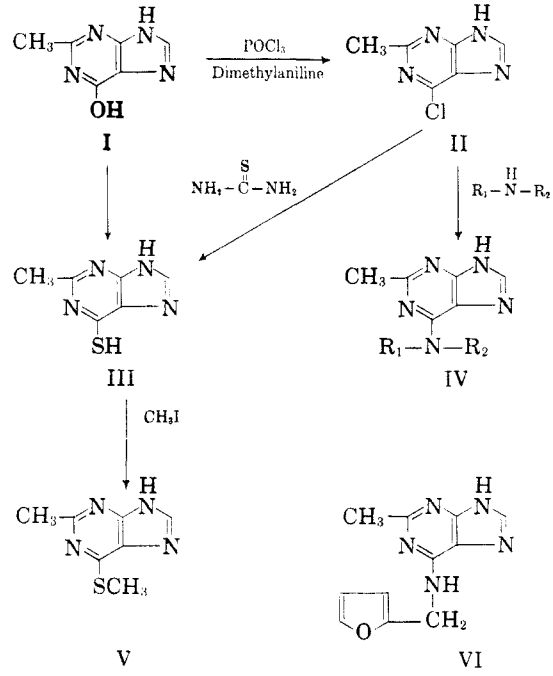
The recent discovery[2,3] of the natural occurrence of 2-methyl-6-aminopurine and 2-methyl-6-hydroxypurine in pseudovitamin B_{12} has prompted the investigation of the synthesis of several new 2-methyl-6-substituted purines as possible purine antagonists in biological systems. The starting material for these derivatives was 2-methyl-6-hydroxypurine (I) which was prepared by the formamide cyclization of the sulfate salt of 2-methyl-4,5-diamino - 6 - hydroxypyrimidine according to the method of Robins, *et al.*[4]

The chlorination of I was accomplished with phosphorus oxychloride and dimethylaniline to give 2-methyl-6-chloropurine (II) in a similar manner to that employed for the conversion of hypoxanthine to 6-chloropurine.[5]

It was discovered that treatment of II with various primary or secondary amines in alcoholic or aqueous solution heated on the steam-bath resulted in the preparation of the 2-methyl-6-substituted-aminopurines (IV) listed in Table I. Of this group 2-methyl-6-furfurylaminopurine (VI) is of particular interest since it can be considered a structural homolog of "Kinetin," 6-furfurylaminopurine, a recently isolated growth factor.[6]

As with 6-chloropurine,[5] treatment of 2-methyl-6-chloropurine (II) with thiourea in boiling ethanol resulted in replacement of the chlorine atom by a mercapto group to give 2-methyl-6-mercaptopurine (III). Thiation of 2-methyl-6-hydroxypurine with phosphorus pentasulfide in tetralin also yielded III in good yield. This preparation is similar to that employed by Elion for 6-mercaptopurine.[7] Methylation of 2-methyl-6-mercaptopurine (III) with methyl iodide gave 2-methyl-6-methylmercaptopurine (V).

The ultraviolet absorption maxima of several of these purine derivatives are listed in Table II. As



REACTION SCHEME

might be expected the ultraviolet absorption spectra of the 2-methyl-6-substituted purines resemble rather closely the spectra of the corresponding 6-substituted purines;[5,7] however, there appears to be a very slight general bathochromic shift of the absorption maxima in acid solution due to the 2-methyl group.

EXPERIMENTAL[8]

Preparation of 2-methyl-6-chloropurine (II). Dimethylaniline (mono free), 21 ml., was added to a suspension of 7.0 g. of 2-methyl-6-hydroxypurine[4] in 200 ml. of phosphorus oxychloride. The solution was refluxed 30 minutes and the excess phosphorus oxychloride was distilled off under reduced pressure using a steam-bath as a source of heat. The syrupy residue was poured onto ice and the aqueous solution cautiously was made basic with concentrated sodium hydroxide and extracted with ether to remove

(1) This investigation was supported in part by research grant C-2845 from the National Cancer Institute, of The National Institutes of Health, Public Health Service.

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(5) Bendich, Russell, and Fox, *J. Am. Chem. Soc.*, **76**, 6073 (1954).

(6) Miller, Skoog, Von Saltza, and Strong, *J. Am. Chem. Soc.*, **77**, 1392 (1955); *J. Am. Chem. Soc.*, **77**, 2662 (1955).

(7) Elion, Burgi, and Hitchings, *J. Am. Chem. Soc.*, **74**, 411 (1952).

(8) Melting points taken on a Fisher-John's block and are uncorrected.