[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF BRITISH COLUMBIA]

The Occurrence of 2-*O*-(4-*O*-Methyl-D-glucopyranosiduronic Acid)-D-xylose in Jute Hemicellulose[1]

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The uronic acid in jute hemicellulose has been shown to be 4-*O*-methyl-D-glucuronic acid and not 3-*O*-methyl-D-glucuronic acid as originally thought.

Since the initial discovery of 4-*O*-methyl-D-glucuronic acid in mesquite gum[2,3] this acid has been isolated from many natural sources, notably gums and hemicelluloses.[4,5] It was therefore of particular interest to note that Das Gupta and Sarkar claimed to have isolated 3-*O*-methyl-D-glucuronic acid from jute.[6] Their claim was based on periodate studies on the hemicellulose and the chromatographic identification of a monomethylglucose, obtained from the acid by lithium aluminum hydride reduction, as 3-*O*-methyl-D-glucose. In view of the lack of crystalline derivatives and the well known difficulty of unequivocally identifying any monomethylglucose by paper chromatography, we have reinvestigated the constitution of the aldobiouronic acid obtained from jute hemicellulose.

Jute sacking was ground in a Wiley mill, solvent extracted, and delignified by treatment with sodium chlorite.[7]

Alkaline extraction of the holocellulose gave a brown solution from which the hemicellulose could be isolated by pouring into two volumes of ethanol acidified with acetic acid. The crude product could be purified *via* its copper complex or by washing with alcohol containing hydrochloric acid. The latter method gave the purer product. Direct alkaline extraction of the jute gave approximately a 5% yield of hemicellulose, whereas extraction after delignification afforded a 16% yield.

Jute hemicellulose was hydrolyzed with sulfuric acid and the acidic components and the neutral sugars separated by ion exchange resins.[8]

Paper chromatographic examination of the acidic fraction in solvent A (see Experimental) showed it to contain mainly aldobiouronic acid together with some free uronic acid and higher oligosaccharides and this was confirmed analytically. This fraction was used for the subsequent steps, but a small sample of chromatographically pure aldobiouronic acid was obtained by separation on Whatman 3MM paper using solvent A and this sample was found to have $[\alpha]^{20}$D + 146.5° (*c* 0.75 in H_2O).

A portion of the acid fraction was cleaved with 10% methanolic hydrogen chloride and the uronic acid moiety identified as methyl 4-*O*-methyl-α-D-glucopyranosiduronamide.[9]

The major portion of the acidic fraction was converted to the neutral disaccharide by lithium aluminum hydride reduction.[9] The two sugars obtained by hydrolysis of the disaccharide were separated on paper using solvent A. The slower moving component was shown to be D-xylose by obtaining the crystalline sugar and the crystalline dibenzylidene dimethyl acetal.[10] The faster component was shown to be a glucose derivative by demethylation,[11] and was characterized as 4-*O*-methyl-D-glucose by the preparation of the crystalline osazone[3] and the crystalline *N*-phenylglucosylamine.[12]

In an attempt to find other derivatives which would enable a simple comparison of 3- and 4-*O*-methyl-D-glucoses to be made, the dibenzyl mercaptals were prepared. That of the former sugar was obtained crystalline only with difficulty and had m.p. *ca.* 66–69°, whereas the latter derivative melted sharply at 158–159° and was obtained in good yield. The latter compound has been reported previously, made by an indirect method and was doubtless impure (m.p. 73° and 96–98°).[13,14]

The point of attachment of the uronic acid to the D-xylose was determined by hydrolysis of the fully methylated neutral disaccharide. There were obtained 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose. The former was characterized mainly by chromatography and also as the *N*-phenylglucosylamine derivative,[15] although this was only obtained in a relatively impure state due to contamination with a small amount of a second fast moving compound, tri-*O*-methyl-L-rhamnose(?). The xylose component was characterized as the crystalline 3,4-di-*O*-methyl-D-xylono-δ-lactone.[16]

This evidence shows conclusively that the uronic acid is 4-*O*-methyl-D-glucuronic acid and that the aldobiouronic acid is 2-*O*-(4-*O*-methyl-D-glucopyranosiduronic acid) -D-xylose. The work of Gorin and Perlin[17] confirms that the linkage is of the α-type. These results further substantiate the generalization tha 4-*O*-methyl D-glucuronic acid is usually joined to position 2 of D-xylose.[18]

As this work was being completed there appeared a preliminary communication by Srivastava and

(1) This work is abstracted from a thesis submitted by I. H. Rogers for the M.Sc. degree, November, 1958, and was supported by the National Research Council of Canada to whom we express our thanks.

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(6) P. C. Das Gupta and P. B. Sarkar, *Textile Research J.*, **24**, 705, 1071 (1954).

(7) L. E. Wise, M. Murphy and A. A. D'Addieco, *Paper Trade J.*, **122** [2], 35 (1946).

(8) G. G. S. Dutton and K. Hunt, THIS JOURNAL, **80**, 4420 (1958).

(9) M. Abdel-Akher and F. Smith, *Nature*, **166**, 1037 (1950).

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(13) R. Schinle, *Ber.*, **65**, 315 (1932).

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(15) J. C. Irvine and A. M. Moodie, *ibid.*, **93**, 95 (1908).

(16) S. P. James and F. Smith, *ibid.*, 739 (1945).

(17) P. A. J. Gorin and A. S. Perlin, *Can. J. Chem.*, **36**, 999 (1958).

(18) G. A. Adams and C. T. Bishop, THIS JOURNAL, **78**, 2842 (1956).

Adams[19] reporting the identification o 2-*O*-(4-*O*-methyl-D-glucopyranosiduronic acid)-D-xylose in jute hemicellulose and the isolation of a crystalline trisaccharide. We are grateful to Dr. G. A. Adams for his kindly agreeing to our joint publication.

Experimental

All evaporations were carried out *in vacuo* at a bath temperature not exceeding 40°. All optical rotations were recorded at 20 ± 2° using sodium light. Unless otherwise stated all chromatography was carried out on Whatman No. 1 paper: solvent A, ethyl acetate–glacial acetic acid–45% formic acid–water (18:3:1:4); solvent B, 1-butanol–ethanol–water–concd. ammonia (40:10:49:1); solvent C, butanone–water–azeotrope; solvent D, 1-butanol–benzene–pyridine–water (5:1:3:3); solvent E, 1-butanol–glacial acetic acid–water (4:1:5); all mixtures in parts by volume.

Jute Hemicellulose.—Jute sacking from the Beamis Bag Co., Vancouver, was reduced to a powder in a Wiley mill and extracted with benzene–ethanol (1:1). The dried fiber was delignified in 100 g. batches by one treatment with sodium chlorite and acetic acid and the average yield of white holocellulose was 90%. The holocellulose (*ca.* 90 g.) was extracted overnight with aqueous sodium hydroxide (1 l., 9.3%), filtered and the residue re-extracted with alkali (500 ml.) for several hours. The combined extracts were acidified with acetic acid and the hemicellulose isolated by the addition of two volumes of ethanol. The crude hemicellulose (*ca.* 16 g. from 100 g. of jute) was purified *via* its copper complex and yielded hemicellulose I (3.2 g. from 5 g. of crude) having ash 2.3% [α] −45.1° (*c* 0.15 in 2 *N* NaOH), neut. equiv. 953 equivalent to 21.7% anhydrouronic acid.

A second batch of crude hemicellulose (5 g.) was suspended in ethanol (150 ml.) containing concentrated hydrochloric acid (5 ml.) and centrifuged after standing overnight. Chloride ion was removed by washing with ethanol and, after drying by solvent exchange with ether and petroleum ether as before, hemicellulose II was obtained, yield 3.5 g., having ash 0.3% [α] −47.1° (*c* 0.5 in 2 *N* NaOH), neut. equiv. 1005 equivalent to 20.6% anhydrouronic acid, OMe 3.2% equivalent to an equiv. wt. of 975 or 21.3% anhydrouronic acid. This method of purification was used for all subsequent batches.

An attempt to prepare a pure sample of hemicellulose by electrodialysis failed because alcohol precipitated the polysaccharide as a gel from which the last traces of water could not be removed by solvent exchange.

Direct alkaline extraction of the jute without delignification gave a 5% yield of hemicellulose having [α] −47.0° (*c* 1.3 in 2 *N* NaOH).

Hydrolysis of Hemicellulose.—Preliminary experiments showed that hydrolysis with sulfuric acid (1 *N*) yielded four main spots having R_{xylose} (R_x) values of 1.44, 1.00, 0.69 and 0.30 corresponding to uronic acid, xylose, aldobiouronic acid and higher oligosaccharides, respectively, when chromatographed on paper in solvent A.

Hydrolysis on the steam-bath with formic acid (45%) for 12 hours yielded similar results but with an increased amount of the component having R_x = 0.30 − 0.35. Further hydrolysis with sulfuric acid (1 *N*) removed this component with a corresponding increase in the concentration of the other components.

Hemicellulose (15 g.) was hydrolyzed on the steam-bath with sulfuric acid (400 ml., 1 *N*) and a constant rotation of +57° was reached after 7 hours. The acidic and neutral components were separated on ion exchange resins as previously described.[8] The neutral fraction was obtained as a pale yellow syrup (9.98 g.) which crystallized spontaneously. The solid was recrystallized twice from methanol to give D-xylose, m.p. and mixed m.p. 143–145° and [α] + 17.0° (equil., *c* 1 in H_2O). It was further identified as the dibenzylidene dimethyl acetal derivative, m.p. and mixed m.p. 211°.[10] When a solution of the crude neutral sirup was heavily streaked on Whatman 3MM paper and irrigated with solvent A there was obtained a trace of a second component. When this was extracted with 80% aqueous methanol and chromatographically examined in solvents A, C and D it appeared to be identical with a sample of L-rhamnose.

The acidic fraction (3.6 g.) was a pale yellow glass having [α] +124.4° (*c* 0.5 in H_2O); OMe 8.1%, neut. equiv. 314; calcd. for $C_{12}H_{20}O_{11}$ OMe 9.1% and neut. equiv. 340. It was shown chromatographically to be mainly aldobiouronic acid contaminated with uronic and tri-(?)-uronic acid. In one instance a sample of the acidic fraction (4.65 g.) was heavily streaked on Whatman 3MM paper (approximately 8 mg. per cm.) and irrigated in solvent A. By extraction of the appropriate zone (R_x = 0.7) with 80% aqueous acetone there was obtained a chromatographically pure sample of the aldobiouronic acid (0.95 g.) having [α] +146.5° (*c* 0.75 in H_2O). In a similar way a chromatographically pure sample of the uronic acid (R_x = 1.44) had [α] +55.0° (*c* 1.25 in H_2O).

Identification of 4-*O*-Methyl-D-glucuronic Acid.—A portion of the acid fraction was refluxed overnight with methanolic hydrogen chloride (10%). The sirup obtained after neutralization ($PbCO_3$) was treated at 5° for two days with saturated methanolic ammonia. Evaporation yielded a crude solid product (m.p. 204–213°) which, after four recrystallizations from aqueous ethanol, had m.p. 234.5–236° unchanged on mixing with methyl 4-*O*-methyl-α-D-glucopyranosiduronamide.[3]

Methyl 2-*O*-(4-*O*-Methyl-α-D-glucopyranosyl)-D-xylopyranoside.—A portion (2.21 g.) of the acidic fraction was refluxed with 3% methanolic hydrogen chloride and a constant rotation of [α] +112° was attained after 5 hours. Neutralization ($PbCO_3$) and evaporation yielded 2.70 g. of glycoside methyl ester which was reduced with lithium aluminum hydride (2.8 g.) in dry tetrahydrofuran (120 ml.). The reduced disaccharide was isolated as the acetate (3.68 g.) and deacetylation[5] gave the methyl glycoside of the disaccharide (2.09 g.) as a viscous sirup having [α] +98.5° (*c* 0.75 in H_2O) and OMe, 19.7%; calcd. for $C_{13}H_{24}O_{10}$, OMe, 18.2%.

Hydrolysis of Disaccharide.—The disaccharide glycoside (850 mg.) was hydrolyzed with sulfuric acid (50 ml., 1 *N*) on a steam-bath and a constant rotation of [α] +44.5° was reached in 14 hours. Neutralization ($BaCO_3$) and evaporation yielded a sirup (820 mg.) which was examined chromatographically in solvents A, B, C, D and E. The best separation of the two components was obtained in solvent A, R_x = 0.98 and 1.24 equivalent to xylose and 3- or 4-*O*-methyl-D-glucose. It was not possible to distinguish unequivocally between 3- and 4-*O*-methyl-D-glucose in any of these five solvents. Attempted separation on a cellulose column with solvent A gave only a partial separation, but chromatographically pure fractions could be obtained in 16 hours by carrying out the separation on prewashed Whatman 3MM paper using solvent A.

Identification of D-Xylose.—The component with R_x 0.98–1.00 was extracted with 80% aqueous methanol and crystallized spontaneously two days after removing the solvent. The aqueous solution had [α] + 22.9° (equil.) and the dibenzylidene dimethyl acetal, after recrystallization from benzene-petroleum ether, had m.p. 211° unchanged when mixed with a sample prepared from the authentic D-xylose.[10]

Identification of 4-*O*-Methyl-D-glucose.—A portion (20 mg.) of the component having R_x 1.24 was demethylated with 48% hydrobromic acid.[11] Chromatographic examination in solvent D, after removal of the acid by Duolite A-4 resin, showed the presence of glucose and unreacted starting material.

A second portion of the monomethyl glucose (55 mg.) was dissolved in water (1.8 ml.) containing acetic acid (1.2 ml., 20%), freshly distilled phenylhydrazine (0.18 ml.) and sodium bisulfite (60 mg.). After two hours heating at 70–80° the osazone separated as small yellow crystals which were recrystallized from aqueous ethanol and had m.p. 159–160°.[3] The osazones of authentic samples of 3- and 4-*O*-methyl-D-glucose were prepared similarly. The latter had m.p. 158.5–159.5°, but the former did not give a sharp melting point even after several recrystallizations from aqueous ethanol. The best value obtained was m.p. 168–172° while the literature reports 178° and 185°.[20] A mixed melting point of the osazone from the jute monomethylglucose with authentic 3-*O*-methyl-D-glucosazone was 146–164° and with authentic 4-*O*-methyl-D-glucosazone was 158.5–159.5°. As additional confirmation the *N*-phenyl-4-*O*-methyl-D-glucosylamine was prepared and had m.p. 158–160° when

(19) H. C. Srivastava and G. A. Adams, *Chemistry & Industry*, 920 (1958).

(20) E. J. Bourne and S. Peat in "Advances in Carbohydrate Chemistry," Academic Press, Inc., New York, N. Y., Vol. V, 1950, p. 155.

recrystallized from ethyl acetate. A sample prepared from authentic 4-*O*-methyl-D-glucose had m.p. 156–158° and a mixed melting point of 156–159°.

In an attempt to make other derivatives of 3- and 4-*O*-methyl-D-glucose for comparison, the dibenzyl mercaptals were made by the usual procedure from authentic specimens. That from 3-*O*-methyl-D-glucose was recrystallized with difficulty from ethanol containing a few drops of water and had m.p. 66–69°. The mercaptal from 4-*O*-methyl-D-glucose readily crystallized from absolute ethanol, and had m.p. 158–159°.

Methyl 2-*O*-(2,3,4,6-Tetra-*O*-methyl-α-D-glucopyranosyl)-3,4-di-*O*-methyl-D-xylopyranoside.—To a portion (864 mg.) of the neutral disaccharide methyl glycoside was added sodium hydroxide (6 ml., 40%) and dimethyl sulfate (0.5 ml.). The mixture was stirred at high speed at a temperature of 45–55° and 0.5-ml. portions of dimethyl sulfate added every 15 minutes for 1.5 hours. This treatment was repeated twice and after 4–5 hours the bath temperature was raised to 80° for 0.5 hours. This cycle was repeated twice and finally after neutralization to *p*H 8.9 with sulfuric acid the product was isolated by continuous extraction with chloroform overnight. The partly methylated sirup (675 mg.), which showed only weak infrared absorption in the hydroxyl region, was dissolved in methyl iodide (20 ml.) and Drierite (*ca.* 1 g.) added. Silver oxide (5 g.) was added in small portions over 9 hours to the refluxing and stirred solution. After three such methylations the infrared spectrum showed no hydroxyl absorption. The distilled methylated sugar (517 mg.) had b.p. 170–180° (bath temp.) at 0.01 mm. and OMe, 55.7%; calcd. $C_{18}H_{34}O_{10}$; OMe, 52.9%. We are indebted to Mr. Y. Tanaka for the infrared spectra.

Hydrolysis of Methylated Disaccharide.—The methylated disaccharide (437 mg.) was dissolved in sulfuric acid (25 ml., 1 *N*) and hydrolyzed on the steam-bath for 11 hours, [α] 84.8°, constant. The sirup (396 mg.) obtained after neutralization ($BaCO_3$) showed two components of R_f 0.68 and 0.83 in solvent A. The mixture was separated on prewashed Whatman 3MM paper using the same solvent. The faster component was extracted with ether and the slower one with ethanol.

Identification of 2,3,4,6-Tetra-*O*-methyl-D-glucose.—Careful chromatographic examination of the fraction having R_f 0.83 showed the presence of a small amount of a faster component (tri-*O*-methyl-L-rhamnose ?) and because of this it was not possible to obtain the tetra-*O*-methyl-D-glucose crystalline. A small amount of *N*-phenyl-2,3,4,6-tetra-*O*-methyl-D-glucosylamine was obtained with m.p. 122–132° and when mixed with an authentic sample 130–136°.

Identification of 3,4-Di-*O*-methyl-D-xylose.—The component with R_f 0.68 was chromatographically pure and was oxidized with bromine in the ordinary way for 48 hours. The derived 3,4-di-*O*-methyl-D-xylono-δ-lactone distilled at 140–160° (bath temp., 0.01 mm.), crystallized spontaneously and had m.p. and mixed m.p. 64–66°.

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The Specific Polysaccharide of Type VI Pneumococcus. I. Preparation, Properties and Reactions[1]

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Purified specific polysaccharide of type VI pneumococcus, S VI, [α]D +129°, contains D-galactose, D-glucose, L-rhamnose, a polyol definitely identified as ribitol and phosphorus in approximately equimolar amounts. The P occurs as a labile diester, and alkaline hydrolysis yields a stable, serologically inactive monoester, [α]D +106°. Periodate oxidation of S VI produces formaldehyde but no formic acid; the galactose is attacked, but the glucose and rhamnose are resistant. After alkaline hydrolysis of S VI, glucose and rhamnose are still attached to the phosphorus-containing portion in periodate-resistant linkages in the original ratio, but now, besides formaldehyde, formic acid is liberated, apparently from the galactose.

S VI seems to be a linear polymer of the unit —[—4 or 2-O-D-galactopyranosyl-(1 → 3)-O-D-glucopyranosyl-(1 → 3)-O-L-rhamnopyranosyl(1 → 1 or 3)-ribitol-3 or 1-O-P(=O)(OH)-O—]$_x$—.

Pneumococci of the serological Types II, V and VI form a triad, of which Types V and VI were originally considered sub-types of II.[2] Because the serological relationships reflect differences and similarities in the capsular specific polysaccharides which, among pneumococci, are the principal determinants of immunological type specificity,[3] a study of the fine structures of these three carbohydrates[4] is considered likely to clarify the as yet obscure relation of chemical constitution to the immunological specificities and cross reactivities involved and to serve as a model for similar investigations. The chemistry of S II and S V is being unravelled by Prof. Maurice Stacey and Dr. S. A. Barker, respectively, of the University of Birmingham, England, and some progress with S II has already been recorded.[5] The present paper is concerned with initial stages of the chemical study of S VI.

Experimental

Materials and Methods.—S VI, isolated by the phenol process,[6] was generously supplied by E. R. Squibb and Sons, through the kindness of Mr. T. D. Gerlough. Type VI anti-pneumococcal horse serum was furnished by the Division of Laboratories, New York State Department of Health. Thanks are also due to Dr. N. K. Richtmyer for many of the samples of polyols and to Dr. F. Smith for the sample of L-threitol.

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(3) M. Heidelberger and O. T. Avery, *ibid.*, **38**, 73 (1923); **40**, 301 (1924); O. T. Avery and M. Heidelberger, *ibid.*, **38**, 81 (1923); **42**, 367 (1925); M. Heidelberger, W. F. Goebel and O. T. Avery, *ibid.*, **42**, 727 (1925).

(4) Hereinafter referred to as S II, S V and S VI.

(5) K. Butler and M. Stacey, *J. Chem. Soc.*, 1537 (1955).

(6) T. D. Gerlough, U. S. Pat. 2,340,318 (Feb. 9, 1944); J. W. Palmer and T. D. Gerlough, *Science*, **92**, 155 (1940).