[CONTRIBUTION FROM THE DIVISION OF INDUSTRIAL AND CELLULOSE CHEMISTRY, MCGILL UNIVERSITY, AND THE WOOD CHEMISTRY DIVISION, PULP AND PAPER RESEARCH INSTITUTE OF CANADA]

The Polysaccharides of White Birch (*Betula papyrifera*). IV. The Constitution of the Hemicellulose[1]

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Hydrolysis of a fully methylated hemicellulose from white birch (*Betula papyrifera*) has yielded a mixture of 2-*O*- and 3-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, 2,3,4-tri-*O*-methyl-D-xylose and 2-*O*-(2,3,4-tri-*O*-methyl-D-glucopyranosyluronic acid)-3-*O*-methyl-D-xylose in a molar ratio of 3:116:1:11. The degree of polymerization of the native and the methylated xylan was 190 and 110, respectively, as determined by osmometry. On the basis of these and previous results it is suggested that white birch hemicellulose is a linear polysaccharide containing a minimum of 110 and a maximum of 190 β-D-xylopyranose residues linked together by 1,4-glycosidic bonds and with, on the average, every eleventh anhydroxylose unit carrying a single terminal side chain of 4-*O*-methyl-D-glucuronic acid joined by a glycosidic bond to the 2-position of the xylose residues. The chemical and physical non-uniformity of the polymer is discussed briefly.

In a previous study[2] it was shown that partial hydrolysis of a hemicellulose isolated from white birch (*Betula papyrifera*) gave a mixture of sugars containing xylose, 2-*O*-(4-*O*-methyl-D-glucopyranosyluronic acid)-D-xylopyranose, 4-*O*-methyl-D-glucuronic acid, D-galacturonic acid, and traces of galactose, glucose, arabinose and rhamnose. The present paper deals with the constitution of the hemicellulose.

The high pentosan content (88%) of the hemicellulose together with the fact that xylose was the predominant component of the sugar mixture obtained on hydrolysis, made it probable that the polysaccharide was composed of xylose building units. While the glacturonic acid was considered as being derived from occluded pectic material, isolation of 4-*O*-methyl-D-glucuronic acid and the above aldobiouronic acid suggested that the polysaccharide contained 4-*O*-methyl-D-glucuronic acid attached glycosidically to the xylose residues. If this were so, the uronic anhydride content of the hemicellulose (10.88%) corresponded to 10.8 anhydroxylose units per acid side group while the methoxyl content gave a value of 10.3. Direct titration of the acid groups with standard alkali indicated an equivalent weight of 1530, corresponding to 10.3 pentose residues per acid side chain. Oxidation with a buffered solution of trisodium paraperiodate gave a value of 11.4.[3,4]

For removal of minor impurities, the hemicellulose was precipitated by complexing with Fehling solution. The purified polysaccharide, which was obtained in its acidic form, contained pentosan (87.9%), uronic anhydride, (11.0%), methoxyl (2.16%) and no Klason lignin. The treatment had not caused any depolymerization. Examination by paper chromatography indicated only slight traces of galacturonic acid and rhamnose besides xylose, 4-*O*-methyl-D-glucuronic acid, and the aldobiouronic acid.

The purified hemicellulose was methylated six times in an atmosphere of nitrogen with aqueous alkali and dimethyl sulfate after which it was dissolved in tetrahydrofuran and methylated twice with powdered sodium hydroxide and dimethyl sulfate.[5] Treatment with methyl iodide gave the methyl ester of the fully methylated acidic xylan. It was now completely soluble in chloroform and carbon tetrachloride, exhibited a methoxyl content required by theory (39.2%), and had an infrared spectrum which showed the absence of any hydroxyl groups.

A portion of the methylated hemicellulose was subjected to methanolysis under conditions known to effect no cleavage of the aldobiouronic acid. The glycosides were quantitatively separated into acidic and neutral components on an ion-exchange resin. The acid fraction contained only one compound which was characterized as methyl 2-*O*-(2,3,4-trimethyl-D-glucopyranosyluronic acid)-3-O-methyl-D-xylopyranoside. The fact that the 4-position was unsubstituted in the above compound but was substituted when the aldobiouronic acid itself was methylated,[2] indicated that its xylose part was joined to other residues through position 4. When the reduced glycoside was hydrolyzed, only 2,3,4-tri-*O*-methyl-D-glucose and 3-*O*-methyl-D-xylose could be found in the hydrolyzate. This fact, together with the absence of any trace of a 3,4-di-*O*-substituted xylose derivative, indicated that the 4-*O*-methyl-D-glucuronic acid was linked as single side chains through the 2-position of the xylose residues.

The neutral glycoside fraction was hydrolyzed to the corresponding mixture of reducing sugars, and this was resolved on a carbon column by a gradient elution technique employing ethanol and water.[6] Three fractions were obtained: (I) a mixture of two monomethyl xyloses, (II) a dimethylxylose and (III) a trimethylxylose. Fraction I could be resolved only by paper ionophoresis[7,8] when spots were obtained corresponding in position to 2-*O*-methyl-D-xylose and 3-*O*-methyl-D-xylose, respectively. Fraction II was identified as 2,3-di-*O*-methyl-D-xylose, and was characterized through its crystalline aniline derivative.[9] The 2,3,4-tri-*O*-methyl-D-xylose which formed fraction III crystallized and was identified through comparison of its melting point and X-ray powder dia-

(1) Paper presented before the Division of Cellulose Chemistry at the 132nd Meeting of the American Chemical Society in New York, N. Y., September, 1957.

(2) C. P. J. Glaudemans and T. E. Timell, THIS JOURNAL, **80**, 941 (1958).

(3) J. F. Mahoney and C. B. Purves, *ibid.*, **64**, 9, 15 (1942).

(4) C. C. Gibbons, *J. Textile Inst.*, **47**, T 511 (1956).

(5) E. L. Falconer and G. A. Adams, *Can. J. Chem.*, **34**, 338 (1956).

(6) B. Lindberg and B. Wickberg, *Acta Chem. Scand.*, **8**, 569 (1954).

(7) H. Bouveng and B. Lindberg, *ibid.*, **10**, 1283 (1956).

(8) A. B. Foster, *J. Chem. Soc.*, 982 (1953).

(9) H. A. Hampton, W. N. Haworth and E. L. Hirst, *ibid.*, 1739 (1929).

gram with an authentic specimen. A portion of the mixture of methylated xyloses also was separated on the paper chromatogram and the amounts of sugars present were determined by a spectrophotometric method.[10] The relative amounts obtained (Table I) agreed fairly well with those calculated from the weight of the fractions.

TABLE I

SUGARS OBTAINED FROM THE METHYLATED HEMICELLULOSE

Component	Weight, mg.	Molar ratio I[a]	Molar ratio II[b]
2-*O*- and 3-*O*-methyl-D-xylose	79	2.7	3.7
2,3-Di-*O*-methyl-D-xylose	3765	116	126
2,3,4-Tri-*O*-methyl-D-xylose	35	1	1
Methyl 2-*O*-(2,3,4-tri-*O*-methyl-D-glucopyranosyluronic acid)-3-*O*-methyl-D-xyloside	801	11.1	...

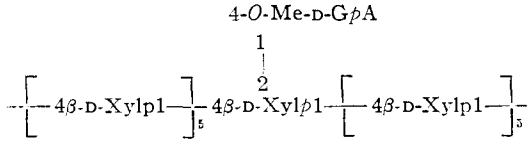
[a] From weight of fractions. [b] By quantitative paper chromatography.

The large quantity of 2,3-di-*O*-methyl-D-xylose isolated indicated that the main portion of the hemicellulose was composed of xylopyranose residues linked together through positions 1 and 4, while the high negative rotation of the polysaccharide suggested that the anhydroxylose units were present in the β-configuration. The 2,3,4-tri-*O*-methyl-D-xylopyranose evidently originated from the non-reducing end groups of the polysaccharide. It had been shown earlier that the side chains consisted of 4-*O*-methyl-D-glucuronic acid groups, linked glycosidically through the 2-position of the xylose residues. No monouronic acids could be detected when the methylated hemicellulose was subjected to methanolysis, and all side groups were accordingly recovered in the form of the partially methylated aldobiouronic acid. The data (Table I) indicate that one mole of the methylated aldobiouronic acid was obtained per 11.9 moles of methylated pentoses. This agrees reasonably well with the values found earlier for the number of anhydroxylose units per acid side group. The monomethylxyloses found in the hydrolyzate could therefore not have been formed by a removal of acid side groups during the methanolysis, particularly as some 2-*O*-methyl-D-xylose also was present together with the 3-*O*-methyl derivative. These two sugars accordingly either represented an artifact or indicated possible branching points in the glucuronoxylan molecule.

While the fact that the amount of mono-*O*-methylxyloses in the hydrolyzate exceeded that of 2,3,4-tri-*O*-methyl-D-xylose strongly indicated that the former of the above alternatives was true, more direct evidence was deemed desirable. The amount of tri-*O*-methylxylose obtained showed that the xylan polymer contained one non-reducing end group per 130 anhydroxylose units. The number-average molecular weight of the fully methylated hemicellulose was determined osmotically with a Zimm–Myerson–Stabin[11,12] osmometer. With a Cellophane membrane and with chloroform as the solvent no diffusion was observed and equilibrium was established within a few hours. The molecular weight was 19,000 ± 300. Assuming one 4-*O*-methyl-D-glucuronic acid group to be associated with 11 xylose residues, the weight of the repeating unit is calculated as 1978. Ten such units were accordingly present per chain molecule, and the number average degree of polymerization was accordingly 110. This value was somewhat lower than that found from the number of non-reducing end-groups present, and definitely precluded the possibility of any branching of the xylan portion of the polysaccharide. The 2-*O*- and 3-*O*-methyl-D-xyloses were therefore artifacts with little or no structural significance. Their presence was most likely caused by random demethylation during the degradation of the methylated hemicellulose. Similar observations have been made in connection with other studies on xylan polysaccharides.[13–16]

The degree of polymerization of 110 obtained for the methylated xylan does not, of course, represent the average chain length of the original polysaccharide, the molecular weight of which was lowered during the methylation. Preliminary data obtained by osmometry with the acetyl and butyryl derivatives indicate a number-average degree of polymerization of 190 ± 5 for the native xylan molecule.[17]

From the above evidence a simplified structure can be suggested for the white birch xylan consisting of a minimum of 110 and a maximum of 190 β-D-xylopyranose residues linked together by 1,4-glycosidic bonds and with, on the average, every eleventh anhydroxylose unit carrying a single terminal side chain of 4-*O*-methyl-D-glucuronic acid joined by a glycosidic bond to the 2-position of the xylose residues. The state of the carboxyl group in the native xylan is uncertain. In the wood, the xylan probably is associated with acetyl groups,[18] the exact manner of attachment of which is presently being studied.[19] When the hemicellulose is isolated by alkaline extraction, these ester groups are, naturally, removed. Recent results[20] indicate that wood xylans are not chemically combined with lignin, although strong evidence to the contrary[21] has also been presented.



The constitution of the white birch xylan studied here is the same as that found previously for a xylan isolated from European beech.[13] A xylan from

(10) T. E. Timell, C. P. J. Glaudemans and A. L. Currie, *Anal. Chem.*, **28**, 1916 (1956).

(11) B. H. Zimm and I. Myerson, THIS JOURNAL, **68**, 911 (1946).

(12) J. V. Stabin and E. H. Immergut, *J. Polymer Sci.*, **14**, 209 (1954).

(13) G. O. Aspinall, E. L. Hirst and R. S. Mahomed, *J. Chem. Soc* 1734 (1954).

(14) G. O. Aspinall and M. L. Carter, *ibid.*, 3744 (1956).

(15) G. G. S. Dutton and F. Smith, THIS JOURNAL, **78**, 2505, 3744 (1956).

(16) G. A. Adams, *Can. J. Chem.*, **35**, 556 (1957).

(17) C. P. J. Glaudemans and T. E. Timell, unpublished results.

(18) E. Hägglund, B. Lindberg and J. McPherson, *Acta Chem. Scand.*, **10**, 1160 (1956).

(19) T. E. Timell, unpublished results.

(20) R. Nelson and C. Schuerch, *J. Polymer Sci.*, **22**, 435 (1956); *Tappi*, **40**, 419 (1957).

(21) A. Björkman, *Svensk Papperstidn.*, **59**, 477 (1956); **60**, 158, 243, 285, 335 (1957).

Finnish birch (*Betula verrucosa*)[22] has been stated to contain 22 anhydroxylose units per 4-*O*-methyl-D-glucuronic acid group, a figure later changed to 15.[23] Softwood xylans, in addition to L-arabofuranose units, also contain side chains of the same uronic acid, in this case, however, with only 4 or 5 xylose residues per acid group.[14,15,24] Acidic side chains other than 4-*O*-methyl-D-glucuronic acid have not been observed for wood xylans, but in at least one case[25] the linkage is not the usual 1,2 but instead 1,3. With one possible exception[16] all wood hemicelluloses of this type so far studied have been linear polymers containing only single side chains. In this respect they differ from the xylans of many non-woody plants such as esparto,[26] corn cob,[27] wheat straw[27-30] and milkweed floss[31] which have been found to contain branched chain molecules.

Although the hemicellulose studied here appeared to be homogeneous when subjected to electrophoresis in a borate buffer solution,[7,8] later evidence might invalidate this conclusion.[32] Recent experiments[11] have indicated that native white birch xylan is non-uniform with respect to both chemical properties and degree of polymerization, and the acidic side chains appear to be distributed at random along the chain molecules. These results, together with complete data for the average molecular weight of the native polysaccharide, will be reported later.

Experimental

General experimental conditions, analytical procedures and solvents used to separate sugars were the same as in the previous paper.[2]

Isolation and Purification of the Hemicellulose.—The hemicellulose was isolated by alkaline extraction of a chlorine holocellulose as described previously[2] in a yield of 27.1% of the wood. The material contained uronic anhydride, 10.88; OMe, 2.00; $[\alpha]^{20}D$ −83° (*c* 1.0 in 5% sodium hydroxide).

The crude hemicellulose which still contained traces of galactose, glucose, arabinose and rhamnose sugar residues, was purified by complexing with Fehling solution.[26] Hemicellulose (23 g.) was dissolved in 4% sodium hydroxide (1500 ml.) in an atmosphere of nitrogen, and freshly prepared Fehling solution (750 ml.) was added. The precipitate was removed by centrifuging, washed once with water, and then stirred at 0° with 0.5 *N* hydrochloric acid (2500 ml.) for 4 hr. The precipitate was washed with 0.1 *N* hydrochloric acid in a 40:60 (v./v.) mixture of water and acetone, and the material was finally dried from anhydrous methanol *in vacuo*. This treatment was repeated twice to yield a product which contained only xylose and uronic acid residues (16.0 g.). A second portion of the hemicellulose was prepared in a similar way to yield the same amount of pure product. *Anal.* pentosan, 87.9; uronic anhydride, 11.0; OMe, 2.16; lignin, nil. The intrinsic viscosity in *M* cupriethylenediamine was identical before and after the purification (0.94 dl./g.).

Methylation of the Hemicellulose.—The purified hemicellulose (15 g.) was stirred in water (75 ml.) overnight and 40% (w./w.) aqueous sodium hydroxide (150 ml.) was added in an atmosphere of nitrogen, after which the mixture was stirred for 4 hr. Dimethyl sulfate (135 ml.) was added dropwise over a period of 10 hr. while the mixture was cooled in ice-water. This treatment was repeated three times. The mixture was heated in a boiling water-bath for 2 hr. and was then neutralized, first with sulfuric acid and then with acetic acid to a *p*H of 5. The partially methylated product was collected by filtration through cloth and was washed with boiling water (5 l.). The washings were acidified and extracted with chloroform to give a small additional amount of methylated material.

A fifth methylation was carried out by dissolving the material in 90% aqueous tetrahydrofuran (600 ml.) to which 40% sodium hydroxide (350 ml.) was added, after which dimethyl sulfate (150 ml.) was introduced at room temperature over a period of 10 hr. This treatment was repeated once after which the material (13 g.) was isolated as before. *Anal.* OMe, 35.4.

Two additional methylations were carried out by the method of Falconer and Adams.[5] The methylated hemicellulose was dissolved in a mixture of chloroform and ethanol (1:1 v./v.) and precipitated by pouring into petroleum ether (b.p. 30–60°) to yield a fibrous product which was suspended in anhydrous tetrahydrofuran (300 ml.). Powdered sodium hydroxide (200 g.) was added to the swollen material followed by dimethyl sulfate (135 ml.) which was introduced dropwise over a period of 10 hr. This treatment was repeated once and the tetrahydrofuran was then removed by distillation. The methylated hemicellulose which had separated in lumps, was isolated, dissolved, and reprecipitated as described above; total yield 12.0 g. A portion (1.0 g.) of the product was converted to the methyl ester of the methylated hemicellulose by treatment with methyl iodide (5 ml.) at room temperature for 40 hr. The product was dissolved by addition of dimethylformamide, methyl iodide was removed *in vacuo* at 60°, and chloroform was added. Dimethylformamide was removed by extraction with water, the chloroform solution was dried over sodium sulfate, and the product was precipitated by pouring into petroleum ether.

The methylated hemicellulose was completely soluble in both chloroform and carbon tetrachloride. A 1% solution in the latter solvent gave an infrared spectrum which showed no hydroxyl band at 3500 $cm.^{-1}$.

Anal. Calcd. for the methyl ester of a completely methylated glucurono-xylan containing 11 anhydroxylose units per 4-*O*-methyl-D-glucuronic acid group: OMe, 39.2. Found: OMe, 39.0; $[\alpha]^{20}D$ −56° (*c* 1.0 in chloroform).

Methanolysis of the Methylated Hemicellulose and Separation of the Acidic Component.—The fully methylated hemicellulose (5.0 g.) was subjected to methanolysis by refluxing in methanol containing hydrogen chloride (2%) until the rotation became constant (13 hr.). The solution was neutralized with silver carbonate and colloidal silver was removed with hydrogen sulfide. Filtration and evaporation of the solvent yielded a clear, yellow sirup, which was dissolved in aqueous barium hydroxide (25 ml., saturated at room temperature) and heated at 60° for 2 hr.[15] The barium hydroxide was removed with carbon dioxide and the mixture was heated at 80° for 15 min. and then filtered. Barium ions were removed with Amberlite cation exchange resin IR 120[33] and the free acids in the mixture were adsorbed on a column of Dowex 1-X4 anion exchange resin[34] (bicarbonate form), which was washed with water until a Molisch test was negative. The washings were combined and evaporated to give a colorless, thick sirup (4.105 g.) of methyl glycosides.

The acid fraction was displaced from the column with *N* sulfuric acid, which was neutralized with barium hydroxide. The filtered solution was passed through a column of Amberlite IR 120[33] for removal of barium ions and the eluent was concentrated to a thick, yellow sirup (0.801 g.) of the methyl glycoside of the partially methylated aldobiouronic acid.

Anal. Calcd. for $C_{16}H_{28}O_{11}$: equiv. wt., 396. Found: equiv. wt., 370.

(22) J. Saarnio, K. Wathén and Ch. Gustafsson, *Acta Chem. Scand.*, **8**, 825 (1954).

(23) Ch. Gustafsson, paper presented at the 128th Meeting of the American Chemical Society, Minneapolis, Minn., September, 1955.

(24) J. Saarnio, *Suomen Kemistilehti*, **B29**, 35 (1956).

(25) D. J. Brasch and L. E. Wise, *Tappi*, **39**, 581, 768 (1956).

(26) S. K. Chanda, E. L. Hirst, J. K. N. Jones and E. G. V. Percival, *J. Chem. Soc.*, 1289 (1950).

(27) I. Ehrenthal, R. Montgomery and F. Smith, THIS JOURNAL, **76**, 5509 (1954).

(28) G. O. Aspinall and R. S. Mahomed, *J. Chem. Soc.*, 1731 (1954).

(29) G. O. Aspinall and E. G. Meek, *ibid.*, 3830 (1956).

(30) C. T. Bishop, *Can. J. Chem.*, **33**, 1073 (1955).

(31) F. W. Barth and T. E. Timell, unpublished results.

(32) B. A. Lewis and F. Smith, THIS JOURNAL, **79**, 3929 (1957).

(33) A product of Rohm and Haas Co., Philadelphia, Pa.

(34) A product of Dow Chemical Co., Midland, Mich.

Preparation, Reduction and Hydrolysis of the Methyl Ester Methyl Glycoside of the Partially Methylated Aldobiouronic Acid.—The partially methylated aldobiouronic acid was refluxed with 2% methanolic hydrogen chloride to give the methyl ester of the compound, which was subsequently reduced with lithium aluminum hydride as described earlier.[2] The disaccharide was treated with methanol (50 ml.) containing hydrogen chloride (5%). After refluxing until constant rotation, the methanol was evaporated and the glycosides were hydrolyzed by refluxing for 6 hr. with *N* hydrochloric acid. After removal of the acid, evaporation gave a sirup which, when examined by paper chromatography, was found to contain a tri-*O*-methylglucose and a mono-*O*-methylxylose in approximately equimolar amounts.

Characterization of 2,3,4-Tri-*O*-methyl-D-glucose and 3-*O*-Methyl-D-xylose.—The above mixture was resolved on large sheets of filter paper with solvent system C. Examination by paper chromatography of the faster moving component showed it to give a spot in the same position as an authentic sample of 2,3,4-tri-*O*-methyl-D-glucose in solvent systems A, B and C. When examined by paper electrophoresis it showed no movement as could be expected for a sugar with no adjacent hydroxyl groups.[8]

Anal. Calcd. for $C_9H_{18}O_6$: OMe, 41.9. Found: OMe, 41.3.

The slower moving component was identical with 3-*O*-methyl-D-xylose on paper ionophoresis in a borate buffer solution,[7,8] moving twice as fast as a sample of 2-*O*-methyl-D-xylose. It was further characterized through the crystalline aniline derivative, 3-*O*-methyl-*N*-phenyl-D-xylosylamine, m.p. and mixed m.p. 136°, $[\alpha]^{20}D$ +120° (*c* 0.3 in ethyl acetate).

Anal. Calcd. for $C_6H_{12}O_5$: OMe, 18.9. Found: OMe, 18.5.

Separation of the Neutral Components of the Methylated Hemicellulose.—The sirup (0.4105 g.) containing the methyl glycosides of the methylated sugars was converted to the reducing sugars and was added to the top of a charcoal–Celite[35] column[36] (30 × 8 cm.). The gradient elution technique of Lindberg and Wickberg[6] was used, with the eluent consisting of aqueous ethanol containing a gradually increasing amount of the alcohol (zero to 45%). Fractions of 20 ml. were collected at 30-min. intervals. Three sugar fractions were obtained, the middle of which predominated. Concentration yielded three sirups which were thoroughly dried and weighed (Table I).

Spectrophotometric Analysis of the Neutral Components of the Methylated Hemicellulose.—A minor portion of the mixture of methylated sugars was resolved on strips of paper with solvent system C and the appropriate sections were eluted with water to a suitable volume. The amount of sugar present in the solution was determined by the *o*-aminobiphenyl method,[11] the relation between concentration and absorbance being determined with authentic samples. The following amounts (in γ/ml.) were required to produce an absorbance of unity at 380 mμ: 3-*O*-methyl-D-xylose, 265; 2,3-di-*O*-methyl-D-xylose, 500; 2,3,4-tri-*O*-methyl-D-xylose, 510. The data presented in Table I are the averages of triplicate determinations.

Characterization of Mono-*O*-methylxyloses.—This fraction, which could not be induced to crystallize, had a methoxyl content corresponding to that of a monomethylxylose and a specific rotation suggesting that it contained sugars belonging to the D-series. Ionophoresis in a borate buffer solution[7] revealed the presence of two compounds corresponding in position to authentic samples of 3-*O*-methyl-D-xylose and 2-*O*-methyl-D-xylose, the former of which predominated. Attempts to resolve the mixture by paper chromatography with a number of different solvent systems all failed. Since it had been shown that the presence of this fraction was an artifact, no further attempts were made to characterize it.

Identification of 2,3-Di-*O*-methyl-D-xylose.—The clear, colorless sirup obtained from the column could not be induced to crystallize.

Anal. Calcd. for $C_7H_{14}O_5$: OMe, 34.8. Found: OMe, 34.8; $[\alpha]^{20}D$ +23° (*c* 2.5 in water).

(35) A product of Johns-Manville Co., New York, N. Y.

(36) R. L. Whistler and D. F. Durso, THIS JOURNAL, **72**, 677 (1950).

The aniline derivative, 2,3-di-*O*-methyl-*N*-phenyl-D-xylosylamine, was recrystallized from ethyl acetate–petroleum ether and had m.p. and mixed m.p. 125–126°, $[\alpha]^{23}D$ +190° (*c* 1.0 in ethyl acetate).

Identification of 2,3,4-Tri-*O*-methyl-D-xylose.—Since the trimethylxylose fraction was contaminated by traces of the dimethyl derivative, it was purified by chromatography on sheets of filter paper using solvent system C. The separated dimethylxylose was added to fraction II. The trimethylxylose (35 mg.) now crystallized spontaneously. The crystals were collected on a porous tile and dried over calcium chloride for several days; m.p. and mixed m.p. 89°. An X-ray powder diagram was identical to that of an authentic specimen.

Anal. Calcd. for $C_8H_{16}O_5$: OMe, 48.4. Found: OMe, 48.0; $[\alpha]^{20}D$ +19° (*c* 0.5 in water).

Periodate Oxidation of the Hemicellulose.—Portions of hemicellulose (100 mg.) were oxidized with a solution (75 ml.) of 0.05 *M* trisodium paraperiodate, buffered to a *p*H of 4.1 with acetic acid to minimize over-oxidation.[3,4] After having been shaken in the dark for the desired length of time, the remaining periodate was determined in the usual way with arsenite at 24-hour intervals for 9 days. Consumption of periodate was plotted against time and the later, almost linear part of the curve was extrapolated to zero time. The amount of oxidant consumed was 11.4 moles per repeating unit of the xylan, the calculated value being 11.0.

Hypoiodite Oxidation of the Hemicellulose.—The oxidation was carried out with 0.1 *N* iodine in a solution buffered to *p*H 10.6 as described by Hirst, Hough and Jones.[37] The amount of iodine consumed corresponded to one reducing group per 71 anhydroxylose units. In view of the uncertainties of the method, this result was not considered significant.

Paper Electrophoresis of the Hemicellulose.—A glass-fiber paper[38] (30 × 10 cm.) was wetted with the electrolyte solution which contained 7.50 g. of boric acid and 4.0 g. of sodium hydroxide per liter.[7] A 5% solution of the hemicellulose in 2.5% aqueous sodium hydroxide was applied in a fine line. Electrophoretic separation was carried out at 600 volts and 230 milliamperes for 7½ hr. The paper was dried in a horizontal position and was sprayed with a solution of acetic acid (100 ml.) containing *o*-aminobiphenyl (3.0 g.), concentrated sulfuric acid (3.0 ml.) and water (3.0 ml.). After heating for 20 min. a single, large band appeared.

Determination of the Molecular Weight of the Methylated Hemicellulose.—The osmometer used was a Zimm-Myerson[11] instrument, as modified by Stabin.[12] Gel Cellophane membranes[39] (diameter 5 cm.) which had never been dried were used. No leakage of the cell was noticed and equilibrium was attained in a few hours. The temperature was 30 ± 0.01° and the static method of measuring the osmotic pressure was employed. In spite of the relatively low molecular weight of the methylated polysaccharide, hardly any diffusion could be observed. The osmotic pressure was determined at four different concentrations (Table II) and the values for the reduced osmotic pressure, π/C, were extrapolated to zero concentration, thus yielding the number-average molecular weight of the polymer.

TABLE II

OSMOMETRY DATA OBTAINED FOR THE METHYLATED HEMICELLULOSE

C^a	π^b	π/C
0.448	4.558	10.18
.316	3.176	10.05
.284	2.830	9.96
.142	1.315	9.26
0	...	8.85

[a] Concentration in g./100 ml. chloroform. [b] Osmotic pressure in cm. chloroform.

(37) E. L. Hirst, L. Hough and J. K. N. Jones, *J. Chem. Soc.*, 928 (1949).

(38) D. R. Briggs, E. F. Garner and F. Smith, *Nature*, **178**, 154 (1956).

(39) Kindly supplied by American Viscose Corporation, Marcus Hook, Pa.

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MONTREAL, QUEBEC

[CONTRIBUTION FROM THE DIVISION OF STEROID METABOLISM AND BIOCHEMISTRY, SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Synthesis of 2-Methoxyestrogens[1]

BY JACK FISHMAN

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The synthesis of 2-methoxyestrone, a new urinary metabolite of estradiol, and 2-methoxyestradiol, a possible metabolite, is described.

The isolation of 2-methoxyestrone, a new metabolite of estradiol, from human urine recently was reported from this Laboratory.[2] Since the amount of material isolated precluded degradation studies, the structure of the metabolite was determined by synthesis,[2,3] which resulted in a small quantity of product sufficient only for comparison purposes. In order to satisfy a need for substantial quantities of 2-methoxyestrone for isotopic dilution studies and other contemplated metabolic experiments, a more productive method of synthesis was necessary. In addition, the possibility that other 2-methoxy metabolites might be present in human urine added interest to an alternative synthesis that might yield new products of natural origin. A recently developed method of *o*-hydroxylation of phenols[4] appeared to provide the answer to these requirements. In general the scheme involves reaction of the phenol with 2-chloro-5-nitrobenzophenone, cyclization of the resultant diaryl ether to the xanthylium salt and oxidation of the latter with hydrogen peroxide to give the *o*-hydroxylated product. This could then be cleaved with piperidine to give the pyrocatechol. Methylation prior to cleavage would result in the corresponding guaiacol.

The initial attempt to use this sequence with estrone resulted in a crystalline compound, m.p. 204–207°, the ultraviolet spectrum of which was identical with 2-methoxyestrone. The infrared spectrum, however, showed that the 17-ketone was no longer present. The spectrum indicated that a ring D lactone had been formed by oxidation with the hydrogen peroxide in the acidic medium.[5] Under several conditions the 17-ketone group failed to survive the oxidation step and therefore attention was directed to the use of a compound lacking this group, from which, however, it could subsequently be generated. Estradiol appeared particularly suitable in that it would also lead to 2-methoxyestradiol which was of interest in itself. The etherification of estradiol-17β with 2-chloro-5-nitrobenzophenone proceeded smoothly in ethanolic potassium hydroxide solution. The nitrobenzophenone ether (Ib) was obtained in better than 90% yield, based on the reagent, and the excess unreacted estradiol was separated easily by alkali extraction. The only by-product, 2-ethoxy-5-nitrobenzophenone, was formed in very small amounts by alcoholysis of the 2-chloro-5-nitrobenzophenone, and could be separated readily from the desired product by chromatography. Since the 17-hydroxy group also failed to survive treatment with acidic hydrogen peroxide, Ib was acetylated to give the non-crystalline 17-acetate Ic. The latter was dissolved in a minimum of acetic acid and cyclized with cold concentrated sulfuric acid. The dark red solution of the xanthylium acid sulfate salt II was diluted with more acetic acid and oxidized with an excess of 30% hydrogen peroxide. The resultant precipitate, 2-hydroxy-17β-acetoxy-$\Delta^{1,3,5(10)}$-estratriene 3-(2-benzoyl-4-nitro)-phenyl ether (IIIa), showed carbonyl absorption at 1655 cm.$^{-1}$ in chloroform, interpreted as indicating bonding between the carbonyl and the phenolic groups. Methylation of the phenol with diazomethane gave the methyl ether IIIb, the carbonyl absorption of which was at 1672 cm.$^{-1}$ in chloroform. The choice of diazomethane as a methylating agent in preference to dimethyl sulfate in alkali was dictated by the desire to avoid the complicating possibility of a Smiles rearrangement.[4] Piperidine cleavage of IIIb proceeded smoothly to give 2-piperidino-5-nitrobenzophenone and 2-methoxy-3-hydroxy-17β-acetoxyestra-1,3,5-(10)-triene (IVa). Separation of the two compounds was achieved by chromatography on alumina. Hydrolysis of the acetate IVa with ethanolic potassium hydroxide gave the desired 2-methoxyestradiol-17β (IVb). The same compound (IVb) was obtained directly from IIIb by hot alkaline hydrolysis, the other product being 2-hydroxy-5-nitrobenzophenone. In this case separation was effected readily by extracting the nitrophenol with alkali, the 2-methoxyestradiol remaining in the organic phase.

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(2) S. Kraychy and T. F. Gallagher, THIS JOURNAL, **79**, 754 (1957).

(3) S. Kraychy and T. F. Gallagher, *J. Biol. Chem.*, **229**, 519 (1957).

(4) J. D. Loudon and J. A. Scott, *J. Chem. Soc.*, 265 (1953).

(5) R. P. Jacobseo, *J. Biol. Chem.*, **171**, 61 (1947).