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The Constitution of a Hemicellulose from Milkweed (*Asclepias syriaca*) Floss[1]

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The general chemical composition of the floss and the stalk of the common milkweed (*Asclepias syriaca*, L.) has been determined. Partial hydrolysis of the floss gave galactose, glucose, mannose, xylose, galacturonic acid, 4-*O*-methyl-D-glucuronic acid, 2-*O*-(4-*O*-methyl-D-glucopyranosyluronic acid)-D-xylopyranose and a triouronic acid. Alkaline extraction of the floss yielded a hemicellulose composed of xylose and uronic acid residues. Hydrolysis of the fully methylated polysaccharide gave 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-xylopyranose in a molar ratio of 0.8:38.6:1:2.3. The number-average degree of polymerization of the methylated hemicellulose was 97 and the corresponding value for the native polymer was 172. On the basis of these results it is suggested that the hemicellulose contains approximately 170 β-D-xylopyranose residues linked together by 1,4-glycosidic bonds and with, on the average, one branching point present per molecule. Every 14th anhydroxylose unit carries a single terminal side chain of 4-*O*-methyl-D-glucuronic acid attached by an α-glycosidic bond to the 2-position of the xylose residues. The molecular-weight distribution contains one maximum.

Milkweeds are tall plants containing a milky juice in all their parts which has given them their name. The common milkweed (*Asclepias syriaca*, L.), which is native to eastern North America, is a perennial and multiplies by seeds and creeping roots. The seeds have tufts of silky hair (the so-called floss) and are enclosed by a pod. When the latter opens, the seeds are spread by the wind. The seed fibers consist of a single cell and have occasionally been used as a substitute for kapok.

Previous investigations dealt with the molecular properties of the cellulose component of the floss.[2,3] This paper is concerned with the general chemical composition of the floss and the stalk and with the constitution of a hemicellulose isolated from the former.

The chemical composition of the floss and the stalk is presented in Table I. The difference in cellulose, uronic anhydride, acetyl and xylan contents between the two parts of the plant is considerable. It had previously[3] been shown that the weight-average degree of polymerization of the cellulose present in the floss was 10,500. The corresponding figure for the cellulose in the stalk was 9,300.[11]

TABLE I

CHEMICAL COMPOSITION OF THE FLOSS AND STALK OF THE COMMON MILKWEED[a]

Component	Floss	Stalk
α-Cellulose[4]	39.6	52.6
Pentosan[5]	35.3	19.2
Lignin[6]	15.1	15.6
Ash[7]	0.2	1.4
Acetyl[8]	6.1	3.7
Uronic anhydride[9]	5.6	10.2
Galactan	1.7	2.3
Glucan	40.2	53.0
Mannan	3.8	1.7
Araban	Nil	1.5
Xylan[10]	27.3	10.6

[a] All values in per cent. of extractive-free material.

Partial acid hydrolysis of the floss gave the above neutral sugars in addition to a mixture of uronic acids, part of which was treated with methanolic hydrogen chloride, reduced with lithium aluminum hydride[12,13] and hydrolyzed to yield a mixture of galactose, xylose and 4-*O*-methyl-D-glucose which was resolved on a cocoanut charcoal column. The galactose was isolated and characterized by paper chromatography, the xylose crystallized and the 4-*O*-methyl-D-glucose was identified through its crystalline osazone.[13,14] The main portion of the uronic acids was partially resolved on a column of ion exchange resin,[15] followed by resolution on a charcoal column to yield galacturonic acid, 4-*O*-methyl-D-glucuronic acid, 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose and a triouronic acid. The galacturonic acid was identified chromatographically after reduction to galactose. Reduction of the ester glycoside of the 4-*O*-methyl-D-glucuronic acid with lithium aluminum hydride and subsequent hydrolysis gave 4-*O*-methyl-D-glucose, characterized through its osazone. While this acid probably originated from the pentosan portion of the floss, the galacturonic acid was believed to arise from pectic material.

The methoxyl content and equivalent weight of the main uronic acid fraction corresponded to that of a monomethylated aldobiouronic acid containing a pentose and a hexuronic acid residue. Its infrared spectrum was identical to that of an authentic sample of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose.[16] Reduction of the methyl glycoside with lithium aluminum hydride, followed by hydrolysis, yielded 4-*O*-methyl-D-glucose and xylose. Reduction of the fully methylated acid and hydrolysis gave 3,4-di-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose which were

(1) Paper presented before the Division of Cellulose Chemistry at the 134th Meeting of the American Chemical Society in Chicago, Ill., September, 1958.

(2) T. E. Timell and J. L. Snyder, *Textile Research J.*, **25**, 870 (1955).

(3) T. E. Timell, *ibid.*, **28**, 270 (1958).

(4) "Testing Methods of the Technical Association of the Pulp and Paper Industry," New York, N. Y., *TAPPI*, **T 9** m-54.

(5) *TAPPI*, **T 19** m-50.

(6) *TAPPI*, **T 13** m-54.

(7) *TAPPI*, **T 15** m-54.

(8) T. E. Timell, *Svensk Papperstidn.*, **60**, 762 (1957).

(9) B. L. Browning, *Tappi*, **37**, 336 (1954).

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

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

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

(15) J. K. Gillham and T. E. Timell, *Can. J. Chem.*, **36**, 510 (1958).

(16) C. P. J. Glaudemans and T. E. Timell, THIS JOURNAL, **80**, 941, 1209 (1958).

both characterized as their respective aniline derivatives. This proved the presence of a glycosidic bond through C_2 of the xylose moiety. The infrared spectrum of the fully methylated product was identical with that of an authentic specimen[16] of methyl 2-*O*-(2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronic acid)-3,4-di-*O*-methyl-D-xylopyranoside methyl ester. This aldobiouronic acid has also been obtained from many wood species as well as from a few non-woody plants such as corn cobs,[17] flax straw,[18] oat hulls[19] and kapok.[20]

Hydrolysis of the aldotriouronic acid, which partly crystallized, and chromatographic analysis indicated the presence in the hydrolyzate of aldotriouronic acid, the above aldobiouronic acid, 4-*O*-methyl-D-glucuronic acid and xylose. The methoxyl content suggested the presence of one 4-*O*-methyl-D-glucuronic acid and two xylose residues. A similar aldotriouronic acid has been isolated from the wood of mesquite gum,[21] trembling aspen,[22] western hemlock,[23] Monterey pine,[24] loblolly pine[25] and white elm.[26]

The high pentosan content of the extractive-free seed hairs, which was approximately the same as that of a related species,[27] suggested that the hemicellulose portion was composed of xylose residues. Delignification of the floss with either chlorine[28] or chlorite[29] followed by extraction with alkali, gave a hemicellulose in a yield of 34–35%. Direct alkaline extraction resulted in a yield of 30% and was the preferred method for isolating a less degraded material.

The hemicellulose contained mostly pentosan and yielded only D-xylose and various uronic acids on hydrolysis. The isolation of the above aldobio- and aldotriouronic acids suggested strongly that the polysaccharide possessed residues of 4-*O*-methyl-D-glucuronic acid linked glycosidically to anhydroxylose units. If this were so, the uronic anhydride content corresponded to 14.3 xylose residues per acid side group and the methoxyl content to 13.8. The equivalent weight of the acidic hemicellulose indicated 14.4 anhydroxylose units for each acid group.

The fully methylated[19,30] hemicellulose was subjected to methanolysis under conditions which should not cleave any methylated aldobiouronic acid. The methyl glycosides were quantitatively resolved into an acidic and a neutral fraction on an anion exchange resin.[15] The acid fraction was characterized as methyl 2-*O*-(2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronic acid)-3-*O*-methyl-D-xylopyranoside in the following way. Reduction with lithium aluminum hydride of the ester glycoside gave a mixture of two methylated sugars which was resolved on a charcoal column, yielding 2,3,4-tri-*O*-methyl-D-glucose and 3-*O*-methyl-D-xylose, both identified through their infrared spectra and through their aniline derivatives. The fact that the 4-position was unsubstituted in the methylated aldobiouronic acid fragment but was substituted when the free acid itself was methylated showed that its xylose moiety was linked to other sugar residues through C_4. This, together with the absence of a 3,4-di-*O*-substituted xylose, indicated that the acid side groups in the 1,4-linked hemicellulose were attached directly to the main chain of the macromolecule.

The neutral glycosides were converted to the corresponding mixture of the free sugars which were separated by paper chromatography. Minor quantities of a mono- (I) and a tri-*O*-methyl xylose (III) and a large amount of a di-*O*-substituted xylose (II) were present. Fraction I was resolved by paper ionophoresis[31–33] into two compounds, corresponding in mobility to 2-*O*- and 3-*O*-methyl-D-xylose and present in approximately equal amounts. The 2,3-di-*O*-methyl-D-xylose forming fraction II was identified through its aniline derivative. Fraction III consisted of 2,3,4-tri-*O*-methyl-D-xylose. A portion of the mixture was subjected to quantitative paper chromatography.[10] The results, together with the weight of the acidic fraction, indicated the presence of mono-*O*-methylxylose (0.82 mole), 2,3-di-*O*-methyl-D-xylose (38.6 moles), 2,3,4-tri-*O*-methyl-D-xylose (1.0 mole) and methyl 2-*O*-(2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronic acid)-3-*O*-methyl-D-xylopyranoside (2.29 moles). The quantity of the latter compound corresponded to 18.6 xylose residues per acid group, a value somewhat higher than had been found by other methods. Since no 4-*O*-methyl-D-glucuronic acid could be detected after methanolysis, the reason for this discrepancy was probably a preferential loss during the methylation of uronide material. Similar observations have been made elsewhere.[16,25]

The large amount of 2,3-di-*O*-methyl-D-xylose obtained showed that the main part of the polysaccharide was composed of D-xylopyranose residues linked through positions 1 and 4. The high negative rotation of the unsubstituted (−99°) and the methylated (−77°) hemicellulose suggested that the anhydroxylose units were present in the β-configuration. The non-reducing end-groups apparently gave rise to the 2,3,4-tri-*O*-methyl-D-xylose. The 4-*O*-methyl-D-glucuronic acid residues were attached as single side chains through the 2-position of the anhydroxylose units. None of them was apparently removed during the methanolysis of the methylated polysaccharide and the presence of the two mono-*O*-substituted xyloses was therefore due to either incomplete methylation, demethylation or branching. While demethylation is

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(18) J. D. Geerdes and F. Smith, *ibid.*, **77**, 3569 (1953).
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(22) J. E. Milks and C. B. Purves, THIS JOURNAL, **78**, 3738 (1956).
(23) J. K. Hamilton and N. S. Thompson, *ibid.*, **79**, 6464 (1957).
(24) D. J. Brasch and L. E. Wise, *Tappi*, **39**, 581, 768 (1956).
(25) T. J. Painter, personal communication.
(26) J. K. Gillham and T. E. Timell, unpublished results.
(27) A. Juillet and J. Délga, *Mém. services chim. état (Paris)*, **32**, 378 (1948).
(28) T. E. Timell and E. C. Jahn, *Svensk Papperstidn.*, **54**, 831 (1951).
(29) L. E. Wise, M. Murphy and A. A. D'Addieco, *Paper Trade J.*, **122**, No. 2, 35 (1946).
(30) R. Kuhn, H. Trischmann and I. Löw, *Angew. Chem.*, **67**, 32 (1955).
(31) A. B. Foster, *J. Chem. Soc.*, 982 (1953).
(32) H. Bouveng and B. Lindberg, *Acta Chem. Scand.*, **10**, 1283 (1956).
(33) A. B. Foster in M. L. Wolfrom and R. S. Tipson, *Adv. Carbohydrate Chem.*, **12**, 81 (1957).

known to occur under the conditions prevailing during hydrolysis of polysaccharides,[16,34,35] the possibility of branching could not be excluded.

In an attempt to resolve this problem, the number-average degree of polymerization of the methylated hemicellulose was determined from osmotic pressure measurements.[16,36] The molecular weight was 17,000 ± 300. With one 4-*O*-methyl-D-glucuronic acid residue present for every 14th anhydroxylose unit, this corresponds to a number-average degree of polymerization (number of xylose residues) of 97. The composition of the methylated hemicellulose indicated the presence of only 43 xylose residues per non-reducing end-group. If it be assumed that on the average one branch point is present per macromolecule, a composition such as that presented in Table II would be expected for the methylated polysaccharide. The agreement between calculated and found values is reasonably good and indicates that slightly more than one branch point was present per molecule through either or both of positions 2 and 3. Oxidation with hypoiodite[37] suggested the presence of one reducing end-group per 62 xylose residues. This is a value somewhat lower than that expected. It is well known, however, that over-oxidation easily occurs in this determination.[16]

TABLE II

MOLE RATIOS OF HYDROLYSIS PRODUCTS AND $\bar{P}_n$[a] OF THE METHYLATED POLYSACCHARIDE

	Found	Calcd.
Mono-*O*-methylxylose	1.6	1
2,3-Di-*O*-methyl-D-xylose	82	94
2,3,4-Tri-*O*-methyl-D-xylose	2	2
$\bar{P}_n$	86	97

[a] Number-average degree of polymerization.

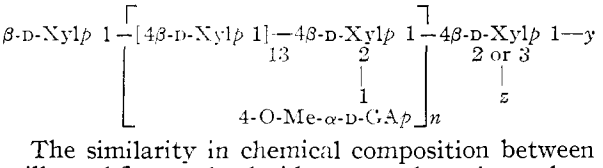
Methylation of another sample of hemicellulose to a slightly lower methoxyl content (35%) and subsequent separation of the methyl derivatives obtained on methanolysis and hydrolysis indicated the same amount of non-reducing end-groups. The molecular weight, as determined by osmometry, was also exactly the same, thus corroborating the above results.

Attempts to determine the molecular weight of the native polysaccharide by osmotic pressure measurements with the acetyl derivative[38,39] failed because of the incomplete solubility of the latter. The intrinsic viscosity of the hemicellulose was estimated in cupriethylenediamine[40] as 0.812 dl./g. Using the relationship developed earlier for a similar polysaccharide,[39] this would correspond to a $\bar{P}_n$ value of 172. For an estimation of its polymolecularity, the hemicellulose was fractionally precipitated from an alkaline solution by gradual addition of ethanol.[39] Fourteen fractions were isolated, ranging in degree of polymerization from 65 to 175. The frequency distribution contained one maximum.

Summarizing the above evidence, a simplified structure can now be proposed for the hemicellulose present in milkweed floss. This polysaccharide evidently contains approximately 170 β-D-xylopyranose residues linked together by 1,4-glycosidic bonds and with, on the average, one branching point present per molecule through either or both of positions 2 and 3. Every fourteenth anhydroxylose unit carries a single, terminal side chain of 4-*O*-methyl-D-glucuronic acid, attached by an α-glycosidic bond to the 2-position of the xylose residues. The acetyl content of the floss is fairly high and from what is now known about similar hemicelluloses from wood[41,42] it appears probable that most of the hydroxyl groups in the native material are esterified with acetic acid. The hemicellulose is polymolecular and the lower D.P. limit is the same as that observed earlier for a methyl glucuronoxylan from white birch,[39] whereas the average D.P. is somewhat lower.

The constitution of the hemicellulose of milkweed floss is similar, albeit not identical, to that of several xylan polysaccharides from other sources. Hemicelluloses containing branched chain molecules include those from American beech,[43] corn cob,[44] esparto grass[45] and wheat straw.[44,46,47] A large number of xylans have been found to contain single side chains of 4-*O*-methyl-D-glucuronic acid, with one exception,[24] invariably linked α-1,2. In this respect, as well as in the absence of any L-arabofuranose residues, the present polysaccharide resembles most closely the linear methyl glucuronoxylans present in many deciduous woods, such as European beech,[34] yellow birch,[42] white birch,[16] white elm[26] or sugar maple.[42] The number of xylose residues per acid group in these hemicelluloses has been found to vary from 7 for elm to 11 for white birch. The corresponding figure for the present polysaccharide, namely 14, indicates the presence of less acid side chains. Kapok, which is also a seed hair, seems to contain a methyl glucuronoxylan with twice as many 4-*O*-methyl-D-glucuronic acid residues[20] as the hemicellulose from the related milkweed floss.

$$x + y + z = 170$$



The similarity in chemical composition between milkweed floss and a deciduous wood species such as

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(35) B. Lindberg, personal communication.

(36) J. V. Stabin and E. H. Immergut, *J. Polmer Sci.*, **14**, 2092 (1954).

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

(38) C. P. J. Glaudemans and T. E. Timell, *Svensk Papperstidn.*, **60**, 869 (1957).

(39) C. P. J. Glaudemans and T. E. Timell, *ibid.*, **61**, 1 (1958).

(40) A. W. Craig and D. A. Henderson, *J. Polymer Sci.*, **19**, 215 (1956).

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

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

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

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

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

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

(47) G. O. Aspinall and E. G. Meek, *J. Chem. Soc.*, 3830 (1956).

white birch (*Betula papyrifera*) is rather striking. Both contain approximately the same amounts of cellulose, hemicellulose and lignin,[48] the molecular weight of the two celluloses is the same[49] and both hemicelluloses are composed of 1,4-linked β-D-xylopyranose residues, carrying single side chains of 4-*O*-methyl-D-glucuronic acid units.[16] One difference appears to be that the hemicellulose from milkweed floss is slightly branched and contains somewhat less acid side groups than the linear birch polysaccharide. Morphologically, there is, of course, little similarity between the two materials. The lignin in the floss is probably distributed throughout the secondary cell wall, while in the birch it is almost exclusively located in the middle lamella.[50] a component entirely missing in the milkweed floss.

Experimental

All specific rotations are equilibrium values and melting points are corrected. Evaporations were carried out *in vacuo* at 40–50°.

Paper Chromatography.—Solvents (v./v.) used for separating the sugars were (A) ethyl acetate–acetic acid–water (9:2:2), (B) 1-butanol–pyridine–water (10:3:3), (C) butan-1-one–ethanol–water (20:5:2), (D) 1-butanol–ethanol–water (40:11:19), (E) 1-butanol–ethanol–water–ammonia (100:25:127.5:2.5), (F) butan-1-one–benzene–ethanol–water (10:10:5:2) and (G) benzene–ethanol–water (169:47:15). Separations were carried out on Whatman No. 1 filter papers by the descending technique. *o*-Aminobiphenyl was used as spray reagent.[10] R_X values are mobilities relative to D-xylose.

Milkweed Floss.—Mature but unopened pods from the common milkweed (approximately 200 kg.) were collected at the end of the 1955 and 1956 growing seasons (September–October). The fibers were manually deseeded and extracted with ethanol–benzene (1:2, v./v.) to yield 2 kg. of floss.

Isolation of Uronic Acids.—Extractive-free milkweed floss (500 g., 20 mesh) was hydrolyzed with sulfuric acid.[51] After neutralization (barium carbonate) to *p*H 5, filtration through Celite[52] and treatment with Amberlite IR 120[53] exchange resin, sugar acids were adsorbed on a column of Dowex 1-X4[54] anion exchange resin (250 g., bicarbonate form). Neutral sugars were removed by washing with water (negative Molisch test) after which the acidic sugars were eluted with *N* sulfuric acid (1.5 l.). The eluate was neutralized (barium hydroxide), filtered through Celite and concentrated to 100 ml. Chromatographic analysis (solvent A) indicated the presence of an aldotriouronic acid, galacturonic acid and 4-*O*-methyl-D-glucuronic acid together with an aldobiouronic acid, the major component.

Reduction, Hydrolysis and Partial Characterization of the Uronic Acid Mixture.—A portion (15 ml.) of the uronic acid mixture was evaporated to dryness and boiled under reflux with 2% methanolic hydrogen chloride (60 ml.) for 5 hr. After neutralization with silver carbonate (5 g.), treatment with hydrogen sulfide and filtration through Celite, concentration yielded a sirup (1.7 g.) which was reduced in tetrahydrofuran with lithium aluminum hydride. Hydrolysis (sulfuric acid) and neutralization (barium hydroxide) of the reduced material gave a sirup containing galactose, xylose and 4-*O*-methylglucuronic acid, as indicated by paper chromatography (solvent C). The mixture was added to a cocoanut charcoal column.[55] Elution with 1.5% aqueous ethanol removed an unchanged portion while 5% ethanol eluted pure 4-*O*-methyl-D-glucose (252 mg.). The two remaining sugars were separated on sheets of filter paper (solvent A). The xylose (188 mg.) crystallized, m.p. and mixed m.p. 144°, $[\alpha]^{20}D$ +18° (*c* 2.0 in water).

Anal. Calcd. for $C_7H_{12}O_6$: OMe, 16.0. Found: OMe, 16.0; $[\alpha]^{20}D$ +52° (*c* 1.0 in water).

Recrystallization of the osazone from ethanol[13,14] yielded needles, m.p. and mixed m.p. 157–158°. An infrared spectrum was identical to that of an authentic specimen of 4-*O*-methyl-D-glucosazone.

Separation of Sugar Acids.—The main portion of the uronic acid mixture was added to a column (5.5 × 110 cm.) of Dowex 1-X4 exchange resin (acetate form) and eluted with *N* and 3 *N* acetic acid.[15] Five fractions were isolated, all of which contained two or more components. The second fraction was resolved on a cocoanut charcoal column (4 × 12 cm., 50–200 mesh) and eluted with 4–15% aqueous ethanol to yield a triouronic acid (0.20 g.), an aldobiouronic acid (2.00 g.) and a mixture of the latter and a mono-*O*-methyluronic acid (0.45 g.). The fourth fraction from the exchange resin column was resolved on a similar carbon column (2 × 8.5 cm.) with 4% ethanol to give a pure mono-*O*-methylmonouronic acid (0.20 g.). The monouronic acid present together with this acid in the fifth fraction (0.54 g.) was not isolated pure.

Anal. Calcd. for $C_{17}H_{28}O_{15}3H_2O$: OMe, 5.9. Found: OMe, 5.8. $[\alpha]^{20}D$ +91° (*c* 3.7 in water). Calcd. for $C_{12}H_{20}O_{11}$: OMe, 9.1; equiv. wt., 340. Found: OMe, 8.7; equiv. wt., 379; $[\alpha]^{20}D$ +108° (*c* 4.3 in water). Calcd. for $C_7H_{12}O_7$: equiv. wt., 208. Found: equiv. wt., 194; $[\alpha]^{20}D$ +43° (*c* 1.2 in water).

The infrared spectrum of the aldobiouronic acid was identical with a sample of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose isolated from white birch wood.[16]

Chromatographic Identification of Galacturonic Acid.—Fraction 5 above was heated with methanolic hydrogen chloride as described and reduced in tetrahydrofuran with lithium aluminum hydride. Hydrolysis (*N* hydrochloric acid) and neutralization (silver carbonate) yielded a sirup. Examination by paper chromatography (solvents A and B) indicated the presence of galactose and 4-*O*-methylglucose. Elution with water gave chromatographically pure galactose.

Identification of 4-*O*-Methyl-D-glucuronic Acid.—Elution of the charcoal column referred to above with ethanol yielded 4-*O*-methyl-D-glucose (183 mg.).

Anal. Calcd. for $C_7H_{12}O_6$: OMe, 16.0. Found: OMe, 16.0; $[\alpha]^{20}D$ +57° (*c* 2.8 in water).

The 4-*O*-methyl-D-glucosazone had m.p. and mixed m.p. 157–158°. Its infrared spectrum was identical with that of an authentic synthetic specimen.

Reduction and Hydrolysis of the Aldobiouronic Acid.—The aldobiouronic acid (1.10 g.) was boiled under reflux with 2% methanolic hydrogen chloride for 8 hr. After neutralization a portion was reduced with lithium aluminum hydride and hydrolyzed to yield 4-*O*-methylglucose and xylose, which were identified chromatographically.

Methylation, Reduction and Hydrolysis of the Aldobiouronic Acid.—The remaining portion of the ester glycoside of the aldobiouronic acid was methylated three times with methyl iodide (5 ml.) and silver oxide (10 g.) in the presence of calcium sulfate (Drierite) and dimethylformamide[30] to yield a sirup (1.11 g.). Its infrared spectrum showed no absorption for hydroxyl groups and was identical with an authentic specimen of methyl 2-*O*-(2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronic acid)-3,4-di-*O*-methyl-D-xylopyranoside methyl ester obtained from birch wood.[16] The dry sirup was reduced in anhydrous diethyl ether with lithium aluminum hydride, extracted with chloroform and evaporated to give a disaccharide, methanolysis and hydrolysis of which yielded a sirup (564 mg.). Examination by paper chromatography (solvent D) suggested the presence of a tri-*O*-methylglucose and a di-*O*-methylxylose.

Separation and Identification of 2,3,4-Tri-*O*-methyl-D-glucose and 3,4-Di-*O*-methyl-D-xylose.—The sugar mixture was added to a cocoanut charcoal column (2 × 10 cm.) and eluted with aqueous ethanol. Elution with 5% ethanol yielded small amounts of lower substituted sugars, while 10% gave a di-*O*-methylxylose (123 mg.) Ionophoresis[31,33] indicated that it was not the 2,3-di-*O*-methyl derivative since it migrated toward the anode.

Anal. Calcd. for $C_7H_{14}O_5$: OMe, 34.8. Found: OMe, 34.6; $[\alpha]^{20}D$ +22° (*c* 1.0 in water).

(48) T. E. Timell, *Tappi*, **40**, 568 (1957).

(49) T. E. Timell, *Svensk Papperstidn.*, **60**, 836 (1957).

(50) P. W. Lange in E. Treiber, "Die Chemie der Pflanzenzellwand," Springer Verlag, Berlin, 1957, pp. 250–280.

(51) J. F. Saeman, W. E. Moore, R. L. Mitchell and M. A. Millett, *Tappi*, **37**, 336 (1954).

(52) A product of Johns–Manville Co., New York, N. Y.

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

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

(55) Product of Fisher Scientific Co., Fair Lawn, N. J.

The 3,4-di-*O*-methyl-*N*-phenyl-D-xylosylamine was recrystallized from petroleum ether–ethyl acetate (9:1 v./v.), m.p. 115°.

Elution with 25% ethanol gave pure tri-*O*-methylglucose as a sirup (298 mg.).

Anal. Calcd. for $C_9H_{18}O_6$: OMe, 41.9. Found: OMe, 41.6; $[\alpha]^{20}D$ +77° (*c* 2.6 in water).

The 2,3,4-tri-*O*-methyl-*N*-phenyl-D-glucosylamine had m.p. and mixed m.p. 145–146°.[56]

Preliminary Characterization of the Aldotriouronic Acid.—The aldotriouronic acid obtained from the charcoal column was concentrated to a sirup which partly crystallized. A portion (5 mg.) was partially hydrolyzed to yield a mixture of sugars which was resolved by paper chromatography (solvent A). Qualitative examination suggested the presence of unchanged aldotriouronic acid (R_X 0.31), the above aldobiouronic acid, 4-*O*-methyl-D-glucuronic acid and xylose.

Isolation of the Hemicellulose.—Milkweed floss (280 g.) was extracted with 0.5% ammonium oxalate for removal of pectic material. The extractive-free product was shaken with 24% (w./w.) potassium hydroxide (5 l.) in an atmosphere of nitrogen for 2 hr. After filtration through sintered glass, the filtrate was poured into a mixture of methanol (25 l.), acetic acid (3 l.) and acetone (1 l.) previously cooled to −16°. The precipitate was washed three times each with 80% ethanol, anhydrous ethanol and anhydrous diethyl ether and dried *in vacuo;* yield 83 g. (30%). Examination by paper chromatography (solvent A) revealed the presence of xylose and uronic acids only; OMe, 1.51; uronic anhydride, 8.55; Klason lignin, 1.59%; equiv. wt., 2090; $[\alpha]^{20}D$ −99° (*c* 1.0 in 5% potassium hydroxide).

Identification of D-Xylose.—A portion of the hemicellulose was hydrolyzed[51] and the uronic acids in the hydrolyzate were removed with Amberlite IR-45 anion exchange resin.[53] The resulting sirup yielded crystalline D-xylose, m.p. and mixed m.p. 145° after recrystallization from methanol; $[\alpha]^{20}D$ +18° (*c* 3.0 in water).

The dimethyl acetal of the dibenzylidene-D-xylose[57,58] had m.p. and mixed m.p. 211–212°.

Methylation of the Hemicellulose.—The hemicellulose (20 g.) was stirred overnight with water (100 ml.), and 40% (w./w.) aqueous sodium hydroxide (200 ml.) was added in an atmosphere of nitrogen, followed by dropwise addition of dimethyl sulfate (180 ml.) over a 12-hr period. This treatment was repeated twice. In two subsequent, similar treatments solid sodium hydroxide (200 g.) was substituted for the aqueous alkali. The mixture was neutralized with acetic acid and the partially methylated product was isolated in the usual way. A sixth methylation was applied by adding 40% sodium hydroxide (300 ml.) and dimethyl sulfate (180 ml.) to a suspension of the material in tetrahydrofuran[19] (500 ml.). This treatment was repeated once after which two similar methylations were carried out with solid sodium hydroxide (200 g.). The product was dissolved in 90% tetrahydrofuran (500 ml.) and was methylated three times as above to yield a product which was dissolved in anhydrous tetrahydrofuran. Dimethylformamide[30] (500 ml.), methyl iodide (10 ml.) and silver oxide (10 g.) were added and the reaction was allowed to proceed at room temperature for 24 hr. The recovered,[30] fully methylated polysaccharide was poured into petroleum ether (b.p. 65–110°). The product was washed with petroleum ether and dried *in vacuo*.

Anal. Calcd. for the sodium salt of a completely methylated glucuronoxylan containing 14 anhydroxylose units per 4-*O*-methyl-D-glucuronic acid residue: OMe, 37.9. Found: OMe, 37.9; $[\alpha]^{20}D$ −77° (*c* 1.3 in chloroform). The infrared spectrum exhibited no hydroxyl band absorption.

Methanolysis of the Methylated Hemicellulose and Separation of the Acidic Component.—The fully methylated hemicellulose (6.5 g.) was boiled under reflux with methanol containing 2% hydrogen chloride for 10 hr. After neutralization (silver carbonate) and purification (hydrogen sulfide), the resulting sirup was heated at 60° in aqueous barium hydroxide (25 ml.) for 2 hr.[59] Barium hydroxide was removed with carbon dioxide and barium ions with Amberlite IR-120 exchange resin. The sugar acids were adsorbed on a column of Dowex 1-X4 exchange resin (acetate form) which was washed with water to yield a mixture of methylated sugars. The acid fraction was displaced from the column with 3 *N* acetic acid and concentrated to a sirup (739 mg.).

Preparation, Reduction and Hydrolysis of the Ester Glycoside of the Partially Methylated Aldobiouronic Acid.—The acidic glycoside fraction was boiled under reflux with 2.5% methanolic hydrogen chloride (50 ml.) for 6 hr. to yield the ester glycoside (600 mg.). The material was dissolved in dry ethyl ether (20 ml.) and was reduced with lithium aluminum hydride (1 g.) in the same solvent (20 ml.). The disaccharide (570 mg.) was hydrolyzed with *N* sulfuric acid (40 ml.) for 3.5 hr. The solution was adsorbed on a cocoanut charcoal column and the acid was removed with 1.5–2% ethanol (1.5 l.). Elution with 5 and 15% aqueous ethanol yielded compounds which corresponded in rate of movement on the paper chromatogram (solvents E, F and G) with 3-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose.

Characterization of 3-*O*-Methyl-D-xylose and 2,3,4-Tri-*O*-methyl-D-glucose.—The component forming the first fraction (165 mg.) moved at the same rate as an authentic sample of 3-*O*-methyl-D-xylose on ionophoresis in a borate buffer and 1.7 times faster than a sample of 2-*O*-methyl-D-xylose. The infrared spectrum was identical with that of an authentic specimen of 3-*O*-methyl-D-xylose.

Anal. Calcd. for $C_6H_{12}O_5$: OMe, 18.9. Found: OMe, 18.3; $[\alpha]^{20}D$ +15° (*c* 5.5 in water).

The 3-*O*-methyl-*N*-phenyl-D-xylosylamine, when recrystallized from ethyl acetate, had m.p. and mixed m.p. 134–135°.[60] The infrared spectrum of the second fraction was identical with that of 2,3,4-tri-*O*-methyl-D-glucose.

Anal. Calcd. for $C_9H_{18}O_6$: OMe, 41.9. Found: OMe, 41.0; $[\alpha]^{20}D$ +70° (*c* 1.9 in water).

The 2,3,4-tri-*O*-methyl-*N*-phenyl-D-glucosylamine had m.p. and mixed m.p. 146°.[56]

Separation of the Neutral Components of the Methylated Hemicellulose.—The neutral glycosides were hydrolyzed with *N* sulfuric acid (50 ml.) for 7 hr. After neutralization (barium hydroxide) and deionization, the solution was evaporated to a sirup (5.87 g.). Examination by paper chromatography (solvents D, F and G) revealed the presence of three spots, a heavy one corresponding to 2,3-di-*O*-methyl-D-xylose and two faint ones corresponding to 2,3,4-tri-*O*-methyl-D-xylose and a mono-*O*-methylxylose.

Isolation and Preliminary Characterization of the Mono-*O*-methylxyloses.—The mono-*O*-methylxyloses were separated from the remainder of the mixture of neutral sugars on large sheets of filter paper (solvent F) and were eluted from the sheets with water. After concentration, the sirup was dissolved in chloroform, and polysaccharides from the paper[61] were removed by filtration. Paper ionophoresis suggested the presence of approximately equal amounts of 2-*O*- and 3-*O*-methyl-D-xylose.

Isolation and Identification of 2,3-Di-*O*-methyl-D-xylose.—The di-*O*-methylxylose was separated from the neutral hydrolyzate on large sheets of filter paper (solvent G). After purification a sirup was obtained (808 mg.) which could not be induced to crystallize; $[\alpha]^{20}D$ +21° (*c* 1.4 in water).

The 2,3-di-*O*-methyl-*N*-phenyl-D-xylosylamine had m.p. and mixed m.p. 125–126°, $[\alpha]^{20}D$ +180° (*c* 0.6 in ethyl acetate). An infrared spectrum of the aniline derivative was identical with that of an authentic sample.

Isolation and Partial Characterization of 2,3,4-Tri-*O*-methyl-D-xylose.—The tri-*O*-methylxylose was separated from the other two xylose derivatives by chromatography on large sheets of filter paper and also by extraction of the hydrolyzate with chloroform (solvent C). Neither the sugar itself nor its aniline derivative could be induced to crystallize. The latter gave an infrared spectrum identical with that of an authentic sample of 2,3,4-tri-*O*-methyl-*N*-phenyl-D-xylosylamine.

Spectrophotometric Analysis of the Neutral Components of the Methylated Hemicellulose.—A small portion of the

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mixture of methylated neutral sugars was applied to strips of filter paper, pretreated with water, and was resolved with solvent system F. Appropriate sections were located with guide strips, eluted with water and diluted to a suitable volume. The concentration of the solutions was determined by the *o*-aminodiphenyl method.[10] the relation between concentration and absorbance for each sugar having been determined previously.[16] The average value of three determinations was used.

Hypoiodite Oxidation of the Hemicellulose.—The oxidation was carried out with a 0.1 *N* iodine solution buffered to a *p*H of 10.6.[19] The amount of iodine consumed after 2.5 hr. in the dark corresponded to one reducing end-group per 62 anhydroxylose units.

Determination of the Molecular Weight of the Methylated Hemicellulose.—Osmotic pressure measurements were carried out with the osmometer of Zimm and Myerson[62] as improved by Stabin and Immergut.[36] Gel cellophane membranes[63] which had never been allowed to dry, were used, the solvent was chloroform–ethanol (9:1 v./v.) and the temperature was 30 ± 0.01°. The osmotic pressure was determined at six different concentrations by the static method and the values of the reduced osmotic pressure, h/w, as plotted against w, were extrapolated to zero concentration (Table III). The number-average molecular weight, $\bar{M}_n$, was calculated from the relationship $\bar{M}_n = 25{,}700/(h/w)_0$ and the corresponding degree of polymerization, $\bar{P}_n$, from the equation $\bar{P}_n = \bar{M}_n \times n/M_R$, where n was the number of xylose residues present per acid side group and M_R was the molecular weight of the repeating unit of the fully methylated polysaccharide (2457).

TABLE III

OSMOMETRY DATA OBTAINED FOR THE METHYLATED HEMICELLULOSE

w[a]	h[b]	h/w
4.815	9.371	1.946
4.415	7.897	1.789
3.814	6.718	1.761
3.511	6.061	1.726
2.760	4.835	1.752
1.904	3.168	1.664
0	...	1.5 1

[a] Concentration in g./kg. solution. [b] Osmotic height in cm. solvent.

Determination of the Intrinsic Viscosity of the Hemicellulose.—Reduced viscosities (η_{sp}/C) of the potassium salt of the hemicellulose were determined at seven different concentrations with a Craig–Henderson[40] viscometer in *M* cupriethylenediamine. Extrapolation to zero concentration according to Huggins[64] gave an intrinsic viscosity, $[\eta]$, of 0.812 dl./g. and a value of k' of 0.428. Kinetic energy corrections were negligible. According to the relationship $\bar{P}_n = 212[\eta]$, developed earlier for a similar polysaccharide[39] this would correspond to a number-average degree of polymerization of 172.

Estimation of the Polymolecularity of the Hemicellulose.—Hemicellulose (4 g.) was dissolved in 5% aqueous potassium hydroxide (200 ml.) and the solution was diluted to 700 ml. with water and ethanol, until the polysaccharide barely remained in solution. Fractionation was carried out at 25° by gradual addition of ethanol until the solution became cloudy, after which stirring was continued for 10 min. Fourteen fractions were collected on the centrifuge and purified in the same way as the original hemicellulose. Final drying was from petroleum ether (b.p. 30–60°) *in vacuo*. The fractions were weighed and their intrinsic viscosity was determined. The frequency distribution had one maximum located at a $\bar{P}_n$ value of 170 and exhibited a slight negative skewness.

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MONTREAL, QUEBEC

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Acetohalogeno Derivatives and Glycosides of D-Galactosamine[1]

BY ZOFIA TARASIEJSKA AND ROGER W. JEANLOZ[2]

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2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl chloride and bromide have been synthesized from D-galactosamine α- and β-pentaacetates. Under various conditions, they afforded methyl and ethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosides, which were further hydrolyzed into methyl and ethyl 2-acetamido-2-deoxy-β-D-galactopyranosides. The 1-chloro derivative was shown to transpose into 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-galactopyranose hydrochloride. Opening of the 1,6-anhydro ring of 2-acetamido-3,4-di-*O*-acetyl-1,6-anhydro-2-deoxy-β-D-galactopyranose with concomitant acetylation was successfully achieved.

Investigations on the metabolism of aminosugars have demonstrated the need for the availability of phosphate esters of D-galactosamine possessing known chemical structures.[3] The prerequisite intermediates in the synthesis of the 1-phosphate esters of D-galactosamine are the acetohalogeno derivatives. Whereas synthesis of this type of derivative in the *N*-acetyl-D-glucosamine series has been the subject of recent numerous publications,[4–13] the analogous derivatives of

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