Anal. Calcd. for $C_{17}H_{30}O_{11}$: OMe 45.3; equiv. wt., 410. Found: OMe 45.0; equiv. wt., 425.

Methanolysis of Methyl 2-*O*-(2,3,4-Tri-*O*-methyl-D-glucuronosyl)-3,4-di-*O*-methyl-D-xyloside.—A solution of the fully methylated aldobiouronic acid in methanol containing hydrogen chloride (8%) was heated (sealed tube) for 8 hours at 110–115°. The reaction mixture was neutralized with silver carbonate, filtered, and the filtrate evaporated to a sirup. The material was extracted with ether, the ether extract concentrated and hydrolyzed by heating with *N* sulfuric acid on a boiling water-bath until the rotation became constant. The hydrolysate was neutralized with barium carbonate, filtered, concentrated *in vacuo* to a volume of 10–15 ml. and passed successively through a column of ion-exchange resin Amberlite IR120 and of Duolite A4 to remove the acid component. The eluate was evaporated to give the neutral sugar component. Paper chromatographic examination of this neutral sugar fraction using methyl ethyl ketone–water azeotrope as the irrigating solvent revealed a heavy spot having an R_f value of 0.57. Comparison standards of 2,3-di-*O*-methyl-D-xylose and 3,5-di-*O*-methyl-D-xylose gave spots having R_f values of 0.56 and 0.63, respectively. The wide variation in R_f values between 3,5-di-*O*-methyl-D-xylose and the sugar in question discounted the possibility that the former was the derivative obtained from the fully methylated aldobiouronic acid. When sprayed with *p*-anisidine–trichloroacetic acid[18] the 2,3-di-*O*-methyl-D-xylose gave a dark reddish-brown spot while the spot produced by the neutral sugar from the aldobiouronic acid was light tan in color. Heavy spotting on paper chromatograms indicated possible contamination with mono-*O*-methyl-D-xylose so the material was purified by sheet paper chromatography. The 3,4-di-*O*-methyl-D-xylose so produced was a sirup, $[\alpha]^{25}D$ +22.1° (*c* 0.7, in methanol).

A solution of the 3,4-di-*O*-methyl-D-xylose (15 mg.) in water (1 ml.) from the fully methylated aldobiouronic acid was oxidized with bromine (0.1 ml.). The reaction mixture was allowed to stand in the dark for 50 hours after which time the di-*O*-methyl-D-xylose had disappeared from the solution as determined by descending paper chromatography. The excess bromine was then removed by aeration, the hydrogen bromide neutralized with silver carbonate and the resulting solution filtered. Residual silver ions were removed by passing hydrogen sulfide through the solution which was then filtered and evaporated *in vacuo* to a sirup. The latter was heated at 95–100° for 3 hours at 15 mm. pressure in order to bring about lactonization. Upon nucleation of the resulting sirup with 3,4-di-*O*-methyl-D-xylono-δ-lactone complete crystallization took place at once. Recrystallization from ether yielded long colorless needles having m.p. and mixed m.p. 67°, $[\alpha]^{23}D$ −51° (25 minutes); −23° (69 hours, constant).[10]

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ST. PAUL, MINN.

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

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

The Constitution of the Hemicellulose of the Straw of Flax (Linum Usitatissimum Sp.). II. Hydrolysis of the Methylated Hemicellulose

BY J. D. GEERDES AND F. SMITH[1,2]

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The hemicellulose isolated from delignified flax straw by extraction with alkali has been shown to be a branched chain 4-*O*-methyl-D-glucurono-L-rhamnoxylan. The side chains are composed of single units of 4-*O*-methyl-D-glucuronic acid which are attached to position 2 of a D-xylopyranose unit of the xylan molecular framework of which L-rhamnopyranose units are also an integral part. The methylated flax straw hemicellulose gives upon hydrolysis: 2,3,4-tri-*O*-methyl-D-xylose (1 mole), 2,3-di-*O*-methyl-D-xylose (105 moles), 2-*O*-methyl-D-xylose (2 moles), 3-*O*-methyl-D-xylose (15 moles), 2,4-di-*O*-methyl-L-rhamnose (2 moles) and 2,3,4-tri-*O*-methyl-D-glucuronic acid (15 moles). The general structural features of the polysaccharide are discussed.

An aldobiouronic acid, 2-*O*-(4-*O*-methyl-D-glucuronosyl)-D-xylose, has been shown[3] to be a component of the hemicellulose of flax straw. This paper is concerned with the main structural features of the hemicellulose itself.

Paper chromatographic examination of the sugars produced from the acid hydrolyzed hemicellulose showed D-xylose to be the principal component with a small amount of a second neutral sugar component having an R_f value which corresponded to that of L-rhamnose. In addition there was an acidic component consisting of 2-*O*-(4-*O*-methyl-D-glucuronosyl)-D-xylose.[3]

When the hemicellulose was hydrolyzed with acid there was a marked increase in optical rotation. This is generally attributed to the cleavage of β-glycosidic bonds which would be in agreement with the proposed linkage in other xylans.[4,5] The high proportion of xylose in the hemicellulose was shown by the fact that crystalline D-xylose could be obtained readily from the neutral fraction of the hydrolysate of the flax hemicellulose.

In order to ascertain the mode of union of the building units of the flax hemicellulose the latter was acetylated[6] and then methylated with methyl sulfate and alkali. Fractional precipitation with the usual solvents gave products which appeared to be homogeneous as determined by specific rotation, methoxyl values, and neutralization equivalents. Following methanolysis with 2% methanolic hydrogen chloride under conditions which retain the sugar acid in the form of a disaccharide, the glycosides were separated into neutral and acidic components using ion-exchange resins. The acidic component was shown[3] to be methyl 2-(2,3,4-tri-*O*-methyl-D-glucuronosyl)-3-*O*-methyl-D-xyloside.

(1) Paper No. 3311, Scientific Journal Series, Minnesota Agricultural Experiment Station.

(2) Extracted from a thesis submitted by J. D. Geerdes to the graduate faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of Ph.D., 1953. This paper was presented at the 125th A.C.S. meeting in Kansas City, 1954.

(3) J. D. Geerdes and F. Smith, THIS JOURNAL, **77**, 3569 (1955).

(4) W. N. Haworth and E. G. V. Percival, *J. Chem. Soc.*, 2850 (1931).

(5) R. J. Mellroy, *ibid.*, 121 (1949).

(6) J. F. Carson and W. D. Maclay, THIS JOURNAL, **70**, 293 (1948).

Furthermore, the introduction of a methyl group by methylation into the 4-position of the xylose unit of the isolated methyl aldobiouronic acid established that in the polysaccharide the xylose moiety is joined to other units through position 4. Since it is also joined through its reducing group, this particular xylose residue clearly constitutes a branch point in the molecular complex. It may be further deduced that the 4-*O*-methyl-D-glucuronic acid is attached as a single side chain unit to the 2-position of xylose by a glycosidic bond in the original polysaccharide molecule since only the 3-*O*-methyl-D-xylose is obtained from the methylated aldobiouronic acid of the methylated polysaccharide.

The mixture of neutral sugar glycosides obtained from the methanolysis of the methylated flax straw hemicellulose was hydrolyzed and resolved into its components on a cellulose–hydrocellulose column.[7] The mixture was found to contain 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose, 3-*O*-methyl-D-xylose, 2,4-di-*O*-methyl-L-rhamnose, a small amount of D-xylose and 4-*O*-(2,3-di-*O*-methyl-D-xylopyranosyl)-2,3-di-*O*-methyl-D-xylose.

The 2,3,4-tri-*O*-methyl-D-xylose was identified by qualitative chromatography, by rotation and by the fact that its anilide had the same R_f value as an authentic specimen of 2,3,4-tri-*O*-methyl-D-xylose anilide. The identity of the 2,3-di-*O*-methyl-D-xylose was established by its transformation into the characteristic crystalline anilide.[8] The identity of the xylobiose, 4-*O*-(2,3,di-*O*-methyl-D-xylopyranosyl)-2,3-di-*O*-methyl-D-xylose, was established by the observation that hydrolysis of it gave 2,3-di-*O*-methyl-D-xylose. When the monomethyl sugar fraction obtained from the column separation was seeded with 2-*O*-methyl-D-xylose, crystallization occurred, yielding this same crystalline sugar. From the mother liquor of the 2-*O*-methyl-D-xylose there was also isolated a small amount of crystalline 3-*O*-methyl-D-xylose.

The component (no. 3, Table III) leaving the column between the 2,3-di-*O*-methyl-D-xylose, and the 2,3,4-tri-*O*-methyl-D-xylose fractions had an R_f value which corresponded to that of a di-*O*-methyl-L-rhamnose. Although it could not be induced to crystallize it readily gave a crystalline anilide. Since 3,4-di-*O*-methyl-L-rhamnose crystallizes very readily it was deduced that the unknown di-*O*-methyl rhamnose was probably not the 3,4-di-*O*-methyl derivative, a view supported by the fact that 3,4-di-*O*-methyl-L-rhamnose gave a sirupy anilide. That the compound was a di-*O*-methyl derivative of L-rhamnose was demonstrated by the fact that direct methylation of the crystalline anilide with silver oxide and methyl iodide[9] afforded the known crystalline 2,3,4-tri-*O*-methyl-L-rhamnose anilide. The possibility that it was 2,3-di-*O*-methyl-L-rhamnose was next examined. The melting point of the anilide of 2,3-di-*O*-methyl-L-rhamnose was almost the same as the anilide of the unknown di-*O*-methyl-L-rhamnose anilide but the mixed melting point showed a depression of 16°. This left only the 2,4-di-*O*-methyl-L-rhamnose to be tested; a specimen of the anilide of 2,4-di-*O*-methyl-L-rhamnose was found to give no depression when mixed with the unknown di-*O*-methyl-L-rhamnose anilide obtained from the flax hemicellulose. The X-ray powder diffraction patterns of the two anilides offered further confirmation that the di-*O*-methyl-L-rhamnose compound under examination was indeed the 2,4-di-*O*-methyl derivative.

From the above experimental evidence, certain structural features of the hemicellulose can be deduced. It is clear that the 2,3,4-tri-*O*-methyl-*d*-xylose is derived from terminal xylopyranose units in the polysaccharide and from the large amount of 2,3-di-*O*-methyl-D-xylose obtained it is concluded that the main body of the polysaccharide consists of D-xylose units of the pyranose type linked, as already pointed out, through positions 1 and 4. The 2-*O*-methyl-D-xylose is derived from units of xylose which form branch points in the molecule; clearly these units are joined through positions 1 and 3. The isolation of 2,4-di-*O*-methyl-L-rhamnose indicates that this sugar is mutually joined to other units of the polysaccharide through positions 1 and 3.

Considering the mole ratios of the cleavage products of the methylated hemicellulose (see Table I) it will be apparent that the polysaccharide molecule has one aldobiouronic acid residue associated with 7 D-xylose units. Discounting for the present the minor components, the average "repeating unit" consists of one 4-*O*-methyl-D-glucuronic acid unit attached as a side chain to a position 2 of one of eight D-xylose units. This would correspond to a "repeating unit" weight of 1487 for the methylated polysaccharide which is in accord with the value (1485) of the neutralization equivalent. The number of terminal units (2,3,4-tri-*O*-methyl-D-xylose) found indicates there are 15 of these repeating units in a single molecule having as the reducing ends D-xylose or L-rhamnose. The L-rhamnose units if not in a terminal reducing position are located in the main chain of the molecule. The exact position of these rhamnose residues is not known but it is clear that they cannot arise either from branching points or from non-reducing terminal positions. The amount of 2-*O*-methyl-D-xylose obtained from the methylated polysaccharide indicates that there is an average of 2 xylose units at which branching occurs in the xylose chain in addition to the 15 xylose units of the chain which form branch points for the attachment of 4-*O*-methyl-D-glucuronic acid side chains.

TABLE I

THE HYDROLYSIS PRODUCTS OF METHYLATED FLAX STRAW HEMICELLULOSE

Sugar derivative	Mole ratio (approx.)
2,3,4-Tri-*O*-methyl-D-xylose	1
2,3-Di-*O*-methyl-D-xylose	105
2-*O*-Methyl-D-xylose	2
3-*O*-Methyl-D-xylose	15
2,4-Di-*O*-methyl-L-rhamnose	2
2,3,4-Tri-*O*-methyl-D-glucuronic acid	15

Although the trace amounts of xylose formed by hydrolysis of the methylated polysaccharide could

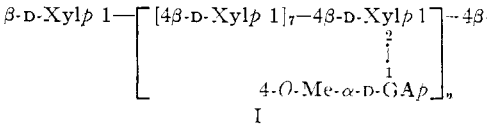
(7) J. D. Geerdes, Bertha A. Lewis, R. Montgomery and F. Smith, *Anal. Chem.*, **26**, 264 (1954).

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

(9) I. Ehrenthal, M. C. Rafique and F. Smith, *ibid.*, **74**, 1341 (1952).

conceivably arise from demethylation[10] or incomplete methylation,[11] the possibility should not be overlooked that it represents a unit in the molecule at which multiple branching occurs.

From these facts a simplified structure I for flax straw hemicellulose may be tentatively proposed.



I

The rhamnose units being joined by 1–3 bonds must form part of the main chain in which case this portion of the molecule would be indicated by

—4-D-Xyl*p* 1—3-L-Rha*p* 1—4-D-Xyl*p* 1—

For convenience the molecule is written with a free reducing group. This may or may not be the case for it is conceivable that the structure depicted may be joined to another one and thus may provide a branching xylose unit which would give rise to the formation of the 2-*O* methyl-D-xylose upon methylation.

This additional information concerning the hemicellulose of flax straw together with that already known about the hemicellulose of esparto grass[10,12] wheat straw,[8,13] corn cobs,[8] pear cell-wall[10] and *Phormium tenax*[5] emphasizes the view that in all these polysaccharides there is present a framework consisting of D-xylopyranose units. The properties of these hemicelluloses vary considerably which would now seem to be due to the nature of the side chains which are attached to the main structural framework of xylopyranose units and to the extent of regularity or irregularity of the branching. In esparto grass hemicellulose the pure polysaccharide contains few if any other sugar units[10] but in the case of the wheat straw hemicellulose[8,13] the side chains consist of L-arabinose units. In the wheat straw hemicellulose studied by Adams[13] the side chains consist of L-arabinose and D-glucuronic acid units, while corn cob hemicelluloses have been obtained which have L-arabinose units[8] and glucuronic acid units[14] attached to the basic anhydroxylose framework. In the case under discussion, the side chains consist almost entirely of units of 4-*O*-methyl-D-glucuronic acid.

Experimental[15]

Hydrolysis of Flax Straw Hemicellulose.—After removing the acidic compound from the selectively acid-hydrolyzed hemicellulose with ion-exchange resins (see preceding paper) the neutral sugar fraction crystallized spontaneously upon removal of solvent and gave D-xylose m.p. and mixed m.p. 146°, $[\alpha]^{25}D$ +18.6° (*c* 1.2, in water). Qualitative paper chromatography of the mother liquor using solvent A gave spots having R_f values of 0.49 and 0.65 corresponding to D-xylose and L-rhamnose, respectively.

Acetylation of Flax Hemicellulose.—A solution of the hemicellulose (7.2 g.) in formamide (100 ml.) was treated with pyridine (65 ml.) followed by acetic anhydride (50 ml.), the latter being added gradually with stirring to keep the temperature below 45°. Two hours after the addition of the acetic anhydride the reaction mixture was poured into a 1% hydrochloric acid solution (1 l.) with stirring, whereupon the acetylated polysaccharide precipitated as a gel. This was allowed to remain in the acidic solution overnight to leach out pyridine and formamide from the gel. The acetylated product was filtered, washed with water, resuspended twice in absolute alcohol, filtered and dried. Ignition showed that the product contained very little, if any, ash. The yield of crude acetylated hemicellulose was 95% of theory.

Methylation of Flax Hemicellulose Acetate.—A solution of the hemicellulose acetate (13 g.) in 1,4-dioxane (100 ml.) was treated simultaneously with 45% potassium hydroxide (250 ml.) and methyl sulfate (75 ml.). The reagents were added during a 3-hr. period with vigorous stirring and the temperature was kept at 45°, acetone being added when necessary to keep the polysaccharide in solution. The reaction was completed by heating for 1 hr. in a boiling water-bath to decompose the excess methyl sulfate and to expel the excess of the acetone, whereupon the partially methylated product separated. Sufficient boiling water was then added to dissolve the salts and the product was filtered. The methylated hemicellulose was dissolved in acetone and remethylated in the same manner as before. After 5 methylations had been applied in this way using acetone as the solvent the methylated product was dissolved in aqueous acetone (the minimum amount of acetone was used) and the solution treated with dilute hydrochloric acid to *p*H 5 whereupon the methylated polysaccharide was precipitated. Further addition of acid failed to precipitate more product. After removing the methylated flax straw hemicellulose thus precipitated, by filtration, it was dried *in vacuo* and dissolved in acetone (70 ml.). When the acetone solution was poured slowly with stirring into light petroleum ether (10 vol.), the methylated flax straw hemicellulose was obtained as an amorphous almost white powder which after filtration and drying *in vacuo* amounted to 9.9 g. and had $[\alpha]^{25}D$ −46.2° (*c* 1.0, in chloroform), equiv. wt. 1485.

Anal. Calcd. for a methylated polysaccharide with the repeating unit proposed (formula I): OMe, 37.6. Calcd. for a methylated pentosan: OMe, 38.7. Found: OMe, 36.6.

The methylated hemicellulose (8.3 g.) was dissolved in acetone (160 ml.) and light petroleum ether (b.p. 30–60°) was added slowly in increasing amounts with stirring to effect fractional precipitation. The properties of the five fractions obtained are given in Table II.

TABLE II

FRACTIONAL PRECIPITATION OF METHYLATED FLAX STRAW HEMICELLULOSE

Fraction	Total petroleum ether added (ml.)	Wt., g.	$[\alpha]^{25}D$ $CHCl_3$ (*c* 1.0)	OCH_3 (%)	Equiv. wt.
I	7[a]	0.25	.....	..	..
II	34	3.35	−54.8°	35.5	1490
III	56	1.25	−53.2°	35.5	1430
IV	65	1.78	−53.2°	36.5	1410
V	80	0.83	−54.1°	36.6	1470

[a] Fraction I, a yellow-grey precipitate, was discarded since it contained inorganic material.

Hydrolysis of Methylated Flax Straw Hemicellulose.—A solution of the methylated hemicellulose (3.086 g.) in methanol (60 ml.) containing 2% hydrogen chloride was refluxed for 12 hours until the rotation became constant. The solution was neutralized with silver carbonate, filtered and evaporated *in vacuo* to a thick sirup of the methyl glycosides (3.425 g.).

The glycosides were dissolved in barium hydroxide solution (50 ml., saturated at room temperature) and the methyl ester of the acid component saponified by warming the mixture for 2 hours at 60°. The excess barium hydroxide was neutralized with carbon dioxide and the solution heated for 15 minutes at 85° to decompose the barium hydrogen car-

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

(11) S. K. Chanda, E. L. Hirst and E. G. V. Percival, *ibid.*, 1240 (1951).

(12) W. N. Haworth, E. L. Hirst and Elsie Oliver, *ibid.*, 1917 (1934).

(13) G. A. Adams, *Can. J. Chem.*, **30**, 698 (1952).

(14) R. L. Whistler and L. Hough, THIS JOURNAL, **75**, 4918 (1953).

(15) For the partition chromatographic analyses the following solvents were used: A, phenol saturated with water; B, methyl ethyl ketone:water azeotrope; C, 1-butanol–ethanol–water (4:1:5).

TABLE III

COLUMN CHROMATOGRAPHIC SEPARATION OF NEUTRAL COMPONENTS OF THE HYDROLYSATE OF METHYLATED FLAX STRAW HEMICELLULOSE

Collection tube no.		Component			
Separation 1	Separation 2	No.	Identity	Wt. (mg.)	$[\alpha]^{23}D$
11–15	10–15	1	2,3,4-Tri-*O*-methyl-D-xylose	20.3	+18.7°(MeOH)
16–19		1 + 2			
20–24	17–22	2	4-*O*-(2,3-Di-*O*-methyl-D-xylopyranosyl)-2,3-di-*O*-methyl-D-xylose	34.1	+100°(MeOH)
25–26		2 + 3			
27–34	24–39	3	2,4-Di-*O*-methyl-L-rhamnose	37.3	+42°(MeOH)
35–42		3 + 4			
43–95	40–70	4	2,3-Di-*O*-methyl-D-xylose	1929.5	+23.5°(H_2O)
113–140		5	2-*O*-Methyl-D-xylose	29.2	+30°(H_2O)
			3-*O*-Methyl-D-xylose	10.5	
151–210		6	D-Xylose	6.0	
			Recovery = 2066.9 or 95%		

bonate. After filtering the solution it was evaporated *in vacuo* to yield a sirup (3.353 g.) in which the acidic component was present in the form of its barium salt. The mixture of sugar glycosides was dissolved in water and passed through a column of cation-exchange resin, Amberlite IR 120,[16] to convert the barium salt into the corresponding free acid glycoside. Concentration of the acidic eluate under reduced pressure gave a sirup (3.117 g.). The acid component of the sirupy glycoside mixture was selectively removed by passing an aqueous solution of the mixture through an anion-exchange resin (Duolite A-4).[17] Evaporation of the neutral eluate *in vacuo* at 40–50° to constant weight yielded a mixture of methylated sugar glycosides (2.434 g.). The acidic glycoside (0.608 g.) was recovered from the resin by elution with alkali followed by treatment with a cation-exchange resin, Amberlite IR 120. The details of the procedure are quoted in the previous paper.[3]

Separation of the Components of the Non-acidic Fraction from Methylated Flax Straw Hemicellulose.—When a solution of the neutral sugar methyl glycosides (2.434 g.) in *N* sulfuric acid (120 ml.) was heated on a boiling water-bath, the specific rotation of the solution changed from $[\alpha]^{23}D$ +56.0° to +25.2° (constant value). The solution was neutralized with barium carbonate, filtered and concentrated *in vacuo* to give the sirupy methyl sugars (2.284 g.).

A portion of the methylated sugar mixture (2.177 g.) was dissolved in methyl ethyl ketone–water azeotrope solution and placed on a column (40 × 3 cm.) packed with a mixture of hydrocellulose and cellulose (1:1).[7] The same solvent was used as the chromatographic developer. The effluent from the column was collected in tubes automatically changed every 10 minutes for the faster moving components (tubes 1 to 90) and every 30 minutes for the slower moving components with lower R_f values (tubes 90 to 210). The tubes were numbered from the time the irrigating solvent had passed through one length of the adsorbent, this being determined by placing a small amount of dye (Sudan IV) on the column with the sugar mixture to be separated. The presence of sugars in the various tubes was determined by spotting a small amount of eluate from each tube on filter paper and spraying with *p*-anisidine–trichloroacetic acid reagent.[18] When spotting indicated possible overlapping of components, the effluents were analyzed by descending paper chromatography (solvent B). Those eluates from tubes containing mixtures were combined, evaporated to a sirup and again placed on the column for further separation. The results are shown in Table III.

Paper chromatographic examination indicated complete separation was obtained in the second pass through the column.

Assuming that component 2 affords the theoretical amount of 2,3-di-*O*-methyl-D-xylose upon hydrolysis and taking into account the weight (680 mg.) of methyl-2-*O*-(2,3,4-tri-*O*-methyl-D-glucuronosyl)-3-*O*-methyl-D-xyloside separated by ion-exchange resins (see preceding paper), then the cleavage products of methylated flax straw hemicellulose are as follows: 2,3,4-tri-*O*-methyl-D-xylose (20.3 mg., 1 mole), 2,3-di-*O*-methyl-D-xylose (1963.6 mg., 105 moles), 2-*O*-methyl-D-xylose (29.2 mg., 2 moles), 3-*O*-methyl-D-xylose (262.4 mg., 15 moles), 2,4-di-*O*-methyl-L-rhamnose (37.3 mg., 2 moles) and 2,3,4-tri-*O*-methyl-D-glucuronic acid (301.7 mg., 15 moles).

Identification of the Components. (a) **Component 1; 2,3,4-Tri-*O*-methyl-D-xylose.**—The thick yellow sirup (20.3 mg.) showed $[\alpha]^{23}D$ +18.7° (*c* 0.9, in methanol) and had the same R_f value on paper as 2,3,4-tri-*O*-methyl-D-xylose when tested with solvent B and solvent C. It failed to crystallize directly when nucleated with an authentic specimen of 2,3,4-tri-*O*-methyl-D-xylose but after distillation (b.p. 120°, 0.002 mm.) it did so. The crystalline anilide obtained from the distillate had the same R_f value as that of an authentic specimen of the anilide of 2,3,4-tri-*O*-methyl-D-xylose but there was insufficient for purposes of isolation.

(b) **Component 2; Identification of 2,3-Di-*O*-methyl-D-xylose.**—This component (34.1 mg.), having $[\alpha]^{21}D$ +100° in methanol (*c* 1.7) was hydrolyzed with *N* sulfuric acid in a sealed tube on a boiling water-bath for 13 hours. Neutralization of the hydrolysate with barium carbonate, followed by filtration and evaporation, gave a yellow sirup. The hydrolyzed compound no longer had the original R_f value of 0.71 but one of 0.54 (solvent B) that corresponded to 2,3-di-*O*-methyl-D-xylose. The sirupy hydrolysate had $[\alpha]^{28}D$ +23.7° in water (*c* 0.9) which is close to the value for 2,3-di-*O*-methyl-D-xylose, and upon treating it (17 mg.) with aniline (12 mg.) in boiling ethanol (1 ml.) for 2.5 hours the crystalline anilide of 2,3-di-*O*-methyl-D-xylose was obtained m.p. and mixed m.p. 126°, after recrystallization from ethyl acetate.

The component was, therefore, most likely 4-*O*-(2,3-di-*O*-methyl-D-xylopyranosyl)-2,3-di-*O*-methyl-D-xylose and it was added to the weight of the 2,3-di-*O*-methyl-D-xylose (component 4) before computing the molecular proportions of the cleavage fragments of the methylated hemicellulose.

(c) **Component 3; 2,4-Di-*O*-methyl-L-rhamnose.**—This component, a sirup (37.3 mg.) had $[\alpha]^{24}D$ +42° in methanol (*c* 0.9), R_f 0.64 (solvent B) and R_f 0.70 (solvent C). These values agreed with those of 2,4-di-*O*-methyl-L-rhamnose.

Treatment of the compound with *N* sulfuric acid in a sealed tube at 95° for 6 hours failed to affect it (R_f value (solvent B) unchanged). The compound was therefore not an oligosaccharide but a simple methyl sugar.

When the compound (30 mg.) was treated with absolute ethanol (1.3 ml.) containing aniline (35 mg.) in the usual way a crystalline anilide (needles), was produced, m.p. 133–134°, $[\alpha]^{25}D$ + 137° in methanol (*c*, 0.7), after recrystallization from ether. It gave no depression of the m.p. when mixed with an authentic specimen of the anilide of 2,4-di-*O*-methyl-L-rhamnose[19] but it gave a depression of the m.p. when in admixture with 2,3-di-*O*-methyl-L-rhamnose anilide.[20]

The R_f value (solvent B) of the 2,4-di-*O*-methyl-L-rhamnose anilide proved to be identical with that of the authentic

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

(17) A product of the Chemical Process Co., Redwood, Calif.

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

(19) Kindly provided by Professor M. Stacey, F. R. S., University of Birmingham, England.

(20) Kindly provided by Dr. E. E. Percival, University of Edinburgh, Scotland.

specimen. A comparison of the X-ray diagram of the 2,4-di-*O*-methyl-L-rhamnose anilide with that of an authentic specimen of this anilide revealed that the two were identical.[21]

When a solution of the 2,4-di-*O*-methyl-L-rhamnose anilide (1.8 mg.) in methyl iodide (5 ml.) was treated under reflux with silver oxide (50 mg.) during 18 hours[9] the corresponding fully methylated anilide was formed. Extraction with acetone gave crystalline 2,3,4-tri-*O*-methyl-L-rhamnose anilide m.p. and mixed m.p. 110° (after recrystallization from ether–light petroleum ether).

Of interest also were the characteristic yellow spots shown by the rhamnose derivatives when sprayed with *p*-anisidine-trichloroacetic acid reagent.[18] Di-*O*-methyl-D-xylose derivatives generally show dark brown colors while tri-*O*-methyl-D-xylose or its anilide gave a characteristic red color. These facts helped to eliminate the possibility that component 3 was a methyl xylose derivative.

(d) **Component 4; 2,3-Di-*O*-methyl-D-xylose.**—This sirupy component (1.9295 g.) was 2,3-di-*O*-methyl-D-xylose, $[\alpha]^{23}D$ +23.5° in water (*c* 1.3). *Anal.* Calcd. for $C_7H_{14}O_5$: OMe, 34.8. Found: OMe, 35.1.

The anilide formed in the usual way and recrystallized from ethyl acetate proved to be 2,3-di-*O*-methyl-D-xylose anilide m.p. and mixed m.p. 126°, $[\alpha]^{23}D$ +181° in ethyl acetate (*c* 1.0). *Anal.* Calcd. for $C_{13}H_{19}O_4N$: OMe, 24.5. Found: OMe, 24.5.

(e) **The Isolation of 2-*O*-Methyl- and 3-*O*-Methyl-D-xylose from Component 5.**—Component 5, consisting of a sirup (39.7 mg.), had $[\alpha]^{23}D$ +30° in water (*c* 1.2). Paper chromatographic examination using solvent B indicated this fraction to be a mixture of two sugars corresponding in R_f values to 2-*O*-methyl-D-xylose and 3-*O*-methyl-D-xylose. This fraction was therefore separated into its respective components by sheet paper chromatography.

The sirup from the band having an R_f value corresponding to 2-*O*-methyl-D-xylose crystallized when nucleated with this sugar and recrystallization from ethyl ether yielded 2-*O*-methyl-D-xylose m.p. and mixed m.p. 132–133°.

The sirup from the band corresponding to 3-*O*-methyl-D-xylose crystallized when nucleated with 3-*O*-methyl-D-xylose and had m.p. and mixed m.p. 98–100° (after recrystallization from ethyl acetate).

From the known equilibrium specific rotations in water of 2-*O*-methyl-D-xylose (+35.9°) and 3-*O*-methyl-D-xylose (+14.8°) and the rotation of component 5 the amount of 2-*O*-methyl-D-xylose and 3-*O*-methyl-D-xylose in the mixture was calculated to be 29.2 and 10.5 mg., respectively. Since it is not unlikely that some cleavage of the aldobiouronic acid occurred during the graded hydrolysis of the methylated polysaccharide, the 3-*O*-methyl-D-xylose, was probably derived by partial cleavage of the 3-*O*-methyl-D-xylose unit of the methylated aldobiouronic acid.[3]

(f) **Component 6, (?) D-Xylose.**—This component, a dark yellow sirup amounting to 6.0 mg., appeared to be xylose from its R_f value (solvent A, B and C). It was by no means pure and it may not be of any constitutional significance. It failed to give a dibenzylidene dimethyl acetal derivative.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF CALIFORNIA AT LOS ANGELES]

Synthesis and Properties of Certain Spiro[4.4]nonenes[1]

BY DONALD J. CRAM AND B. L. VAN DUUREN

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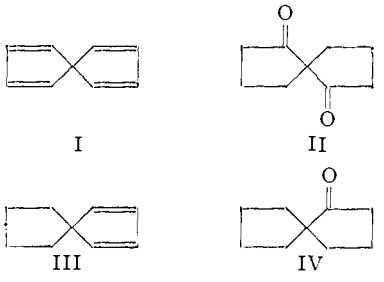
The synthesis of 1-spiro[4.4]nonene (VIII), 1,3-spiro[4.4]nonadiene (III) and 1,6-spiro[4.4]nonadiene (XVIII) are described, as well as the behavior of III as a dienophile in a Diels–Alder reaction.

The objective of this investigation was the exploration of the chemistry of the spiro[4.4]nonenes, the compound 1,3,6,8-spiro[4.4]nonatetraene (I) being the ultimate target for the work. Since 1,6-diketospiro[4.4]nonane (II)[2] was an obvious but expensive starting material, the model synthesis of 1,3-spiro[4.4]nonadiene (III) from the more available 1-ketospiro[4.4]nonane (IV) was developed first.



I II III IV

The monoketone IV[3] was converted to its oxime which was reduced catalytically to provide 1-aminospiro[4.4]nonane[2] (V) which was dimethylated with formaldehyde and formic acid[4] to give 1-dimethylaminospiro[4.4]nonane (VI). This material was converted to the amine oxide VII which on pyrolysis gave 1-spiro[4.4]nonene (VIII).[5] The same olefin was obtained in better yield through the Hofmann elimination reaction, the infrared spectra and physical properties of the two olefin samples being identical.

Olefin VIII was characterized by quantitative catalytic hydrogenation to the known saturated spiro[4.4]nonane (IX).[6] Bromination of olefin VIII with N-bromosuccinimide gave an unstable oil which could not be purified, and which was converted directly to 3-dimethylamino-1-spiro[4.4]-nonene. Alkylation of this material led to the quaternary compound XI which when heated with silver oxide gave diene III. This material was thermally unstable and when distilled at atmospheric pressure appeared to dimerize to give the

(1) This research was conducted under contract AF 33(616)-146 with the United States Air Force, the sponsoring agency being the Aeronautical Research Laboratory of the Wright Air Development Center, Air Research and Development Command.

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