of Sciences of the United States of America. The model compounds used in the paper chromatography were kindly given by Dr. Carlton W. Dence of this College, and initial exploratory work on this reaction was carried out by Mr. William Erby.

SYRACUSE, N. Y.

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

The Constitution of the Hemicelluloses of Sitka Spruce (*Picea sitchensis*). III. Structure of an Arabomethoxyglucuronoxylan[1]

BY G. G. S. DUTTON AND K. HUNT

RECEIVED AUGUST 10, 1959

Methylation of sitka spruce hemicelluloses obtained by potassium hydroxide extraction of a chlorite holocellulose preparation yielded a homogeneous sample after fractional precipitation. Hydrolysis yielded 2,3,4-tri-*O*-methyl-D-xylose (1 mole), 2,3,5-tri-*O*-methyl-L-arabinose (0.4 mole), 2,3-di-*O*-methyl-D-xylose (10 moles), 2-*O*-methyl-D-xylose (0.3 mole), 3-*O*-methyl-D-xylose (1.2 moles) and 2-*O*-(2,3,4-tri-*O*-methyl-D-glucuronosyl)-3-*O*-methyl-D-xylose (3.4 moles). The structural features of the polysaccharide are discussed.

Previous papers in this series have reported on the nature of the aldobiouronic acid[2] and the mannan portion[3] of sitka spruce hemicelluloses. The present paper reports the constitution of an arabomethoxyglucuronoxylan. The current status of research on the constitution of hemicelluloses from coniferous trees has recently been summarized[4] and is not repeated here.

Sitka spruce sawdust was treated with sodium chlorite and the resulting holocellulose extracted with 5% potassium hydroxide.[2] The mixed hemicelluloses were methylated, first with methyl sulfate and sodium hydroxide and finally with Purdie reagents. The partly methylated polysaccharide did not separate from the aqueous solution during the Haworth methylations and had to be recovered by dialysis and evaporation. The fully methylated product was fractionated from chloroform solution with petroleum ether and the results are shown in Table II. Fraction 23b appeared to be homogeneous and was used in the subsequent experiments. Methanolysis with 2% hydrogen chloride in methanol cleaved the polysaccharide which was separated into neutral and acidic components by the use of ion-exchange resins. The methyl ester of the acidic component was reduced with lithium aluminum hydride and yielded crystalline methyl 2-*O*-(2,3,4-tri-*O*-methyl-D-glucopyranosyl)-3-*O*-methyl-D-xyloside identical with that originally obtained from western hemlock[5] and since obtained from sugar maple.[6] The neutral sugars were resolved on a cellulose–hydrocellulose column[7] using butanone–water azeotrope as the solvent.[8] Three fractions were obtained and paper chromatography showed these to contain 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose and two monomethyl-D-xyloses. The first two have very similar R_f values and may be separated either by using a benzene–ethanol–water solvent[9] or by taking advantage of the preferential furanoside formation of the arabinose derivative.[5] The latter method of separation was used and the xylose derivative was characterized as the crystalline 2,3,4-tri-*O*-methyl-N-phenyl-D-xylosylamine[10] and the arabinose derivative by paper chromatography after hydrolysis of the furanoside. The 2,3-di-*O*-methyl-D-xylose, which formed the major component, was identified as the crystalline sugar.[11] The 2- and 3-*O*-methyl-D-xyloses did not separate sufficiently well to enable crystalline derivatives to be prepared but enough of each sugar was obtained chromatographically pure to permit comparison with authentic samples.

From the above experimental evidence it is clear that the main structural feature of the hemicellulose is a chain of D-xylopyranose units linked through positions 1 and 4. Since hydrolysis involves an increase in rotation it is presumed that β-linkages are involved. The isolation of the known crystalline disaccharide glycoside from the acidic component shows that the uronic acid occupies a terminal position and that the uronic acid is joined to C_2 of the main xylose chain thus confirming our previous results.[2] The linkage in the aldobiuronic acid was judged to be α because of the high positive rotation and this now has been proved by lead tetraacetate degradation.[12] Since arabinose was only isolated in the form of 2,3,5-tri-*O*-methyl-L-arabinose it is clear that the arabofuranose units must also occupy terminal positions and strongly suggests that the arabinose is an integral part of the molecule. No free uronic acid was observed after methanolysis and hydrolysis and thus the monomethylxylose units must represent branch points or artifacts caused by incomplete methylation or demethylation during hydrolysis. In view of the relative amounts of the two isomers obtained it is possible that the arabinose is linked to position 3 of the main chain and that there is limited branching at position 2.

(1) Presented at the 136th A.C.S. Meeting, Atlantic City, N. J., Sept., 1959.

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

(3) G. G. S. Dutton and K. Hunt, *ibid.*, **80**, 5097 (1958).

(4) J. K. N. Jones and T. J. Painter, *J. Chem. Soc.*, 573 (1959).

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

(6) T. E. Timell, *Can. J. Chem.*, **37**, 893 (1959).

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

(8) L. Boggs, L. S. Cuendet, I. Ehrenthal, R. Koch and F. Smith, *Nature*, **166**, 520 (1950).

(9) G. A. Adams, *Can. J. Chem.*, **33**, 56 (1955).

(10) R. A. Laidlaw and E. G. V. Percival, *J. Chem. Soc.*, 1600 (1949).

(11) E. G. Meek, *ibid.*, 219 (1956).

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

It may be seen from Table I that the sitka spruce "xylan" has a repeating unit of about 16 xylose units to which are attached three 4-*O*-methyl-D-glucuronic acid side chains at position 2 and an occasional arabofuranose unit at position 3. In this respect the sitka spruce arabomethoxyglucuronoxylan most closely resembles that of western hemlock[5] and apart from the presence of arabinose is similar to that from loblolly pine.[4] European larch[13] appears to have an essentially linear acidic "xylan" containing arabinose whereas that from Norway spruce is linear but without arabinose.[14] The presence of arabinose seems to be a minor and variable quantity and arabinose is absent entirely in the "xylans" from apple wood and cherry wood.[15]

TABLE I

HYDROLYSIS PRODUCTS OF SITKA SPRUCE METHYLATED XYLAN

Sugar derivative	Wt., mg.	Mole ratio (approx.)
2,3,4-Tri-*O*-methyl-D-xylose	28	1
2,3,5-Tri-*O*-methyl-L-arabinose	12	0.4
2,3-Di-*O*-methyl-D-xylose	256	10
3-*O*-Methyl-D-xylose	28	1.2
2-*O*-Methyl-D-xylose	8	0.3
2-*O*-(2,3,4-Tri-*O*-methyl-D-glucuronosyl)-3-*O*-methyl-D-xylose	195	3.4

It is of interest to note that in all cases but one in which the structures of xylose hemicelluloses have been reported the uronic acid has occupied a terminal position and it has invariably been isolated as an aldobiuronic acid because of the difficulty of hydrolyzing the glycosidic bond. The one exception so far noted is that of hemicellulose B from ground nut shells.[16] It is claimed that in this instance the L-arabinose units are linked to position 4 of the glucuronic acid which in turn is glycosidically linked to the main xylose chain. This exception is important since it suggests an alternative biosynthetic pathway and the structure of this hemicellulose is at present being reinvestigated by us.

Experimental[17]

Isolation of Sitka Spruce Hemicellulose.—Chlorite holocellulose was extracted with 5% potassium hydroxide and the hemicelluloses precipitated by the addition of ethanol after acidification with acetic acid. From 300 g. of sawdust there was obtained 23.9 g. of hemicellulose having $[\alpha]^{23}D$ −8° (*c*, 0.6 in water).

Methylation of Sitka Spruce Hemicellulose.—Hemicellulose (18 g.) was dissolved in water (175 ml.) and solid sodium hydroxide (50 g.) added. Methyl sulfate (150 ml.) and sodium hydroxide solution (350 ml.) were added with vigorous stirring over a 3-hr. period and acetone was added periodically to control the foaming. During this period the temperature was maintained at 50° and finally was raised to 90° for 0.5 hr. Since the partly methylated polysaccharide did not separate, the cooled solution was dialyzed overnight against running water and the polysaccharide recovered by evaporation to a thick sirup. The methylation was repeated five more times and dialysis was necessary each time. The solution from the final dialysis was concentrated to *ca.* 350 ml., cooled in ice and acidified with ice-cold hydrochloric acid (6 *N*). The curdy precipitate was dissolved in chloroform which was washed until free of chloride ion and then evaporated to a glassy solid (14 g.). The supernatant remaining after the precipitation of the polysaccharide was neutralized to *p*H 7 and extracted overnight with chloroform. A further quantity (1.5 g.) of polysaccharide was recovered which was not further examined.

The partially methylated hemicellulose (14 g.) was dissolved in acetone (50 ml.) and methyl iodide (100 ml.) added. Silver oxide (25 g.) was added in 5 portions over a 7-hr. period to the stirred and refluxing solution. Two further methylations were carried out using the same quantity of reagents but without the addition of acetone. There was finally obtained a glassy solid (6.7 g.) which was fractionated.

Fractionation of the Methylated Hemicellulose.—The methylated hemicellulose (6.7 g.) was dissolved in chloroform (100 ml.) and diluted with ethyl ether (100 ml.). A small amount of inorganic material was removed by centrifugation and the resulting clear orange solution fractionated by the addition of petroleum ether (30–60°).

Methanolysis of Methylated Hemicellulose.—Fraction 23b was considered to be homogeneous and the methoxyl value indicated a fully methylated pentosan. Accordingly 0.66 g. was dissolved in methanol (30 ml.) containing 2% hydrogen chloride and the solution refluxed until the rotation was constant (9 hr.). The solution was neutralized (Ag_2CO_3) and evaporated to give a sirup consisting of methyl glycosides (0.70 g.). The sirup was dissolved in saturated barium hydroxide solution (20 ml.), allowed to stand overnight at room temperature and finally heated for 1 hr. at 60°. The cooled aqueous solution was then continuously extracted with petroleum ether (30–60°, *ca.* 200 ml.) for 20 hr. The excess barium hydroxide was neutralized with Dry Ice, the solution was filtered and the filtrate passed through a column of Amberlite IR-120 resin to remove barium ions.

Separation and Identification of the Acidic Component.—The acidic eluate was passed through a column of Duolite A-4 resin which removed the acidic component. The neutral eluate was evaporated to a sirup (0.26 g.). The acidic component was eluted with sodium hydroxide (15 ml., 1 *N*) and the deep yellow solution passed through a fresh column of Amberlite IR-120 resin. The colorless eluate was strongly acidic and was evaporated to dryness (0.195 g.).

TABLE II

FRACTIONATION OF METHYLATED HEMICELLULOSE

Fraction	Total petroleum ether added, ml.	Weight, g.	$[\alpha]^{23}D$ ($CHCl_3$)	OMe, %
1	190	0.67	−30.8°	..
2	220	.53[c]	−28.9	38.8
3	260	.55[c]	−29.4	39.2
4	310	.83	−24.1	..
5	410	.70	−20.1	..
6[a]	..	1.42	− 5.4	..
7[b]	..	0.42	....	
23a	40	0.14	−32.9	..
23b	55	.66	−37.5	39.4
23c	80	.10	−22.6	..

[a] By precipitation of mother liquor from fraction 5 into an excess of petroleum ether. [b] By evaporation of mother liquor from fraction 6. [c] Amalgamate fractions 2 and 3; refractionate to give 23a, b, c.

The acidic component was dissolved in methanol (15 ml.) and treated with ethereal diazomethane until a green color persisted. The ester (0.186 g.) was recovered by evaporation and was reduced with lithium aluminum hydride as previously described.[5] There was thus obtained methyl 2-*O*-(2,3,4-tri-*O*-methyl-D-glucosyl)-3-*O*-methyl-D-xyloside, on seeding with an authentic sample, having m.p. and mixed m.p. 164–167° and $[\alpha]^{23}D$ +84° (*c* 0.8 in water).

Separation and Identification of the Neutral Components.—The petroleum ether extract (0.12 g.) and the neutral eluate from the Duolite A-4 resin (0.26 g.) were combined and the mixed glycosides hydrolyzed by heating with sulfuric acid (30 ml., 1 *N*) on a steam-bath until the rotation was

(13) G. O. Aspinall and J. E. McKay, *J. Chem. Soc.*, 1059 (1958).

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

(15) G. G. S. Dutton, S. A. McKelvey and G. Murata, to be published.

(16) B. Radhakrishnamurty and V. R. Srinivasan, *Proc. Indian Acad. Sci.*, **49**, 98 (1959).

(17) All evaporations were carried out at *ca.* 15 mm. and at a bath temperature not exceeding 40°.

constant (8 hr.). The solution was neutralized ($BaCO_3$) and evaporated to a sirup which was extracted once with methanol to remove a small amount of inorganic matter. A mixture of the methylated sugars (0.353 g.) was dissolved in butanone–water azeotrope (2 ml.) containing a few drops of methanol and added to the top of a cellulose–hydrocellulose column (40 × 2.8 cm. i.d.). Two drops of Sudan IV was added to mark the front and the column developed with butanone–water azeotrope. The column was jacketed at 30° and the front time was 3.5 hr. with a rate of flow of 25 ml./hr. Tubes 1 (from the dye)–50 were collected every 15 min. and thereafter at 30-min. intervals, with the results shown in Table III.

TABLE III

SEPARATION OF NEUTRAL SUGARS

Tube number	Component number	Identity
3-11	1	2,3,5-Tri-*O*-methyl-L-arabinose
		2,3,4-Tri-*O*-methyl-D-xylose
13-27	2	2,3-Di-*O*-methyl-D-xylose
60-85	3	2- and 3-*O*-methyl-D-xylose

Component 1.—The sirup (40.3 mg.), which had a very slight positive rotation, was shown readily by paper chromatography (butanone–water) to be a mixture. Using the values $[\alpha]D$ −38.5° (*c* 1 in methanol) for 2,3,5-tri-*O*-methyl-L-arabinose and $[\alpha]D$ 18.5° (*c* 1 in methanol) for 2,3,4-tri-*O*-methyl-D-xylose, it was concluded from the rotation of the sirup that there were present *ca.* 13 mg. of the arabinose derivative and 27 mg. of the xylose derivative.

When the sirup was dissolved in methanol containing 1% HCl, the solution became strongly positive in rotation and reached a constant value in 3.5 hr. After neutralization (Ag_2CO_3) the mixture of neutral sugar and furanoside was separated on the cellulose-hydrocellulose column. The neutral sugar was detected with *p*-anisidine spray in tubes 10–21 (10 min. fractions) and the furanoside in tubes 1–8 (positive Molisch and negative *p*-anisidine).

Component 1a. 2,3,5-Tri-*O*-methyl-L-arabinose.—The sirup obtained in tubes 1–8 was hydrolyzed with sulfuric acid and on chromatographic examination it was shown to consist of a single component and to behave in the same way as an authentic sample of 2,3,5-tri-*O*-methyl-L-arabinose.

Component 1b. 2,3,4-Tri-*O*-methyl-D-xylose.—The sirup from tubes 10–21 (20 mg.) partly crystallized on seeding and was characterized as the crystalline 2,3,4-tri-*O*-methyl-N-phenyl-D-xylosylamine, m.p. and mixed m.p. 98–100°.

Component 2. 2,3-Di-*O*-methyl-D-xylose.—This component (256 mg.) crystallized on seeding and after recrystallization had m.p. and mixed m.p. 75–77°.

Component 3. Mixture of 2- and 3-*O*-Methyl-D-xyloses.—The sirup (36 mg.) had $[\alpha]^{23}D$ +19.8° and was shown by paper chromatography for 20 hr. in butanone–water to be a mixture of monomethylxyloses. From tubes 60–62 and 81–85 it was possible to obtain chromatographically pure samples corresponding to 3- and 2-*O*-methyl-D-xylose, respectively, but not in sufficient quantity to prepare any crystalline derivatives. From the rotation of the sirup it was judged to contain *ca.* 78% 3-*O*-methyl-D-xylose and 22% 2-*O*-methyl-D-xylose.

Acknowledgment.—The authors are grateful to the National Research Council of Canada for continued financial support and to Dr. J. K. N. Jones F.R.S. for the gift of crystalline 2,3-di-*O*-methyl-D-xylose.

VANCOUVER, B. C., CANADA

[CONTRIBUTION FROM THE MERCK, SHARP AND DOHME RESEARCH LABORATORIES]

Alkylated Adrenal Hormones. The Synthesis of 5-Methylated Pregnanes

BY JOHN H. FRIED, GLEN E. ARTH AND LEWIS H. SARETT

RECEIVED AUGUST 14, 1959

The synthesis of 5-methylpregnane-11β,17α,21-triol-3,20-dione acetate and 5-methyl-1-pregnene-11β,17α,21-triol-3,20-dione acetate *via* angular methylation of a suitably protected 6-ketone II is described.

Although there is no evidence that allo-dihydrohydrocortisone exhibits anti-inflammatory activity when administered systemically to animals, local activity has been demonstrated with this compound.[1] The possibility that the lack of systemic activity could be due to rapid reduction of the 3-ketone function, prompted us to prepare the sterically hindered 5-methyl analog. This paper describes the synthesis of 5-methylpregnane-11β,17α,21-triol-3,20-dione 21-acetate and 5-methyl-1-pregnene-11β,17α,21-triol-3,20-dione 21-acetate. Chuman[2] has described the preparation of 5α-methylcholestane-3β,6β-diol by reaction of cholesteryl-β-oxide and methylmagnesium iodide. Attempts to utilize this reaction with 17α,20,20,21-bismethylenedioxy-3-ethylenedioxy-5,6β-oxidopregnane-11β-ol (I) did not give the desired 5α-methyl product but a compound of unknown constitution.

However, 17α,20,20,21-bismethylenedioxy-3-ethylenedioxy-allo-pregnane-6,11-dione (II), recently reported,[3] appeared to be a suitable starting material for the projected synthesis. Although II possesses carbonyl functions at C-6 and C-11, selective alkylation of the 6-ketone was judged to be the more likely possibility due to the relatively unreactive character of saturated 11-ketosteroids[4a]; furthermore, on the basis of the stabilities[4b] of the enolates involved and in the absence of over-riding steric effects, preferential methylation of II at C-5 appeared to be indicated.[5] The two possible enolic forms of the C-6 ketone have double bonds between carbons five and six and six and seven. Turner[6] has shown that in the cholestane series the former is *ca.* 1.5 kcal. more stable than the latter. This is in agreement with the initial formation of a C-5 substituted bromide as the result of bromination of 6-ketosteroids.[7]

Initial attempts to methylate II using either potassium *t*-butoxide in *t*-butyl alcohol or sodium hydride in benzene were unsuccessful; however, addi-

(1) J. L. Hollander, E. M. Brown, Jr., R. A. Jessar, L. Udell, N. Smukler and M. A. Bowie, *Ann. Rheumatic Diseases*, **13**, 297 (1954).

(2) M. Chuman, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **70**, 253 (1949).

(3) J. H. Fried, G. E. Arth and L. H. Sarett, THIS JOURNAL, **81**, 1235 (1959).

(4) (a) Private communication from Dr. R. E. Beyler; *cf.*, however, E. R. H. Jones, G. D. Meakins and J. S. Stephenson, *J. Chem. Soc.*, 2156 (1958), for the methylation of unsaturated 11-ketosteroids; (b) *cf.* H. M. E. Cardwell, *ibid.*, 2442 (1951).

(5) *Cf.* D. A. Peak and R. Robinson, *ibid.*, 1581 (1937).

(6) R. B. Turner, W. R. Meador and R. E. Winkler, THIS JOURNAL, **79**, 4122 (1957).

(7) I. M. Heilbron, E. R. H. Jones and F. S. Spring, *J. Chem. Soc.*, 801 (1937).