Carbohydrate Research, 214 (1991) 107–113
Elsevier Science Publishers B.V., Amsterdam

A novel L-fuco-4-*O*-methyl-D-glucurono-D-xylan from *Hyptis suaveolens**

Gerald O. Aspinall, Peter Capek[†], Roshan C. Carpenter, D. Channe Gowda[‡], and Janusz Szafranek**
Department of Chemistry, York University, North York, Toronto, Ontario M3J 1P3 (Canada)

(Received September 6th, 1990; accepted for publication November 1st, 1990)

ABSTRACT

The acidic polysaccharide from the seed-coat mucilage of *Hyptis suaveolens* is a highly branched L-fuco-4-*O*-methyl-D-glucurono-D-xylan for which a structure is proposed having a 4-linked β-D-xylan backbone carrying side chains of single 4-*O*-methyl-α-D-glucuronic acid residues at O-2 and 2-*O*-L-fucopyranosyl-D-xylopyranose units at O-3. The structural analysis involves base-catalyzed β-elimination of uronic acid residues from the methylated glycan followed by degradation using a modified Svensson oxidation-elimination sequence.

INTRODUCTION

Previous studies by one of us[1] showed that the polysaccharide preparation from the seed-coat mucilage of *Hyptis suaveolens* contains a neutral and an acidic polysaccharide. The acidic glycan was shown to contain residues of L-fucose, D-xylose, and 4-*O*-methyl-D-glucuronic acid in the ratio of 1:2.5:1.1. The infrequent occurrence of L-fucose in glycans from higher plants[2] and the absence of previous evidence for this sugar as a constituent of polysaccharides of the D-xylan family prompted us to undertake a more-detailed examination of the *Hyptis suaveolens* glycan.

DISCUSSION

Examination of the hydrolysis products of the carboxyl-reduced glycan[1] by g.l.c.–m.s. of the derived 1-deuterioalditol acetates showed the presence of a derivative of 4-*O*-methylglucose and confirmed the previously assumed location of the methyl ether substituent in the original uronic acid. Linkage analysis of the methylated carboxyl-reduced glycan (A) by g.l.c.–m.s. of the partially methylated alditol acetates (Table I) established its highly branched character with a variety of linkage types and

* Presented at the 15th International Carbohydrate Symposium, Yokohama, Japan, August 12–17, 1990.
[†] On leave of absence from the Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Czechoslovakia, 1988–1989.
[‡] Department of Biochemistry, Georgetown University Medical School, Washington, D.C. 20007, U.S.A.
** On leave of absence from the Department of Chemistry, University of Gdansk, Poland, 1987–1988.

0008-6215/91/$ 03.50

showed that fucopyranose and glucopyranose (from the uronic acid) residues were present exclusively as non-reducing end-groups. The fragment ions of the 1-deuterio-di-*O*-methylxylitol triacetate fraction showed this to be a mixture of the otherwise enantiomeric 2,3- and 3,4-dimethyl ethers. The fragment ions of the 1-deuterio-mono-*O*-methylxylitol tetraacetate fraction showed it to consist of both 2- and 3-monomethyl ethers arising from two types of branch point, probably with side-chains attached at O-2 and O-3 of 4-linked xylopyranose residues.

Aqueous solutions of the acidic glycan tend to gel in water and ^{1}H-n.m.r. spectra (D_2O) gave only broad signals. ^{13}C-N.m.r. spectra were more informative, showing compositional features from resonances at δ 173.6 (CO_2H), 81.9 (C-4 of 4-Me-Glc*p*A), 59.9 (OCH_3), and 15.5 (C-6 of Fuc*p*). Four major signals among several resonances in the anomeric region gave reasonably well resolved doublets in the proton-coupled spectrum. Resonances at δ 101.8 (J 156 Hz), 100.8 (J 160 Hz), 99.3 (J 175 Hz), and 98.0 (J 173 Hz) may be assigned to two β- and two α-glycosidic residues.

Evidence for the site of attachment of the 4-*O*-methyl-D-glucuronic acid residues as single unit side-chains was obtained by base-catalyzed degradation[34] of the methylated acidic glycan (**B**). Treatment of this methylated glycan with dimsyl base afforded base-degraded methylated glycan **C**. Comparison of the ^{13}C-n.m.r. spectra of the parent (**B**) and base-degraded (**C**) methylated glycans confirmed the removal of uronic acid residues through the disappearance of resonances at δ 170.6 (CO_2Me) and 52.3 (CO_2Me). Whereas the ^{13}C-n.m.r. spectrum of the parent methylated glycan **B** showed *inter alia* two clearly defined anomeric-carbon resonances at δ 97.1 and 96.3, the disappearance of the latter resonance in methylated, base-degraded glycan **C** allows the

TABLE I

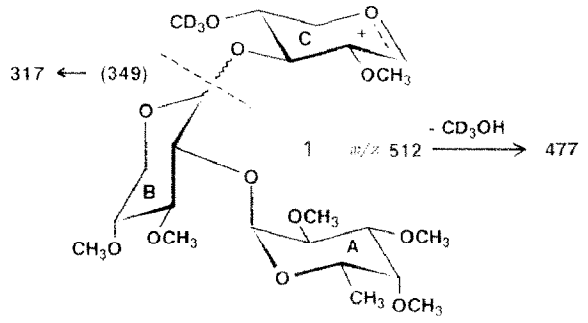
Sugar analysis of methylated *Hyptis suaveolens* glycan derivatives

Methylated alditol acetate	*Relative proportions*[a]		
	(A)[b]	(D)[b]	(E)[b]
2,3,4-Me_3Fuc	18	18	18
2,3,4,6-Me_4Glc	22		
2,3-Me_2Xyl	11	11	13[c]
3,4-Me_2Xyl[d]	11	11	
2*,3-Me_2Xyl[e]		11	
2,4*-Me_2Xyl[e]			4
2-MeXyl[f], 3-MeXyl[f]	37	28[g]	10

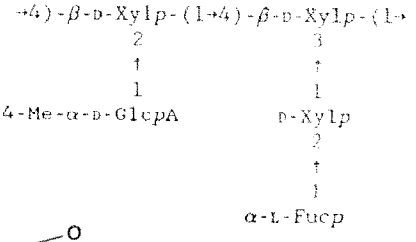
[a] Normalized with respect to Me_3Fuc. [b] (A) Methylated carboxyl-reduced glycan; (D) base-degraded methylated glycan after trideuteriomethylation; (E) base-degraded methylated glycan after oxidation–elimination and trideuteriomethylation. [c] Equimolar proportions as shown by abundances of primary fragment-ions at m/z 117 and 188, 189, and 190. [d] * Indicates location of trideuteriomethyl substituent. Approximate quantitation of Me_2Xyl isotopomers based on ratios of fragment ions at m/z 117, 118, and 121, and changes (relative to [c]) in ratios of ions at m/z 189 and 190. [f] Approximately equimolar proportions based on ratios of ions at m/z 118 and 120. [g] Relative decrease in abundances of ions due to 3-MeXyl component.

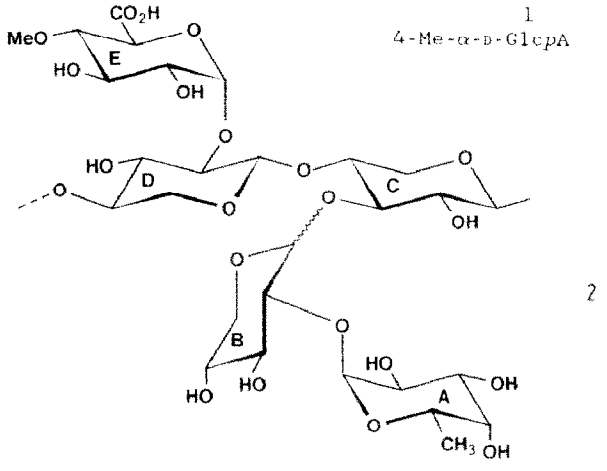
assignment of the α-D-configuration to the original 4-*O*-methyl-D-glucuronic acid residues. Trideuteriomethylation of degraded methylated glycan C furnished methylated glycan D which on sugar analysis (Table I) showed a decrease in the proportion of the mono-*O*-methylxylitol fraction (especially of the 3-*O*-methylxylitol component) and a corresponding increase in the proportion of the di-*O*-methylxylitol fraction. Examination of the mass spectrum of the latter fraction showed the presence of fragment ions of the aforementioned 2,3- and 3,4-di-*O*-methylxylitol acetates and in addition those of 2-*O*-trideuteriomethyl-3-*O*-methylxylitol triacetate with an ion at *m/z* 121 and an increase in the abundance of the ion at *m/z* 189 over that in the di-*O*-methylxylitol fraction from the methylated carboxyl-reduced glycan. These results clearly show that the 4-*O*-methyl-α-D-glucuronic acid residues in the *Hyptis suaveolens* polysaccharide are linked to O-2 of residues in the D-xylan backbone as in the 4-*O*-methyl-D-glucurono-D-xylans from woods and other higher plants[2].

The two unusual features of the *Hyptis* glycan are the terminal L-fucopyranose and the 2-linked D-xylopyranose residues, the first being necessarily and the second considered possibly located in side chains to the 4-linked D-xylan. Evidence for the presence of a disaccharide unit as a side-chain was obtained by the application of a modification[5] of the Svensson degradation[6-8] that takes place with decomposition of only those sugar residues at which oxidation takes place and thus results in minimum loss of structural information. Base-degraded methylated glycan C was oxidized with dimethyl sulfoxide and trifluoroacetic anhydride [Swern oxidation[9]], and then treated with triethylamine. The mixture was reduced with sodium borodeuteride and then heated with cation-exchange resin to effect mild acid hydrolysis of pent-3-enopyranosyl-2-ulose residues, and the product was trideuteriomethylated to give degraded methylated glycan E. We have stressed elsewhere[10] that the conditions required for the modification of structurally related model compounds may not always be simply translated to polysaccharide substrates. In this instance it was not possible to ensure that all the 2-hydroxyl groups of the branch points formerly bearing uronic acid side-chains had been oxidized or that subsequent reactions had gone to completion. Even with incomplete reaction and thus limited chain cleavage (so that the quantitative significance of the compositional analysis of degraded methylated glycan E may be taken with reserve), qualitatively unambiguous conclusions may be drawn from the formation of 2-*O*-methyl-4-*O*-trideuteriomethylxylitol triacetate as the only detectable isotopically labelled methylated sugar and the absence of derivatives from any xylopyranose end groups (Table I). Information on the placing of the sugar residue giving rise to this derivative was obtained from the fast-atom bombardment (f.a.b.) mass spectrum of methylated degraded glycan E which showed prominent fragment ions at *m/z* 189, 317, 477, and 512. The generation of a glycosyl cation **1** at *m/z* 512 may be considered to arise from a terminal trisaccharide unit (residues A, B, and C). Regions in the methylated 4-linked xylan, for which partial structure **2** may be proposed for the parent glycan, could furnish such a terminal trisaccharide unit through a sequence of reactions involving (*i*) base-catalyzed loss of uronic acid (residue E) to give methylated glycan C (**3**); (*ii*) oxidation and β-eliminative cleavage during work-up with triethylamine to **4**;

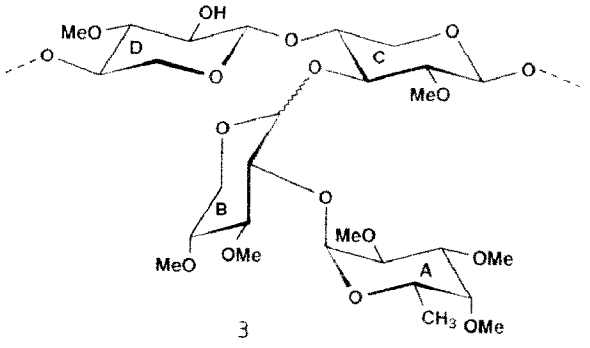

CD3O
C
317 ← (349)
OCH3
- CD3OH
m/z 512
477
B
CH3O
OCH3
OCH3
A
OCH3
CH3
OCH3

1

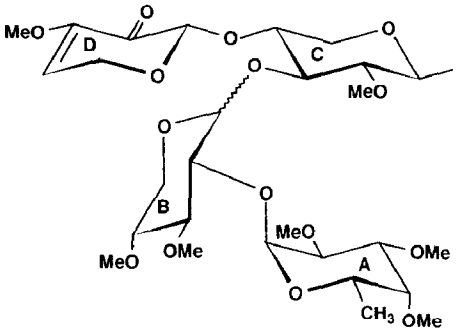

→4)-β-D-Xylp-(1→4)-β-D-Xylp-(1→
2
3
1
1
4-Me-α-D-GlcpA
D-Xylp
2
1
α-L-Fucp


CO2H
MeO
E
HO
OH
HO
D
C
OH
B
HO
HO
HO
OH
A
CH3
OH

2


OH
MeO
D
C
MeO
B
MeO
OMe
MeO
OMe
A
CH3
OMe

3



4

and (*iii*) reduction and mild acid hydrolysis, with loss of residue D, and further alkylation of exposed hydroxyl groups in residue C. Glycosyl cation **1** is the fragment ion of lowest mass in which a trideuteriomethyl group could be recognized and the ready loss of trideuteriomethanol in a characteristic secondary fragmentation places the trideuteriomethyl substituent on residue C which bears the fucopyranosylxylopyranose unit, but was originally present as a branching residue in the xylan backbone.

The *Hyptis suaveolens* polysaccharide provides an interesting addition to the xylan family[2]. Our evidence defines both types of side-chain, single 4-*O*-methyl-D-glucuronic acid and L-fucopyranosyl-D-xylopyranose units, as attached respectively to O-2 and O-3 of different 4-linked xylopyranose residues. It is considered probable that unbranched xylopyranose residues in the backbone are also 4-linked, and that the 2-linked chain residues are more likely to be accommodated in the fucopyranosyl-xylopyranose side-chains. Assignment of anomeric configurations for the different glycosyl residues is certain only for the 4-*O*-methyl-α-D-glucuronic acid residues, and that those of others is not yet complete. The close relationship of this glycan to other 4-*O*-methylglucuronoxylans would strongly suggest that β-D-configurations may be assigned to backbone xylopyranose residues. α-L-Fucopyranose residues are of most frequent occurrence and may be proposed for these units in this glycan. With incomplete resolution of anomeric carbon resonances in the ^{13}C-n.m.r. spectrum, no conclusions can yet be drawn concerning the configuration of the 2-linked D-xylopyranose residues in the disaccharide side-chains.

EXPERIMENTAL

General methods. — Sugars and their methylated derivatives were determined as their alditol acetates by g.l.c.–m.s.[11]. Methylations of glycan derivatives were performed by the Hakomori procedure as described by Jansson *et al.*[11], and the methylated derivatives were hydrolyzed with 2M CF_3CO_2H for 1 h at 120°. Uronic acid determinations were carried out spectrophotometrically with the 3-hydroxydiphenyl reagent[12]. Other experimental methods are described in the accompanying paper[5].

Samples of polysaccharide and the carboxyl-reduced derivative were those described previously[1]. When further quantities of acidic glycan were required samples were purified by ion-exchange chromatography on DEAE-Sephadex A-50 (carbonate form) and elution with 0.3 and 0.5M ammonium carbonate.

Composition of carboxyl-reduced glycan. — Hydrolysis of carboxyl-reduced glycan gave fucose, xylose, and 4-*O*-methylglucose in the molar ratio of 1:3.1:1.2. The acetylated alditol from the reduced uronic acid component gave fragment ions at *m/z* 189 and 262 in confirmation of the location of the methyl ether substituent.

Uronic acid degradation of methylated acidic glycan. — Methylated acidic glycan B (50 mg) was kept in 0.5M sodium sulfinylmethanide (4 mL) for 4 days at 60°. The cooled solution was neutralized with AcOH, was poured into water and extracted with $CHCl_3$. Extracts were washed with water, dried, concentrated, and chromatographed on Sephadex LH-20 to give methylated glycan C (26 mg) which showed no ester carbonyl absorption at 1720–1740 cm^{-1}.

Degradation of methylated glycan C by oxidation–elimination reactions and analysis of the product. Trifluoroacetic anhydride (900 μL) in CH_2Cl_2 (1 mL) was added at −70° to dry Me_2SO (600 μL), in CH_2Cl_2 (4mL), the mixture was stirred for 30 min, and methylated glycan C (26 mg) in CH_2Cl_2 (2 mL) was added with maintenance of temperature below −65°. After 4 days Et_3N (1.6 mL) was added, the mixture was brought to room temperature, washed with saturated NH_4Cl solution, washed with water, dried and concentrated. The residue was kept overnight in CH_2Cl_2 containing ~10% of Et_3N. Concentration of the solution followed by chromatography on Sephadex LH-20 in CH_2Cl_2 furnished a residue (14 mg) whose i.r. spectrum showed absorption at 1710 cm^{-1} (C=O) and 1635 cm^{-1} (C=C). The residue was successively reduced with $NaBD_4$ and heated with Amberlite IR-120 (H^+) resin at 80° as described in the accompanying manuscript, and the product was trideuteriomethylated to give methylated glycan D (4 mg).

ACKNOWLEDGMENTS

The authors thank the Natural Sciences and Engineering Research Council of Canada for financial support, Dr. B. H. Khouw for recording electron-impact mass spectra, and Dr. H. Pang, Carbohydrate Research Centre, Faculty of Medicine, University of Toronto for the fast-atom bombardment mass spectrum.

REFERENCES

1 D. C. Gowda, *Phytochemistry*, 23 (1984) 337–338.
2 A. M. Stephen, in G. O. Aspinall (Ed.), *The Polysaccharides*, Vol. II, Academic Press, New York, 1983, pp. 97–193.
3 B. Lindberg, J. Lönngren, and J. L. Thompson, *Carbohydr. Res.*, 28 (1973) 351–357.
4 G. O. Aspinall and K.-G. Rosell, *Carbohydr. Res.*, 57 (1977) c23–c27.
5 G. O. Aspinall, P. Capek, R. C. Carpenter, and J. Szafranek, *Carbohydr. Res.*, 214 (1991) 95–105.
6 P.-E. Jansson, L. Kenne, B. Lindberg, H. Liunggren, J. Lönngren, U. Rudén, and S. Svensson, *J. Am. Chem. Soc.*, 99 (1977) 3812–3815.

7 S. Svensson, *Methods Enzymol.*, 50 (1978) 33–38.
8 S. Svensson and L. Kenne, *Methods Carbohydr. Chem.*, 8 (1980) 67–81.
9 K. Omura and D. Swern, *Tetrahedron*, 34 (1978) 1651–1660.
10 G. O. Aspinall, *Acc. Chem. Res.*, 20 (1987) 114–120.
11 P.-E. Jansson, L. Kenne, H. Liedgren, B. Lindberg, and J. Lönngren, *Chem. Commun., Univ. Stockholm*, 8 (1976) 1–75.
12 N. Blumenkrantz and G. Asboe-Hansen, *Anal. Biochem.*, 54 (1973) 484–493.