Carbohydrate Research, 52 (1976) 255–258

Note

Linkage of pyruvyl groups in the specific capsular polysaccharide of *Pneumococcus* type IV*

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(Received April 26th, 1976; accepted for publication, June 23rd, 1976)

Pyruvyl groups, linked as an acetal to the 4- and 6-hydroxyl groups of D-galactose, were first shown to occur in a polysaccharide of seaweed by Hirase[1]. They have since been found in many bacterial polysaccharides, usually as an acetal bound to two hydroxyl groups[2–9]. The pyruvyl group is a powerful immunodeterminant when linked to a sugar in this fashion[2], and its removal gives rise to marked changes in immunological specificity[2,10]. The pyruvyl group is a substituent in the capsular specific polysaccharide of pneumococcal type IV[2,11] (S IV), and its linkage is the subject of the present report.

Hydrolysis of methylated S IV yielded 79% of 6-*O*-methyl-D-galactose, 21% of 2,3,6-tri-*O*-methyl-D-galactose, and a trace of 4,6-di-*O*-methyl-D-galactose. Under the same hydrolytic conditions, methylated depyruvylated S IV (dpS IV) gave 98% of 2,3,6-tri-*O*-methyl-D-galactose and about 2% of 2,3,4,6-tetra-*O*-methyl-D-galactose. The identity of the methylated D-galactoses was confirmed by the R_F values on t.l.c. (Table I) and by the retention times of the alditol acetate derivatives of the methylated D-galactoses on g.l.c. (Table II).

The presence of 2,3,6-tri-*O*-methyl-D-galactose in the hydrolyzate of methylated S IV is presumably due to methylation of dpS IV generated from S IV under the alkaline conditions of the methylation. As much as 2% of pyruvic acid was released[11] from S IV by M sodium hydroxide solution for 1.5 h, even at 0°. The 4,6-di-*O*-methyl-D-galactose found in the hydrolyzate of methylated S IV and the 2,3,4,6-tetra-*O*-methyl-D-galactose found in the hydrolyzate of methylated dpS IV were apparently derived from residues of D-galactose located at the nonreducing end of the polysaccharide chain.

It was shown that the pyruvyl group is linked only to D-galactose[11]. Thus, the isolation of 6-*O*-methyl-D-galactose as the major, methylated neutral sugar

*Aided by a grant (BMS 73-00968 A01) from the National Science Foundation (U. S. A.).
† J. Y. Lew also held a training-grant fellowship (AI-00392-04) from the National Institutes of Health (U. S. A.).

from methylated S IV and 2,3,6-tri-*O*-methyl-D-galactose and a trace of 2,3,4,6-tetra-*O*-methyl-D-galactose from methylated dpS IV establishes that the pyruvyl group is linked to O-2 and O-3 of the D-galactose residues.

TABLE I

T.L.C. OF METHYLATED SUGARS ON SILICA GEL AND CELLULOSE

Compound	R_{TMG}[a]			
	Silica gel		*Cellulose*	
	A[b]	*B*[c]	*C*[d]	*D*[e]
2-*O*-Methyl-D-galactose	16	57	60	51
3-*O*-Methyl-D-galactose	16	51	56	46
4-*O*-Methyl-D-galactose	11	47	56	45
6-*O*-Methyl-D-galactose	18	50	58	50
6-*O*-Methyl-D-galactose isolated from methylated S IV	18	50	59	50
2,4-Di-*O*-methyl-D-galactose	47	77	72	65
2,6-Di-*O*-methyl-D-galactose	61	85	75	69
4,6-Di-*O*-methyl-D-galactose	42	78	72	66
2,3,4-Tri-*O*-methyl-D-galactose	78	88	87	85
2,3,6-Tri-*O*-methyl-D-galactose isolated from dpS IV	89	94	89	87
3,4,6-Tri-*O*-methyl-D-galactose	82	89	84	82
2,3,4,6-Tetra-*O*-methyl-D-galactose	98	98	98	87
2,3,4,6-Tetra-*O*-methyl-D-glucose	100	100	100	100

[a]Abbreviation: TMG, 2,3,4,6-tetra-*O*-methyl-D-glucose. [b]Development 3 times in solvent *A*, [c]development twice in solvent *B*, [d]development once in solvent *C*, and [e]development once in solvent *D*

TABLE II

RELATIVE RETENTION TIMES OF ALDITOL ACETATE DERIVATIVES OF METHYLATED SUGARS

Compound	T[a]
2,3,4,6-Tetra-*O*-methyl-D-glucitol	1.00
2,3,4,6-Tetra-*O*-methyl-D-galactitol	0.83
2,3,4-Tri-*O*-methyl-D-galactitol	2.40
2,3,6-Tri-*O*-methyl-D-galactitol from methylated dpS IV	1.80
2,4-Di-*O*-methyl-D-galactitol	4.20
2,6-Di-*O*-methyl-D-galactitol	2.55
4,6-Di-*O*-methyl-D-galactitol	2.75
3-*O*-Methyl-D-galactitol	6.76
6-*O*-Methyl-D-galactitol	3.44
6-*O*-Methyl-D-galactitol from methylated S IV	3.41

[a]Relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

EXPERIMENTAL

General methods. — Silica gel and cellulose, thin-layer sheets were purchased from Eastman Kodak Co., Rochester, N.Y., 14650, preparative, silica gel plates from Brinkmann Instruments Inc., Westbury, N.Y. 11590.

Gas-liquid chromatography was performed in a Hewlett–Packard model 7660 A gas chromatograph equipped with a dual-flame, ionization detector, and a 3 m × 3 mm (o.d.) stainless-steel column, packed with Gas Chrom Q (60–80 mesh), pre-coated with 3% ECN SS-M (Applied Science Labs. Inc., State College, Pa. 16801). Alditol acetate derivatives of methylated sugars were prepared according to Kim *et al.*[12]; the relative proportions were calculated from the relative areas of the g.l.c. peaks (0.5 height × width at this level).

Solvent systems used for chromatography were: (*A*) 1:1 (v/v) acetone–benzene, (*B*) 2:2:1 (v/v) acetone–benzene–ethanol, (*C*) 40:11:2:19 (v/v) butanol–ethanol–pyridine–water, and (*D*) 4:1:5 (v/v) butanol–ethanol–water (organic phase).

Material. — S IV polysaccharide from Merck, Sharp, and Dohme was further purified with alcohol as the Li salt. In gel-diffusion against antibodies to pneumococcal C-substance, it gave a weak positive reaction. S IV was depyruvylated by heating with 5mM H_2SO_4 for 30 min at 80°, followed by exhaustive dialysis against water.

Methylation procedure. — Methylation of S IV (217 mg) and dpS IV (194 mg) was performed according to Conrad's modification of Hakomori's procedure[13]. Each product was dialyzed against running tap-water overnight and extracted 4 times with chloroform. Appropriately combined extracts were washed with water, and the chloroform was removed by distillation under reduced pressure (yields, 117 mg from S IV and 158 mg from dpS IV). Both compounds were free from hydroxyl groups by i.r. absorption analysis. The aqueous solutions of presumably partially methylated products were concentrated *in vacuo* and kept for further examination.

The methylated polysaccharides were first hydrolyzed in sealed tubes under N_2 with 90% formic acid for 4 h at 100° and the solutions evaporated to dryness at 45° under reduced pressure. Each residue was suspended in 0.5M H_2SO_4 and hydrolyzed for 16 h at 100° in a sealed tube, neutralized with a saturated $Ba(OH)_2$ solution, and the $BaSO_4$ was centrifuged off. After concentration *in vacuo* in a rotary evaporator, each hydrolyzate was applied to a preparative silica gel plate and developed 3 times in solvent *B* to separate the methylated neutral and amino sugars. A guide strip on one side was stained with ninhydrin and one on the other side with 3% *p*-anisidine in 95% ethanol. Sections containing the methylated sugars were scraped off the plate and eluted with 95% ethanol.

6-*O*-Methyl-D-galactose isolated from methylated S IV was crystallized from absolute ethanol, m.p. 122–124°(lit.[14]: m.p. 122–123°). A portion was converted into the phenylhydrazone, which was crystallized from absolute methanol, m.p. 176–177° (lit.[14]: m.p. 179°).

The presumed 2,3,6-tri-*O*-methyl-D-galactose from methylated, depyruvylated S IV did not crystallize and was converted into a 1,4-lactone[15,16]. About 1 mg in

water (1 ml) was treated with Br_2–H_2O (1 ml) for 70 h at room temperature. Unreacted Br_2 was removed by aeration and acid was neutralized with Na_2CO_3. Na^+ ions were removed by passage of the solution through a column of Ag 50 W (W-8, H^+) ion-exchange resin. The 2,3,6-tri-*O*-methyl-D-galactono-1,4-lactone formed long needles from peroxide-free ether, m.p. 99–100°; lit.[15]: m.p. 97–98°; lit.[16]: m.p. 96–97°; lit.[17]: m.p. 99°.

ACKNOWLEDGMENTS

We thank Dr. Herbert Kayden, Department of Medicine, New York University Medical Center, New York, N.Y., for use of g.l.c. equipment; Dr. G. G. S. Dutton, Department of Chemistry, University of British Columbia, Vancouver, Canada, Dr. C. P. J. Glaudemans, National Institutes of Health, Bethesda, Maryland, and Dr. Georg Springer, Northwestern University, Evanston, Illinois, for generous gifts of methylated D-galactoses; and Dr. H. E. Conrad, Department of Biochemistry, University of Illinois, Urbana, Illinois, for assistance and laboratory facilities in the preparation of the methylated polysaccharides.

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