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A Method for Gas–Liquid Chromatographic Identification of *O*-Methyl Ethers of 2-Deoxy-2-methylamino-D-glucose and 2-Deoxy-2-methylamino-D-galactose[1]

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A method is described for the g.l.c. identification of *O*-methyl ethers that can be formed on methylation–fragmentation analysis of polymers containing 2-acetamido-2-deoxy-D-glucopyranose or 2-acetamido-2-deoxy-D-galactopyranose units. The possible 2-deoxy-*O*-methyl-2-methylamino-D-glucoses and -D-galactoses and their derived hexitols, were converted to their acetates and trimethylsilyl ethers. The g.l.c. was carried out using a number of liquid phases and the retention times of the derivatives were found to be sufficiently diverse to make positive identifications of unknowns feasible.

Une méthode est décrite pour identifier par c.l.g les éthers *O*-méthylés pouvant se former pendant les analyses par méthylation et fragmentation de polymères contenant soit l'unité acétamido-2 désoxy-2 D-glucopyranose soit l'unité acétamido-2 désoxy-2 D-galactopyranose. Les composés possibles, les désoxy-2 *O*-méthyl méthylamino-2 D-glucoses et D-galactoses ainsi que leurs dérivés hexitols, ont été transformés en acétates et éthers triméthylsililés correspondants. En utilisant un certain nombre de phases liquides, la c.l.g conduit, pour les dérivés, a des temps de retention suffisamment différents pour permettre une bonne identification des composés inconnus.

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Partially *O*-methylated 2-deoxy-2-methylamino derivatives of D-glucose (1) and D-galactose (2) have been synthesized to serve as standards in structural investigations of polymers or oligomers containing 2-acetamido-2-deoxy-D-glucopyranose and 2-acetamido-2-deoxy-D-galactopyranose units, respectively. These fragments can be formed when methylation by the Kuhn (3) (methyl iodide-*N*,*N*-dimethylformamide – silver oxide) or Hakomori (4, 5) (methyl iodide–sodium hydride–dimethyl sulfoxide) procedures are carried out and the product hydrolyzed by acid. Two effective methods for identification of the *N*-methyl hexosamine derivatives are by means of an amino acid analyzer and by g.l.c. as trimethylsilyl (t.m.s.) ethers (1, 2). The former method can be used (6–8) when a 0.2–1.0 mg sample of *O*-methyl ether is available. Each derivative has a characteristic retention time and gives an absorption maximum of 570 mμ on reaction with ninhydrin. When only microgram quantities are available, however, the g.l.c. method must be used. To increase the effectiveness of the g.l.c. analysis of complex mixtures of *O*-methyl sugars a more detailed investigation of the g.l.c. properties of the above standards was made. The present work describes the preparation of a number of new derivatives and the determination of their retention times on a variety of liquid phases.

A previous g.l.c. examination of the t.m.s. ethers of 2-deoxy-*O*-methyl-2-methylamino-D-glucoses (1) and their D-galactose isomers (2) showed that most of the hexoses can be distinguished, even though each compound generally gives rise to two peaks, arising from the α- and the β-anomer. However, Perry and Webb (9) have obtained a superior separation by g.l.c. of the acetates of derived 2-amino-2-deoxy-*O*-methyl-D-glucitols using a column containing neopentyl glycol sebacate (NPGSb) at 245°, where only one peak is formed from each parent hexitol. Although the acetates of 2-deoxy-*O*-methyl-2-methylamino-D-hexitols could neither be eluted from the column under these conditions nor from a column containing diethylene glycol adipate – diethylene glycol (4:1), a separation was obtained using a UC W-98 (a methyl vinyl silicone) liquid phase at 210° (Table 1). Different emergence orders were observed using the t.m.s. ethers on UC W-98 liquid phase at 160° and NPGSb liquid phase at 140° (Table 1). The acetates were prepared using acetic anhydride – pyridine at 100°, a process which results in formation of *O*-acetyl, *N*-acetyl derivatives. Treatment of the hexitols with trimethylsilyl chloride – hexamethyldisilazanepyridine at 100° (10) should result in formation of *O*-, but

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TABLE 1. Retention times, in minutes, of derivatives of 2-deoxy-*O*-methyl-2-methylamino derivatives of D-glucitol and D-galactitol

O-Methyl derivative of 2-deoxy-2-methyl-amino-D-hexitol	Retention time (min)		
	Trimethylsilyl derivatives on NPGSb at 140°*	Trimethylsilyl derivatives on UC W-98 at 160°†	Acetates on UC W-98 at 210°
Glucitol series			
Unsubstituted	9.4	8.40	3.85
3-	8.3	5.81	3.08
4-	7.8	5.63	3.60
6-	6.8	5.32	2.70
3,4-Di-	8.2	4.08	2.53
3,6-Di-	6.6	3.68	2.10
4,6-Di-	5.8	3.53	2.37
3,4,6-Tri-	6.8	2.62	1.65
Galactitol series			
Unsubstituted	8.8	7.99	4.00
3-	9.2	6.28	3.35
4-	7.6	5.55	3.80
6-	7.2	5.38	2.70
3,4-Di-	9.2	4.58	2.87
3,6-Di-	7.6	4.22	2.20
4,6-Di-	6.7	3.70	2.45
3,4,6-Tri-	9.6	3.05	1.88

*NPGSb, neopentylglycol sebacate.
†UC W-98, a methyl vinyl silicone.

not *N*-trimethylsilyl derivatives by analogy with the trimethylsilylation of galactosamine (11). The acetylation and etherification processes are effective since all of the resulting derivatives gave one peak on g.l.c.

Another series of retention times was obtained using the t.m.s. ethers of 2-deoxy-*O*-methyl-2-methylamino-D-hexoses on UC W-98 liquid phase at 160° and the corresponding acetates on UC W-98 at 210° (Table 2). The emergence orders differ from each other and from those obtained with the t.m.s. ethers on NPGSb at 140° (1, 2; Table 2).

It can be seen from Tables 1 and 2 that by use of the six different g.l.c. conditions a good resolution of the 2-deoxy-*O*-methyl-*N*-methyl-amino derivatives of D-glucose and D-galactose can be obtained together with a wide variance in their emergence orders. It should therefore be possible to methylate a polysaccharide known to contain 2-acetamido-2-deoxy-D-glucose or -D-galactose units, hydrolyze the product, and detect the resulting *O*-methyl-*N*-methyl derivative(s) in the presence of other *O*-methyl ethers. It appears that superimposition of the g.l.c. signals of these two types of compound under six analysis conditions is unlikely. An alternative method of identification of *N*-methyl sugars in a methylation product is possible in which they are concentrated by ion-exchange chromatography prior to g.l.c. A suggested procedure is reduction of the methylation mixture by sodium borohydride followed by preferential absorption of the *N*-methyl hexitol on a column of a cation-type ion exchange resin. It can then be eluted with aqueous ammonium hydroxide and examined by the g.l.c. procedure.

Experimental

Preparation of Derivatives for Gas–Liquid Chromatography

(1) Preparation of 2-Deoxy-O-methyl-2-methylamino-D-hexitols

N-Methyl-hexosamine hydrochlorides (10 mg) were dissolved in water (1 ml) containing sodium borohydride (5 mg). After 3 h excess reagent was destroyed with acetic acid and the solutions evaporated to dryness. Boric acid was removed as trimethyl borate by dissolving the residues in methanol and evaporating, a process that was repeated three times.

(2) Preparation of Acetates

Samples of *N*-methyl-hexosamine hydrochloride and *N*-methyl-hexosaminitol containing approximately 1 mg of material were treated with acetic anhydride – pyridine (0.2 ml:0.2 ml) at 100° for 2 h and the solutions then evaporated *in vacuo*. The residues were dissolved in chloroform (0.5 ml) and applied to the g.l.c. columns in 3 μl aliquots.

TABLE 2. Retention times, in minutes, of derivatives of 2-deoxy-*O*-methyl-2-methylamino derivatives of D-glucose and D-galactose on g.l.c.

O-Methyl derivative of 2-deoxy-2-methyl-amino-D-hexose	Retention times (min)		
	Trimethylsilyl derivatives on NPGSb at 140° (1, 2)	Trimethylsilyl derivatives on UC W-98 at 160°	Acetates on UC W-98 at 210°
Glucose series			
Unsubstituted	12.4(76%), 20.4(24%)	3.95(15%), 5.86(60%) 8.02(25%)	1.72(28%), 3.63(72%)
3-	9.4(81%), 12.8(19%)	3.54(90%), 4.56(10%)	1.39(15%), 3.35(85%)
4-	7.3(73%), 12.3(27%)	3.36(76%), 4.46(24%)	1.42(22%), 3.13(78%)
6-	13.8(78%), 16.6(22%)	4.45(73%), 5.10(27%)	1.17(25%), 2.39(75%)
3,4-Di-	6.5(84%), 9.4(16%)	2.30(99%), 2.70(1%)	1.07(8%), 2.49(92%)
3,6-Di-	10.4	2.59(83%), 2.88(17%)	0.88(8%), 2.17(92%)
4,6-Di-	8.1(80%), 10.6(20%)	2.35(78%), 2.80(22%)	0.90(13%), 1.95(87%)
3,4,6-Tri-	7.3	1.55	1.50
Galactose series			
Unsubstituted	9.2(67%), 12.9(23%)	4.90(71%), 5.40(29%)	1.92(21%), 3.57(39%) 3.73(39%)
3-	9.9 , 11.6	3.68	1.50(17%), 3.02(83%)
4-	9.5(78%), 14.5(22%)	3.60(76%), 4.44(24%)	1.50(35%), 3.29(65%)
6-	8.5(89%), 11.5(11%)	3.33(94%), 3.70(6%)	1.27(16%), 2.45(84%)
3,4-Di-	12.4(76%), 15.8(24%)	2.87(70%), 3.15(30%)	1.19(15%), 2.67(85%)
3,6-Di-	8.7 , 10.2	2.37(95%), 2.94(5%)	2.00(14%), 2.10(86%)
4,6-Di-	7.7(75%), 12.2(25%)	2.20(85%), 2.74(15%)	0.97(27%), 2.08(73%)
3,4,6-Tri-	10.2(76%), 12.8(24%)	1.69(89%), 1.88(11%)	0.74(20%), 1.67(80%)

(3) Trimethylsilyl Ethers

One milligram samples of sugar were converted to the t.m.s. ethers by the method of Sweeley *et al.* (10). Two-tenths of a milliliter of reagent was used and the solutions (3 μl) were applied directly to the g.l.c. column.

Gas–Liquid Chromatographic Procedure

The columns used are as follows: *(1)* a 3 ft × 1/8 in. (i.d.) glass column containing 10% of NPGSb (Applied Science Laboratories, State College, Pennsylvania) on silanized, acid-washed Chromosorb W (80–100 mesh), pretreated with Silyl 8 column conditioner (0.2 ml; Pierce Chemical Company). *(2)* A 4 ft × 1/8 in. (i.d.) glass column containing 3.8% of silicone gum UC W-98 (Chromatographic Specialties Ltd., Brockville, Ontario) on silanized, acid-washed Chromosorb W (80–100 mesh).

The g.l.c. unit was a Hewlett–Packard Model 402 incorporating a hydrogen flame detector operated under the following conditions: H_2 pressure: 12 p.s.i., rotameter reading 2.5; He pressure: 45 p.s.i., rotameter reading 3; air pressure: 30 p.s.i., rotameter reading 3.75.

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